

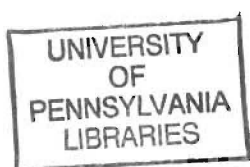
# **Human Tissue Plasminogen Activator**

**Chemical Engineering 459  
University of Pennsylvania  
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**Project Advisor: Dr. Scott Diamond  
Project Suggested by: Dr. Scott Diamond**

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April 18, 2000

Prof. Scott Diamond  
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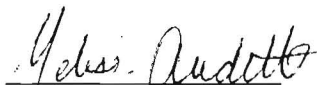
Dear Prof. Diamond and Dr. Kivnick,

Enclosed in this report is the completed design and economic analysis of the proposed human tissue plasminogen activator plant. The plant is designed to be implemented in the U.S. Northeast and will have a capacity to produce 80 kg of tPA per year or 1.28 MM doses per year.


The optimum plant design has 2 individual fermentation and separation trains. The fermentation trains grow the cells that produce our product in a scale-up process consisting of three different size vessels: 40 L, 400 L and the maximum 5000 L. The product is sent to a separation train, which purifies and sterilizes the protein for medical use. The plant also contains a reverse osmosis water purification system that is designed to produce all of the pure, pharmaceutical quality water used to make all of the process liquids and clean out the unit operations during sterilization.

An economic analysis of the plant was performed based on the design data using the spreadsheet provided by Holger Nickisch. The plant described within the report has an IRR of 59.7% based on a \$117 MM initial capital investment. The optimum selling price of the product tPA was determined using market share information provided by Prof. Diamond to be \$500.00 per dose. The group highly recommends construction of the plant.

Sincerely,

  
Melissa Audette

  
Christian Metallo

  
Kasidit Nootong



## **ABSTRACT**

Tissue plasminogen activator (tPA) has long been considered “the flagship product” of the biotechnology industry. This protein is a popular treatment for heart attacks, coronary heart disease, and stroke. Genentech has held a patent for the development of tPA-producing cells and sold it for approximately \$2,000 per 100-mg dose. Within the next few years this patent will expire and the opportunity will exist to produce a generic form of tPA at a fraction of the cost. This design report describes a plant that can manufacture this recombinant protein without spending \$500 MM on research and development costs.

A cheap, generic form of tPA can acquire a large share of the current \$300 MM tPA market. The FDA has recently prohibited the sale Abbott Laboratories’ thrombolytic drug, urokinase. The only other thrombolytic medication available is streptokinase (SK); while it is much cheaper, SK causes more severe side effects and is somewhat less effective than tPA. It is therefore likely that the market for a generic tPA will expand as the price decreases. For this reason we have designed our plant to produce approximately 80 kg per year. Plant calculations were completed by hand and with the use of the SuperPro Designer program (for the Separation Section). Costing was completed using the economic spreadsheet created by Holger Nickisch. Purchase costs were obtained as company quotes or estimates from the design consultants. Our pharmaceutical plant has an investor’s rate of return of 59.7% based on a total capital investment of \$104 MM. Given the profitability of this process we highly recommend construction of this plant.



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# INTRODUCTION

## **Impetus for the Project**

The emergence of the biotechnology industry has created an explosion of new products in the healthcare industry. In 1986 tissue plasminogen activator (tPA) was approved by the FDA for the treatment of acute myocardial infarction and was the first recombinant, therapeutic protein to be produced in mammalian cells. Since that time biotechnology and pharmaceutical companies have attempted to beat competitors to the market with newly invented medications. In the next few years the patent of this popular “clot-busting” drug will be expiring, and competitors will be able to enter the market and create low-cost generic tPA. This report describes a process for the production of such a drug. A cheaper version of tPA can compete with expensive forms such as Genentech’s Activase™ and at the same time compete for market share owned by low-cost alternatives like streptokinase. Reduced healthcare reimbursements to U.S. hospitals and growing scrutiny over excessive drug prices will create a market for generic tPA in the U.S., and the prospect of a safer alternative to streptokinase and urokinase in foreign markets can potentially make it extremely profitable.

## **Applications**

The function of tPA in the human body is to activate plasminogen. This helps the body clear arterial blockage, which is a major cause of heart disease. Cholesterol buildup in arteries is exacerbated by fibrin formations that surround the blockage. Shrinking arterial diameters puts undue strain on the heart, leading to a variety of health problems. Active plasminogen releases plasmin, an enzyme that dissolves these fibrin formations, which hold the clot in place. This in turn can reduce blood pressure and

relieve cardiac stress in individuals with heart disease. The role of tPA in plasminogen activation makes it an extremely effective medication for patients suffering from acute myocardial infarction or cardiac arrest. More recently tPA has been approved for the treatment of deep vein thrombosis, pulmonary embolism, and stroke.

## **Production**

Production of recombinant tPA has been documented in both bacterial and mammalian cells. The use of *Escherichia coli* (bacterial host cell) produces small amounts of protein and makes the separation process much more complicated. Our process utilizes mammalian Chinese Hamster Ovary (CHO) cells, which are commonly used for genetic engineering production and research.

The DNA sequence from which tPA is produced is cloned from human melanoma cells. This sequence is then inserted into the genome of the CHO cells, and the highest producing strains of these tPA-CHO cells are selected and used for our process. The selected tPA-CHO cells are grown in a train of three fermentors, and after twenty days of growth in the three reactors the cell broth is harvested and sent to the Separation Train. During fermentation tPA is produced by the CHO cells and secreted into the liquid media solution. Serum-free media is used because it contains no growth proteins that would interfere with the purification process.

## **Purification**

Purification of biologically active molecules can be very difficult and costly. The Separation section is usually the most expensive part of pharmaceutical processes; this

holds true for our plant as well. The purification of our recombinant tPA exploits differences in density, protein size, and chemical affinity. The heart of the Separation trains is the Affinity Chromatography column, in which our protein is isolated from the thousands of proteins present in the harvest media. At the end, steps are taken to ensure the safety of therapeutic use in humans. Endotoxins that may be present in the solution are removed and the solution is sterilized in a 0.2  $\mu\text{m}$  filter. The final product is then bottled in the correct dosage and lyophilized, creating a stable drug that can be shipped to hospitals and stored indefinitely.

## **Protein Chemistry**

Proteins are chemicals in the body that interact with one another to send messages and complete complex enzymatic reactions. In order to retain their activity (functional ability) they must be treated with care under “gentle” conditions. Proteins lose activity at temperatures above freezing; for this reason the entire Separation section of our process is kept at 4°C by a refrigeration system. Any sudden change in environment can also damage the tPA, so our process is designed to minimize abrupt chemical changes.

The size of tPA is about 66,000 daltons (one dalton = g/mol), the mature full-length protein is made up of 562 amino acids. In high concentrations tPA can form aggregates which are difficult to separate and can be considered inactive protein. To circumvent this problem arginine is added to the tPA solution at a concentration of 2M. The product stream in our process has a 2M arginine concentration during most steps. Our recombinant tPA exactly resembles the protein produced in human melanoma cells and its ability to activate plasminogen has been demonstrated as well.

## **Flowsheet Preparation**

The production method of tPA is straight forward and similar to any other fermentation process in the pharmaceutical industry (i.e. penicillin). The main difficulty lies in the creation of a batch schedule that provides for the completion of 50 batch-runs each year. The separation of tPA centers around affinity chromatography, for which the tPA containing solution must be adequately prepared. Various forms of filtration were investigated including microfiltration, tangential flow ultrafiltration, and gel filtration. Once the unit operations were chosen they were organized and scheduled to provide for two-week batches. In addition to the Fermentation and Separation Trains, a reverse osmosis water purification system is required to provide the sterile water that is needed throughout our plant. The final, detailed process is presented in the following sections. All design considerations relevant to a batch-wise, pharmaceutical process have been taken into account, including scheduling, sterilization, and sanitization requirements.

# MATERIAL BALANCES



## FIGURE 1: Total Plant Flowsheet

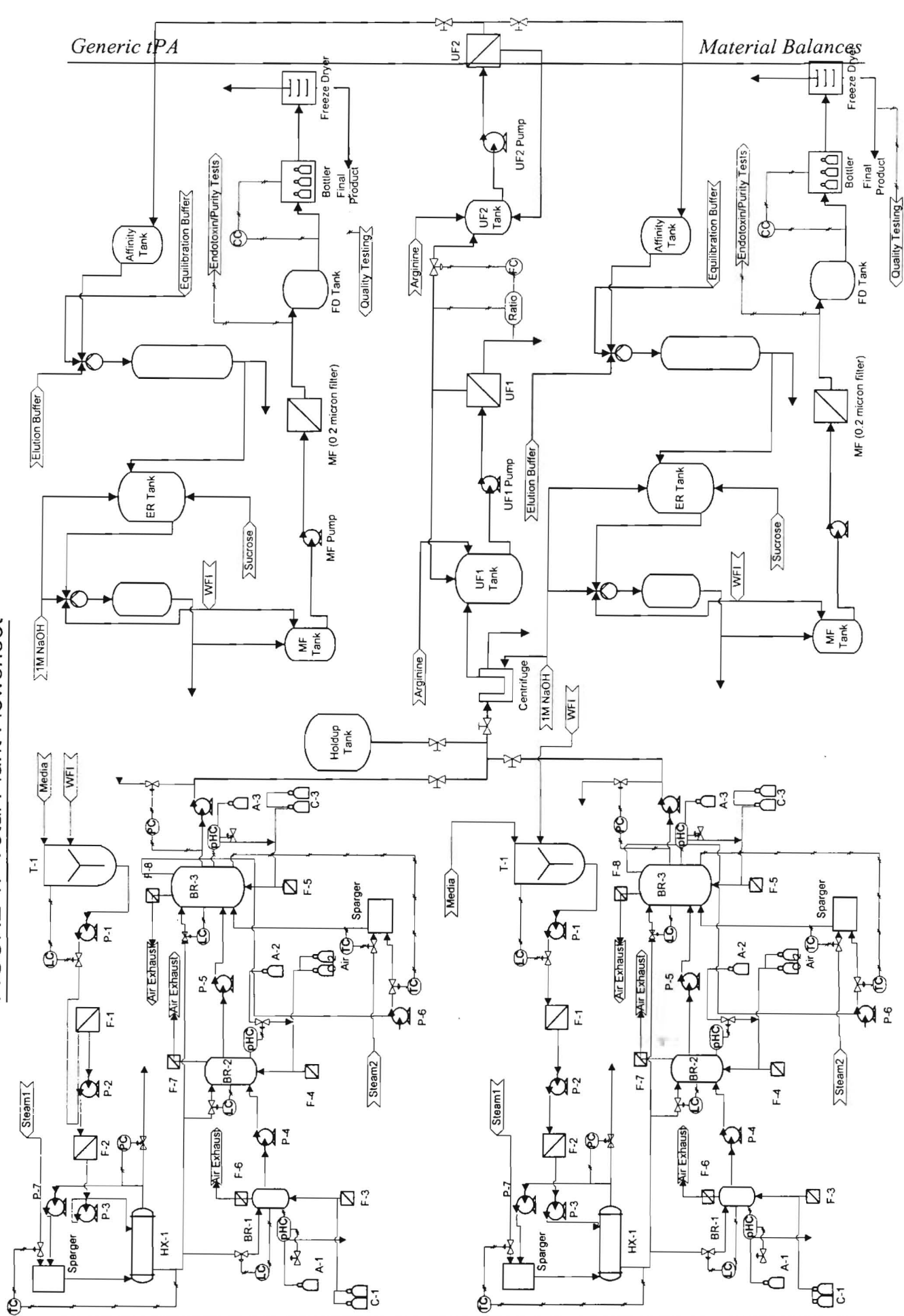
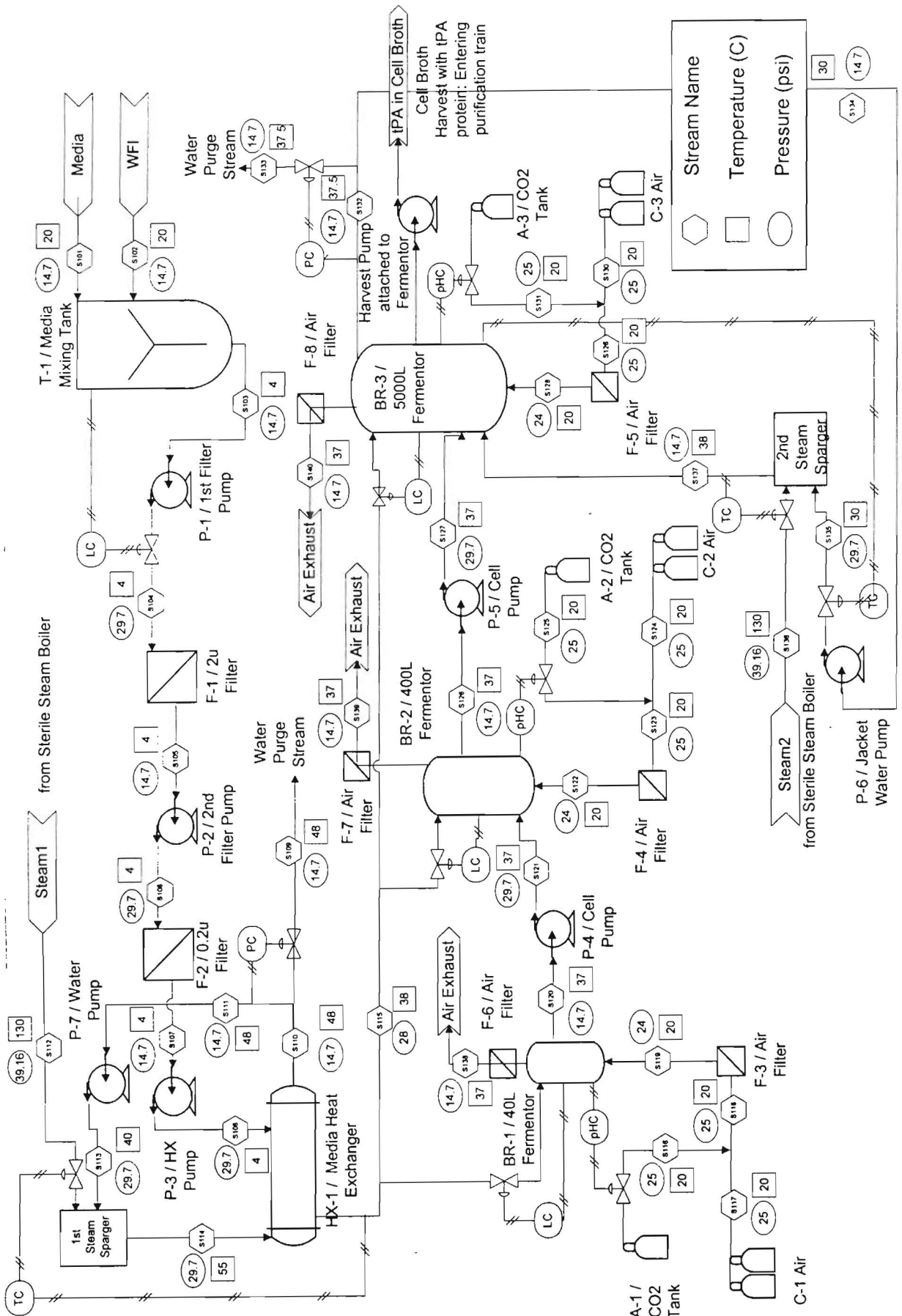


FIGURE 2: Fermentation Section



	S101	S102	S103	S104	S105	S106	S107	S108	S109	S110	S111	S112	S113	S114	S115	S116	S117	S118	S119	S120
Temp. (°C)	20	20	4	4	4	4	4	4	48	48	48	130	20	55	38	20	20	20	20	37
Pressure (psi)	14.7	14.7	14.7	29.7	14.7	29.7	14.7	29.7	14.7	14.7	41.7	39.16	29.7	29.7	28	25	25	25	24	14.7
Phase	liquid	solid	liquid	liquid	liquid	liquid	liquid	liquid	liquid	liquid	liquid	vapor	liquid	liquid	liquid	vapor	vapor	vapor	vapor	liquid
Total Flow (kg/batch)	4500	450	4500	4500	4500	4500	4500	4500	1100	38600	37500	1100	37500	38600	4500	0.02	0.3	0.32	0.32	30
Sterile Water	4500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Water	0	450	0	0	0	0	0	0	1100	38600	37500	0	37500	38600	0	0	0	0	0	0
Powdered Media	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Complete Media	0	0	4500	4500	4500	4500	4500	4500	0	0	0	0	0	0	4500	0	0	0	0	0
Air	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.3	0.3	0.3	0
CO2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.02	0	0.02	0.02	0
Steam	0	0	0	0	0	0	0	0	0	0	0	1100	0	0	0	0	0	0	0	0
Cell Broth	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30

	S121	S122	S123	S124	S125	S126	S127	S128	S129	S130	S131	S132	S133	S134	S135	S136	S137	S138	S139	S140
Temp. (°C)	37	20	20	20	20	37	37	20	20	20	20	37.5	37.5	30	20	130	38	37	37	37
Pressure (psi)	29.7	24	2.5	2.5	2.5	14.7	39.7	24	25	2.5	2.5	14.7	14.7	14.7	29.7	39.16	29.7	14.7	14.7	14.7
Phase	liquid	vapor	vapor	vapor	vapor	liquid	liquid	vapor	vapor	vapor	vapor	liquid	liquid	liquid	liquid	vapor	liquid	vapor	vapor	vapor
Total Flow (kg/batch)	30	4.9	4.9	4.5	0.4	300	300	75.5	75.5	70	5.5	106100	1600	104500	104500	1600	106100	0.35	5	80
Sterile Water	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Water	0	0	0	0	0	0	0	0	0	0	0	106100	1600	104500	104500	0	106100	0	0	0
Powdered Media	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Complete Media	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Air	0	4.5	4.5	4.5	0	0	0	70	70	70	0	0	0	0	0	0	0	0	0	0
CO2	0	0.4	0.4	0	0.4	0	0	5.5	5.5	0	5.5	0	0	0	0	0	0	0	0	0
Steam	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1600	0	0	0	0
Cell Broth	30	0	0	0	0	300	300	0	0	0	0	0	0	0	0	0	0	0	0	0
Cell gas admissions	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.35	5	80

\*\*\*\*All Streams are measured in kg per batch\*\*\*\*

FIGURE 3: Separation Section

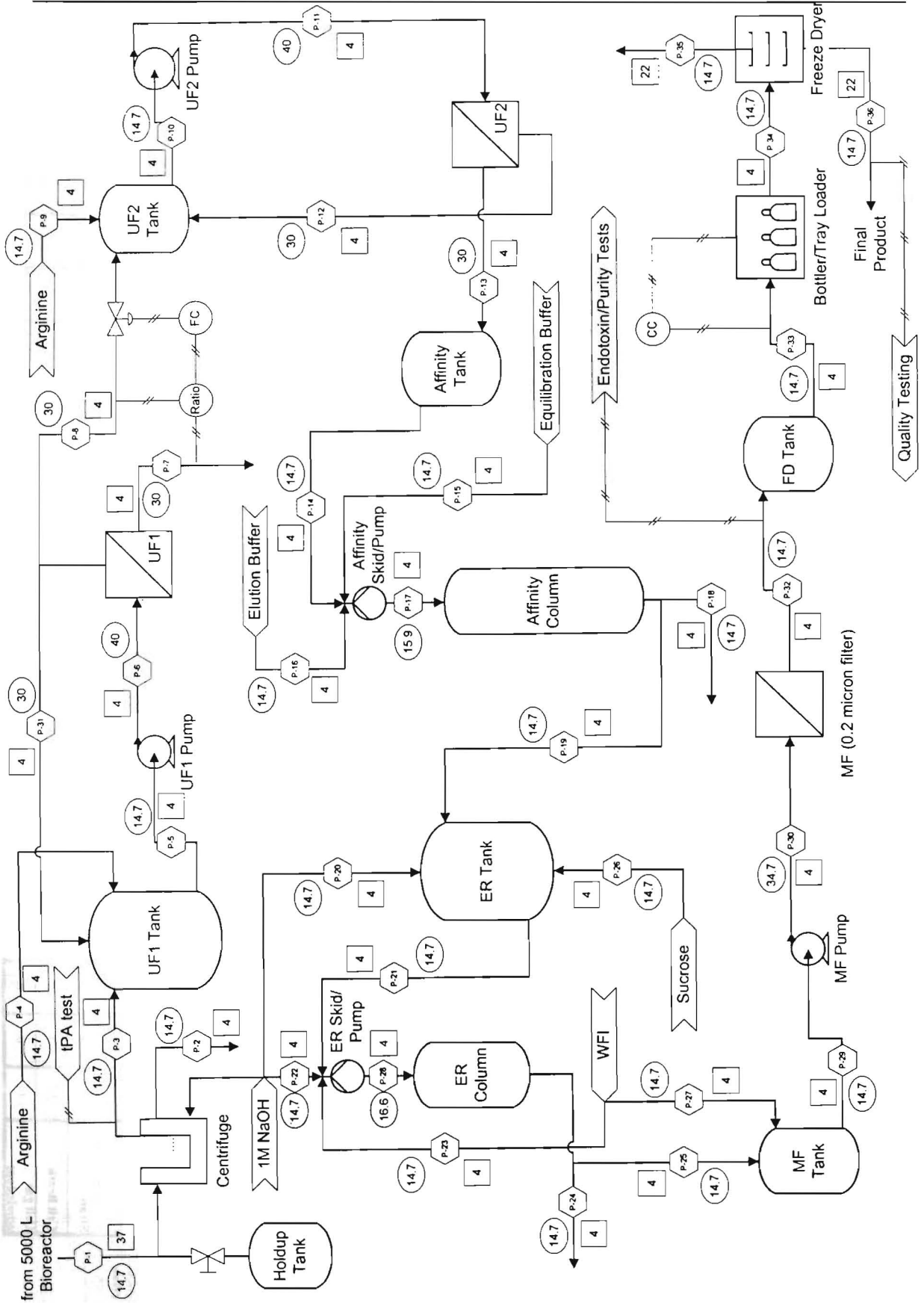


TABLE 2: Material Balances for Separation Section

	P-1	P-2	P-3	P-4	P-5	P-6	P-7	P-8	P-9	P-10	P-11	P-12	P-13	P-14	P-15	P-16	P-17	P-18
Temp. (°C)	37	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Pressure (psi)	14.7	14.7	14.7	14.7	14.7	40	30	30	14.7	14.7	40	30	30	14.7	14.7	14.7	15.9	14.7
Phase	Liquid	Solid cells	Liquid	Solid	Liquid	Liquid	Liquid	Liquid	Solid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
Total kg/batch	4024	458	3566	850	4416	4416	4166	250	67.4	317	317	6.3	311	311	597	523	1132	1174
Arginine	0	0	0	850	850	850	802.2	47.8	67.4	115.2	115.2	2.3	112.9	112.9	0	227.5	239.2	291.7
WFI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	564.6	284.6	849.2	650
Glycine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11.3	11.3	3.39
NaOH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Liq. media	3565	0.3565	3564	0	3564	3564	3364	200.3	0	200.3	200.3	4	196.3	196.3	0	0	0	196.3
CHO cells	457.3	457.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
tPA	2.16	0.11	2.05	0	2.05	2.05	0.11	1.94	0	1.94	1.94	0.05	1.89	1.89	0	0	0	0.28
NaCl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26.6	0	26.6	26.6
PBS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.57	0	5.57	5.57
Sucrose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Components are listed in kg/batch

	P-19	P-20	P-21	P-22	P-23	P-24	P-25	P-26	P-27	P-28	P-29	P-30	P-31	P-32	P-33	P-34	P-35	P-36
Temp. (°C)	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	22	22
Pressure (psi)	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7	16.62	14.7	34.7	14.7	14.7	14.7	14.7	14.7	14.7
Phase	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Solid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Vapor	Solid
Total kg/batch	404	0.34	404	192	47	240	404	0.026	59	644	463	463	1726	463	463	463	278	185
Arginine	175.0	0	175.0	0	0	0	175.0	0	0	175.0	175.0	175.0	329.2	175.0	175.0	175.0	0	175.0
WFI	219.0	0.33	219.0	185.0	47.1	232.2	219.0	0	59	451.1	278.0	278.0	0	278.0	278.0	278.0	278.0	0
Glycine	8.7	0	8.7	0	0	0	8.7	0	0	8.7	8.7	8.7	0	8.7	8.7	8.7	0	8.7
NaOH	0	0.013	0.013	7.39	0	7.39	0.013	0	0	7.40	0.013	0.013	0	0.013	0.013	0.013	0	0.013
Liq. media	0	0	0	0	0	0	0	0	0	0	0	0	1382.4	0	0	0	0	0
CHO cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
tPA	1.61	0	1.61	0	0	0	1.60	0	0	1.61	1.60	1.60	14.16	1.60	1.60	1.60	0	1.60
NaCl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PBS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sucrose	0	0	0	0	0	0	0	0.026	0	0	0	0	0	0	0	0	0	0

Components are listed in kg/batch

**FIGURE 4: Water Purification Section**

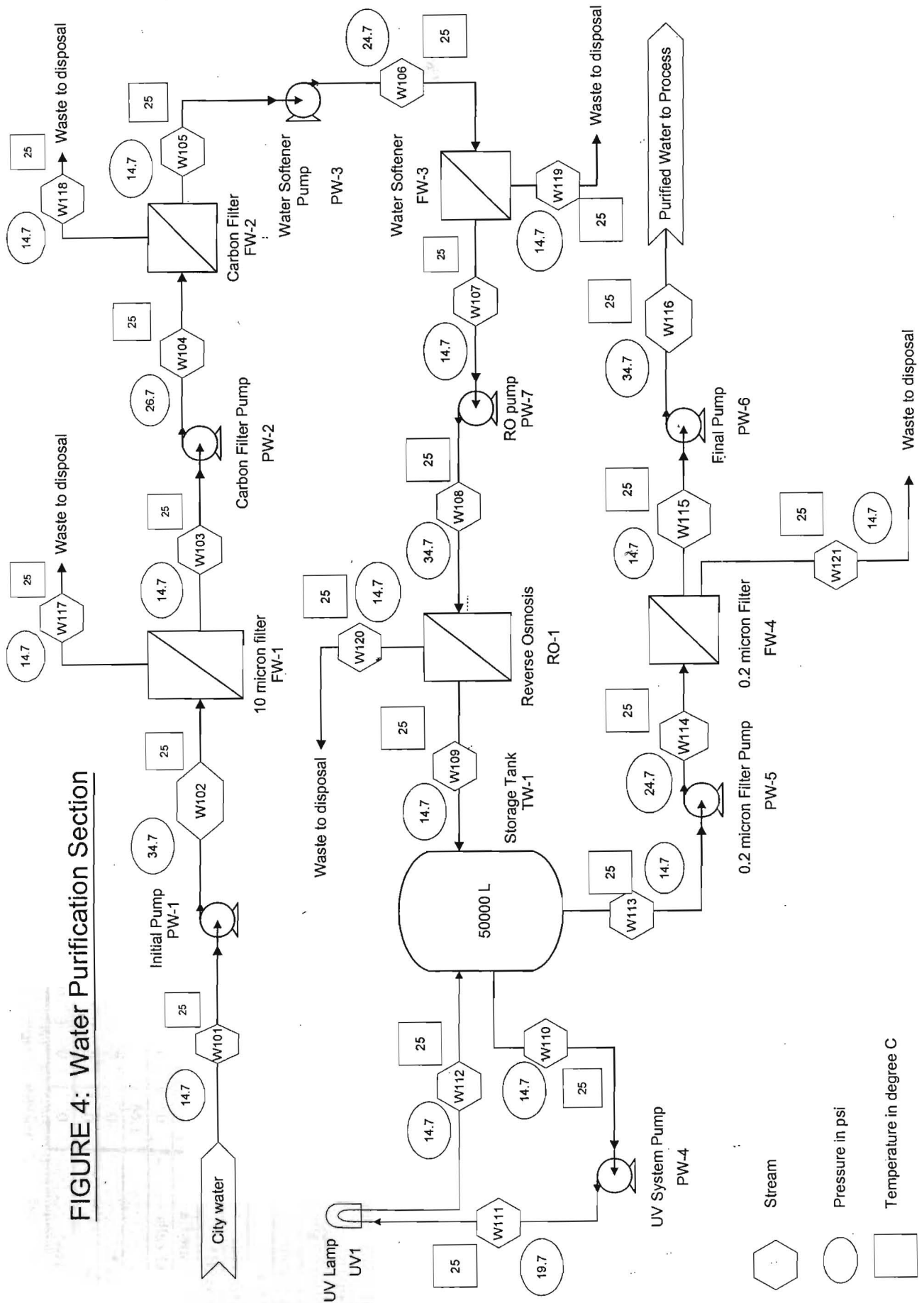


Table: 3 Material Balance for Water purification Section

	W101	W102	W103	W104	W105	W106	W107	W108	W109	W110	W111	W112	W113	W114	W115
Temp ( C )	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
Pressure (psi)	14.7	34.7	14.7	26.7	14.7	24.7	14.7	34.7	14.7	14.7	19.7	14.7	14.7	24.7	14.7
Phase	liquid	liquid	liquid	liquid	liquid	liquid	liquid	liquid	liquid	liquid	liquid	liquid	liquid	liquid	liquid
Total Flow	35.46	35.46	19.34	19.34	77.28	77.28	77.28	77.28	36.74	23.18	23.18	23.18	30.91	30.91	19.31
Waste	0.03	0.03	0.02	0.02	0.01	0.01	0.01	0.01	0.0001	0.0001	0.0001	0.0001	0.0002	0.0002	0
Water	35.43	35.43	19.32	19.32	77.27	77.27	77.27	77.27	36.74	23.18	23.18	23.18	30.91	30.91	19.31

	W116	W117	W118	W119	W120	W121
Temp ( C )	25	25	25	25	25	25
Pressure (psi)	34.7	14.7	14.7	14.7	14.7	14.7
Phase	liquid	solid	solid	solid	solid	solid
Total Flow	19.31	0.01	0.002	0.001	0.01	0.0002
Waste	0	0.01	0.002	0.001	0.01	0.0002
Water	19.31	0	0	0	0	0

\*\*\*\*All Streams are measured in kg per min\*\*\*\*

# PROCESS DESCRIPTION



## **Development of CHO Cell Line**

Before designing the human tissue plasminogen activator (tPA) process plant, a Chinese Hamster Ovary (CHO) cell line that secretes the product protein must be developed in the research and development laboratory. The cell line development process is estimated to take one year based on a suggestion by Prof. Diamond. The strategy for synthesizing tPA from CHO cells is a combination of transfecting the DNA sequence that produces tPA into the CHO cells and amplifying the gene into multiple plasmids. The Bowes melanoma cell line, which naturally produces tPA, is used to isolate the desired DNA sequence. PCR (polymerase chain reaction) is then used to make numerous copies of the tPA sequence that is 2009 bases long. Two specifically designed primers are applied to isolate the tPA gene. The primers are a sequence of nucleotides that form base pairs with the complementary sequences located near the beginning and the end of the desired gene. The primers ensure that only the tPA gene is amplified during the PCR. The sequence can be checked using restriction enzymes to break it down into smaller segments and measure them using the technique of gel-electrophoresis.

The amplified sequence is inserted into a DHFR expression vector with the EcoRV blunt cut restriction enzyme. The vector is placed in a neo-tPA plasmid and transfected into the CHO cells. Those cells that take up the plasmid will produce multiple copies of the gene as they grow and divide. The presence of the gene in the cell will result in the production of tPA. In order to only select the cell culture strains that largely amplify the gene, the cells are exposed to a cytotoxic drug that will kill any cells that possess the DHFR vector. The tPA gene is located within the DHFR vector, so the

desired cultures die most readily in the presence of the drug. The cells that exhibit the most “favorable” response to the drug are grown up in large volumes and frozen at  $-70^{\circ}\text{C}$  to create a cell stock. This cell stock will be used to begin every subsequent batch to ensure that each culture produces large amounts of tPA.

The CHO cell line that is used in our process is estimated to produce 50 pg of tPA per cell per day. Cell growth environments and the storage conditions of the cell line can alter the production rate of the cells, therefore the value used is a slight under estimation of the possible production levels of the cells. In addition to external factors, internal cellular mutations can effect the total production of tPA in any given culture. Mutations result in the production of different, inactive proteins, or cause the cell to cease production of tPA altogether. The research laboratory continuously tests the integrity the CHO cell line for its ability to produce tPA for the life of the process.

## **Raw Materials**

### **Fermentation Materials**

The raw materials for the process include 100% sterile water, powdered media, compressed air, and  $\text{CO}_2$ . The sterile water is produced in the plant with a reverse osmosis water purification system from water that is purchased from the municipal source in the area. The sterile water is mixed with HyQ PF-CHO dual powder media (see Appendix C, page 272) to generate the liquid media that makes up the growth medium for the CHO cells in the bioreactors. The Hyclone media is serum free but contains all of the nutrients, salts and amino acids necessary for cell growth. A serum free media was chosen because the presence of serum in the growth media interferes with the

recombinant protein purification by introducing other proteins to the product stream.

Under the advice of Neil Collins from Hyclone Media Company, the media does not need to be supplemented with any additional proteins such as glutamine. The formulation for this media is not yet available due to patent protection but Mr. Collins verified the presence of important components such as insulin, growth factors and transferrin.

The media also contains a sodium bicarbonate buffer to maintain a pH of 7.3.

The pH is adjusted by the continuous flow of CO<sub>2</sub> into the fermentors. A pH control measures the internal conditions of the fermentor and adjusts the flow of CO<sub>2</sub> accordingly to change the pH. The CO<sub>2</sub> is a part of the air stream that is pumped into the bioreactor to provide the oxygen needed for the cells' metabolism. The CHO cells require 0.06 to  $0.2 \times 10^{-12}$  mol O<sub>2</sub>/hr \* cell (Shuler p.455). The compressed air and CO<sub>2</sub> are purchased in tanks from BOC gases (see Appendix C, page 275). In order to prevent any disturbance in the inlet stream, all of tanks for a given batch are hooked up to the fermentor and opened at the same time to maintain a constant pressure and flow rate for the life of the batch.

## Arginine

Arginine Hydrochloride is used throughout the Separation process to prevent the aggregation of tPA in concentrated solutions. It is first added to the product solution in UF1 Tank before concentration in UF1. The concentration is only brought to 1M here in order to reduce the waste of arginine (the majority would be immediately discarded in UF1 filtrate). Prof. Diamond advised us that a 1M concentration would be enough for the short period of time between UF1 and UF2 Tank. The 200 L volume in UF2 Tank is then

increased to 2M arginine. The final input of arginine comes in the elution of Affinity Column. As tPA is eluted, the concentration of the protein gets quite high in the elution buffer. The glycine based buffer is made to a concentration of 2.4M arginine which results in a final product concentration of 2M in each dissolved dose. Endotoxin-free arginine can be purchased in bulk from Sigma-Aldrich at a price of \$119/kg; we will require about 74,500 kg each year (see Appendix D, pages 283 and 290).

### **Glycine**

Glycine, or aminoacetic acid is the simplest amino acid and is an important ingredient in the Elution buffer for our Affinity Column. We follow Reagan et al. in using 0.5M glycine in our tPA elution. We will be using endotoxin-free glycine to ensure the quality of our final product. Glycine can be purchased in bulk by Sigma-Aldrich at \$43/kg and our yearly needs are about 770 kg (see Appendix D, pages 283 and 290).

### **Sucrose**

The final packaging requirements of lyophilized tPA contain a small amount of sucrose. This biotechnology grade sucrose will be added into the product solution in ER Tank. It is added before ER Column to ensure that any endotoxins introduced will be removed the ER Column. Approximately 3 kg/yr are required and this can be bought for \$29 from Sigma-Aldrich (see Appendix D, pages 283 and 290).

**PBS**

Phosphate buffer solution, or PBS, is the liquid medium for the Equilibration buffer used in the Affinity Column. About 117,000 L of PBS will be used in our process each year. This can be bought in bulk for approximately \$0.80/L from Sigma-Aldrich (see Appendix D, pages 283 and 290).

**NaOH**

Sodium hydroxide, or NaOH is used to adjust the pH in ER Tank after elution in the acidic buffer, and it also acts as a cleaning agent for ER Column and Centrifuge. The yearly requirements are 550 kg and this can be bought in bulk form Sigma-Aldrich for \$17.50/kg (see Appendix D, pages 283 and 290).

**NaCl**

Sodium chloride, or NaCl is present in the Affinity Column Equilibration buffer in a concentration of 0.65M. This adds up to yearly requirements of about 4500 kg. Biotechnology grade NaCl can be purchased in bulk from Sigma-Aldrich at \$18/kg (see Appendix D, pages 283 and 290).

**Fermentation Process**

The fermentation section of the tPA production is a batch process that consists of a scale-up series of 3 individual stainless steel fermentors ranging in size from 40 Liters to 5000 Liters. Each fermentor operates under the same growing conditions: a temperature of 37 °C, a pressure of 14.7 psi, and a pH of 7.3. The vessels contain a 6

blade standard Marine impellor located in the center to mix the contents and disperse the air stream sparged through the bottom. The function of these fermentors is to provide an optimal environment for growth of a CHO cell line that excretes the product tPA protein.

The fermentors are filled with media that is produced in a blending tank T-1 (see specification page 100). Before the media can enter any of the bioreactors, it is heated to 37 °C and extensively filtered. Due to varying batch lengths for the three different fermentors, the media is held in the tank for up to 7 days at 4 °C before it is needed to fill a bioreactor. The cold temperature preserves the quality of the media and prevents the growth of any bacteria. The media is sent through filters F-1 and F-2 (see specification pages 108 and 109) before it is heated to growth temperature. The filters are used to remove 99.9% of the contaminants and to sterilize the media. The purpose of F-1 is to protect the 0.2 micron F-2 from getting clogged too quickly.

The media is pumped through the filters with peristaltic pumps that maintain a flow rate of 2 gal/min and increase the pressure from 14.7 psi up to 29.7 psi. The media is finally pumped through a shell and tube heat exchanger that is designed to heat the media up to 37 °C using a small temperature gradient that will not damage the organic material in the media. A warm water stream heats the media as it enters the shell side of HX-1 (see specification page 99) at a temperature of 55 °C and leaves at 48 °C. The warm water stream is a recycle stream made up of city water that is heated using a steam sparger. The warm media is finally pumped into the fermentors to provide the essential liquid environment for CHO cell growth.

The fermentors are all outfitted with a complete computer control system as well as individual unit operation control units. The internal environment of the bioreactors is

maintained with the aid of a conventional heating jacket on BR-1 and BR-2 (see specification pages 96 and 97) and a 4-inch half-pipe heating jacket surrounding BR-3 (see specification page 98). All the fermentors are also covered in 2 inches of fiberglass insulation to increase the efficiency of the heating jackets. The pH is regulated by a pH control that adjusts a valve on the inlet stream of CO<sub>2</sub>. The amount of CO<sub>2</sub> flowing into the reactor can change the pH of the cell broth (see Dynamic Controls page 44). Finally the reactor is fed with a constant flow of compressed air, supplemented with CO<sub>2</sub>. The gases are supplied to the vessel by tanks that are attached to a sparger located at the base of the fermentor.

In the designed process the CHO cells are grown up for a total of 20 days in the three fermentors. The size of the fermentor influences the length of the cell culture batch. BR-1 only takes 5 days to achieve the desired cell density, while BR-2 takes 7 days to grow and BR-3 takes 8 days to achieve maximum growth. The batch schedule for each fermentor is designed to allow enough time to run 50 batches per year. BR-3 runs continuously through out the year in order to fit in 25-2 week batches in one year, but the smaller fermentors have some down time in between batches (see the complete schedule on page 171). The schedule is also designed to fit in an extra fermentation train for possible future expansion of the plant.

## **Separation Trains**

The Separation trains accomplish the difficult task of purifying tPA from the complex mixture of biochemicals present in the harvest media. Throughout the life cycle

cells produce thousands of proteins of different size, structure, and chemical activity. Each of these differences must be exploited in order to obtain a pure, stable product.

Throughout the Separation process the product solution must be kept as cold as possible to prevent loss of protein activity. High temperatures (room temperature is considered high for proteins) cause proteins to degrade and denature, basically changing the conformation that is essential for correct function. For this reason an air-conditioning system keeps the temperature of the entire Separation section at a temperature of 4°C.

Because there are two Bioreactor production lines running, the separation train will be split to two trains at the outlet of UF2. In effect, there will be two identical units for each of the following: Affinity Tank, Affinity Skid/Pump, Affinity Column, ER Tank, ER Skid/Pump, ER Column, MF Tank, MF Pump, MF, FD Tank, Bottler, and Freeze Dryer. Once the train has been split it is unwise to reintroduce the separated batches because cross contamination could possibly occur, resulting in a loss of both batches.

The Separation Train begins with the draining of BR3. The cell broth is sent directly to Centrifuge (see specification page 119) for clarification unless the machine is down. If more time is needed the cell broth can be stored in Holdup Tank (see specification page 112) until the Centrifuge is ready. The broth is processed at a rate of 400 L/hr and the resulting cell waste is sent to the EarthCare Company for disposal. Given a cell production rate of 50 pg/cell/day, an average cell density in BR-3 of 2 MM cells/mL, a volume of 4000 L and a production time of 6 days, the amount of tPA produced in BR-3 is estimated at 2.4 kg, and this number is reduced by 10% to yield 2.16 kg to account for variability in the cells' ability to produce the recombinant protein. We



assume that another 5% is lost with the cells during clarification in the Centrifuge. After this step the harvest media is sent to UF1 Tank (see specification page 113). The total process time for Centrifuge is about 12 hours.

The harvest media is then stored overnight and mixed with 850 kg of arginine in UF1 Tank. A 1M concentration is needed at this time to prevent aggregation of the tPA when it is concentrated. This concludes Day 1 of the separation. Day 2 consists of ultrafiltration in UF1 (see specification page 125) and UF2 (see specification page 126). Water and other dissolved solids pass through the UF1 membrane and a concentrated tPA solution is collected in the retentate. The 3500 L of harvest media can be processed in UF1 in just over 6 hrs, and the resulting volume is 200 L.

More arginine is added in UF2 Tank to again prevent aggregation. The solution must then be cleaned before application to the chromatography column because microscopic solids may still remain in solution and these particles can damage the chromatographic resins. The first method of pretreatment that we investigated was gel filtration. This chromatography operation is accomplished by applying a given volume of solution to a column and continually supplying sterile process water afterward. Particles of different sizes have different residence times, so one can collect a sample with a specific residence time that will contain the desired product. The downside of this operation is that the large volume of sterile water used causes drastic dilution of the sample, which is extremely undesirable in our purification process.

The method we eventually chose was ultrafiltration with a larger pore size. The 500 kDa pores of UF2 allow tPA to pass through while removing larger debris. The process requires no additional water so dilution does not occur. The 200 L volume can

be ultrafiltered in less than one hour, and it is assumed that 5% of the tPA will not be recovered, leaving 1.89 kg. *(For a more complete description of ultrafiltration theory see Unit Descriptions page 78).*

The tPA is then purified in four cycles in the Affinity Column (see specification page 128). tPA binds to the anti-tPA antibody which is bound to the Affinity Resin (see specification page 129). The protein is then “released” during elution and sent to ER Tank. Dividing the Affinity Column processing into four cycles allows us to cut the resin requirements from about 180 L to approximately 45 L, which cuts our yearly capital costs drastically. Two cycles can be performed each day, so the final elution cycle takes place on Day 4. It is assumed that 15% of the tPA will be lost in this step leaving over 1.6 kg to be recovered. *(For a more complete description of affinity chromatography theory see Unit Descriptions page 82).*

After elution from Affinity Column the solution needs to be neutralized with addition of 1M NaOH to protect the tPA. The final product must also contain a certain amount of sucrose (see Prof. Diamond email page 289). These materials are added to the solution in ER Tank (see specification page 116) before processing in ER Column.

The next step in the separation process is the removal of endotoxins, which are chemicals derived from bacteria that cannot be injected into humans. The first removal technique I investigated was anion exchange based on advice given by a representative at Amersham Pharmacia Biotech. The process required a gel filtration step, followed by an adjustment to an acidic pH before application to an anion exchange column. About 98% of the endotoxins would bind to an anion exchange column (Harrison 1994), which is probably enough for our purposes. I was unsatisfied with this process because it was

very costly (requiring many of Amersham's more expensive products) and forced us to subject our protein to harsh conditions (low pH and ion exchange elution).

My search over the internet then led me to an affinity resin made by Sterogene. The Acticlean Etox resin removes virtually all of the endotoxins in a sample. While it is somewhat expensive, the resin can be reused, can be run at a neutral pH, and will bind to practically none of our protein. The representative said that the resin was ideal for removal of small amounts of endotoxins, while anion exchange is more commonly used for removal of high endotoxin concentration. The ER Column (see specification page 130) simply involves the application of our product solution to ER Resin (see specification page 131). The endotoxin free tPA solution can be collected directly from the column and prepared for lyophilization in MF Tank. The solution will be completely collected on Day 6. The tPA losses are conservatively estimated at 5% in the ER Column, leaving about 1.6 kg to be purified. *(For further information on endotoxin removal see Unit Descriptions page 84).*

The solution volume is then increased to 400 L with addition of WFI in MF Tank in order to set the correct packaging concentrations. The contents are then sterilized by filtration through a 0.2 µm filter, MF (see specification page 127). This filtration will be complete in less than one hour and the filtrate will be collected in FD Tank (see specification page 118). tPA losses in the MF are estimated at 1%, though the filter will be replaced each batch.

FD Tank will store our final product solution overnight before the bottling and freeze drying cycles begin. On Day 7 the first of five bottling/freeze drying cycles will begin. A UV spectrophotometer is used to measure the protein concentration, and this

concentration is used to calculate the volume to be loaded into each vial. The Bottler (see specification page 132) uses this information and charges approximately 3200 100-mL vials with the tPA solution and loads them onto trays in the Freeze Dryer. The charging and loading will take a total of 2 hours based on comments from a representative of the Cozzoli Machine Company.

Finally, the Freeze Dryer (see specification page 133) forms a solid tPA powder in sterile vials and seals the stoppers. The drying time for each cycle is estimated as 24 hours, which is a conservative estimate, though we cannot be certain without experimentation. Given five cycles and allowing for over five hours of cleaning time between each cycle the Separation batch will be complete on Day 12. This is well within the limits of 14 days per batch. In fact, the two Separation trains can handle 75 batches per year (three fermentation trains) if there is enough demand. Approximately 1.6 kg of tPA should be present after lyophilization, and this is enough tPA for 16,000 doses. Our yearly production is then estimated at 1.28 MM doses.

## **Water Purification System**

City water from stream W101 is brought into the water purification system through pump PW-1 (see specification page 135). This water contains small organic molecules, salts, and various types of bacteria, so it must be purified to meet purified water standards.

The water stream W102 is pumped through filter FW-1 (see specification page 136) to separate any particles that are larger than 10  $\mu\text{m}$ . FW-1 is a tangential flow filter, which has the advantage over the dead-end filter such that it can generate higher

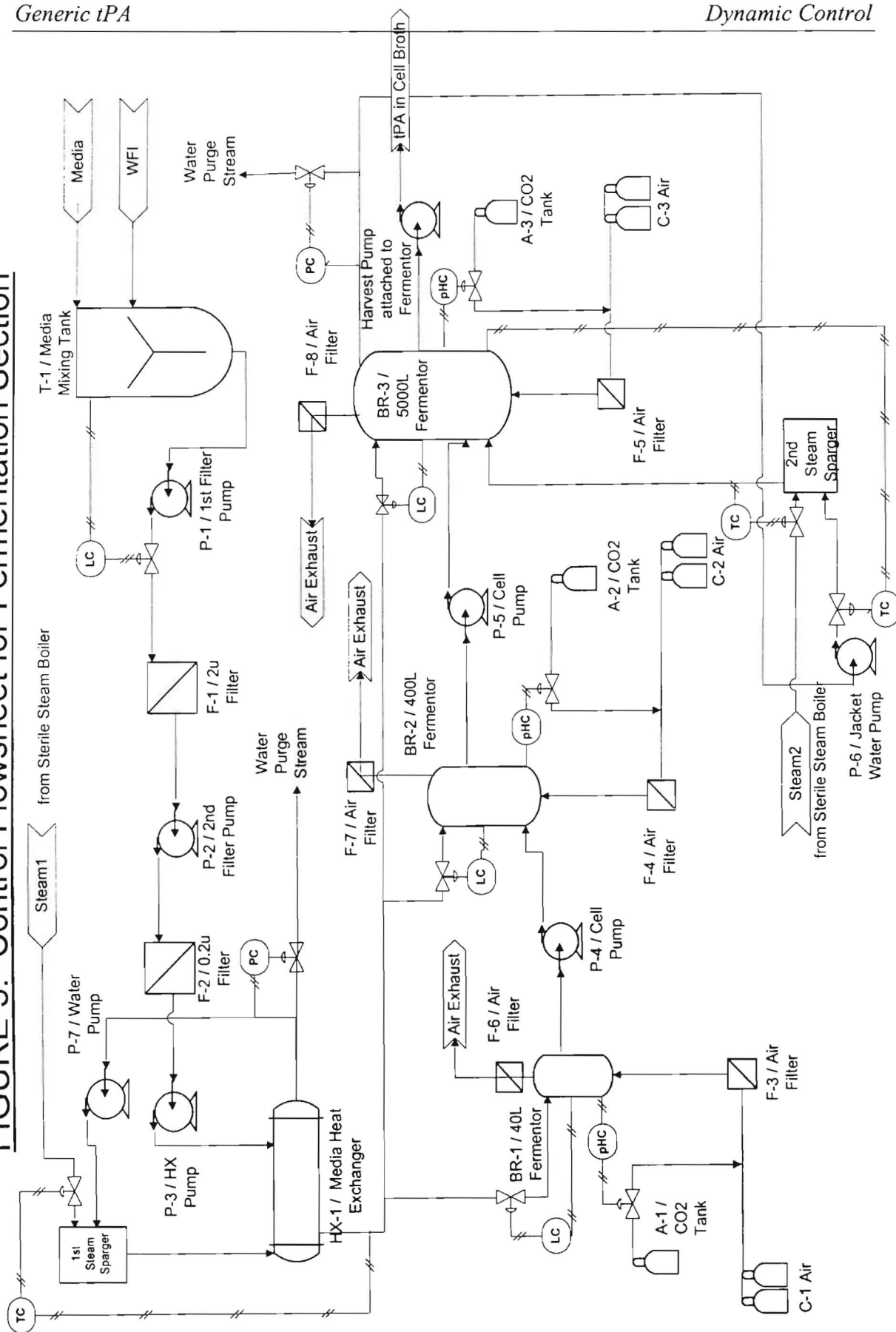
permeate flow. The purpose of FW-1 is not only to separate unwanted particles, but also to protect the membrane of the reverse osmosis system downstream from damaging large particles. Since FW-1 is exposed to many particles and unwanted species, it is easily contaminated, so we will replace it before starting each batch. After FW-1, city water will be pumped through FW-2 (see specification page 138), which is used to remove chlorine and organic molecules. Chlorine must be removed from city water at this point since it is quite erosive to the cation resin in water softener as well as the reverse osmosis membrane and 0.2  $\mu\text{m}$  filter downstream. Organic molecules can also damage the reverse osmosis system membrane. After FW-2, city water is pumped directly to FW-3 (see specification page 139) using pump PW-3 (see specification page 140). Filter FW-3 is present to remove ions from the city water. For this particular softener, the Polystyrene Sulfonate cation is chosen because it binds to positive ions like magnesium and calcium, which are abundant in impure water.

At this point, large particles, chlorine, organic molecules, and positive ions have already been separated from the city water stream. Water in stream W108 will now enter RO-1 (see specification page 142), which is considered the most important piece of equipment in the water purification process. The reverse osmosis system RO-1 is a high-pressure unit that allows only water to flow through its semi-permeable membrane. The membrane is able to filter out any remaining ions, small particulates, organic molecules or bacteria. Purified water RO-1 is then transferred to the storage tank TW-1 (see specification page 143) so that it can be allocated for various uses during the process. During the time that the purified water sits in TW-1, some extremely small organic particles and ions may be settle at the bottom and form larger particles – becoming a

source for bacterial contamination. To prevent this from happening, water is circulated through UV-1 (see specifications page 145), which is capable of breaking down the forming particles and killing any bacteria that may be left in the water. When the water is needed in the fermentation or separation processes, purified water is flows through pump PW-5 (see specification page 146) into filter FW-4 (see specification page 147), which will act as the last line of protection to trap any minute particulates from contaminating the final purified water. Like the 10  $\mu\text{m}$  filter, the 0.2  $\mu\text{m}$  microfilter will be replaced after each batch.

# DYNAMIC CONTROL

FIGURE 5: Control Flowsheet for Fermentation Section





## **Control Considerations**

The calculations for this process were performed assuming steady-state plant conditions. However, the steady-state analysis is not an adequate representation of the plant design feasibility. The tissue plasminogen activator (tPA) production process is a batch process with a batch length of 2 weeks. The plant runs 50 batches a year in two separate fermentation trains. The plant must have a reasonable control structure to regulate the cell growth environments and the process recycle streams. The total yearly production of tPA is only 80 kg/year, therefore the control scheme is important to prevent any disturbances that could kill a batch of cells and significantly reduce the yearly production.

The control scheme designed for the tPA process is based on the suggestions made by Professor William Luyban from Lehigh University. Dr Lyban's nine basic rules of plant-wide control could not be applied to the tPA process because of the batch mode of operation. However, he was able to provide assistance in the design of controls over individual unit operations. The final control scheme was a result of a meeting with Dr. Lyban and Dr. Warren Seider in which he offered suggestions for an optimal structure.

## **Control Objectives**

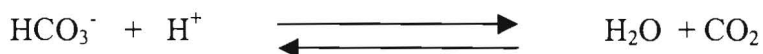
The principle objectives for the control system of a pharmaceutical process that uses cells to produce a product are the preservation of 100% sterile quality through out the entire production line and the maintenance of the growing environment of temperature 37 °C and pH of 7.3. The principle constraint for the process is the sensitivity of the Chinese Hamster Ovary (CHO) cell line. A CHO cell cannot withstand a temperature change of more than  $\pm 2^{\circ}\text{C}$  or a pH change of more than  $\pm 0.2$  units. In

addition to growing conditions, a control system is put in place to regulate the flow rates of steam that is sparged into recycle streams, as well as streams that are used to fill vessels to very specific volumes. The control scheme expresses these specifications using controllers that are designated for the important design considerations.

## **Control of Individual Unit Operations**

### **Control of pH in Fermentors**

The fermentors are filled with a media that contains a sodium bicarbonate buffer that is sensitive to CO<sub>2</sub>. Each vessel is equipped with a pH meter located within the cell broth liquid. The optimum pH for the cell culture is 7.3 with a tolerance of  $\pm 0.2$  units. If a change in the pH is detected, the pH meter will send a signal to adjust the valve attached to the CO<sub>2</sub> stream. In order to stabilize the pH to the optimum value, the CO<sub>2</sub> forms a chemical reaction with the Sodium Bicarbonate buffer in the media. The Sodium bicarbonate ions and CO<sub>2</sub> produce the following chemical reaction:



When the cell broth solution becomes basic, more CO<sub>2</sub> is pumped into the cell broth in order to drive the reaction in the reverse direction to produce more H<sup>+</sup> ions in the solution. The reverse process occurs if the pH becomes too acidic; the CO<sub>2</sub> flow rate is reduced and the reaction moves to the right in order to reduce the number of H<sup>+</sup> ions in the liquid.

### **Level Controls**

All of the tanks and fermentors are equipped with level controls on the inlet flows to ensure the exact volume of water or media is added to the unit. In order to preserve the sterile quality of the water and the media the fluid cannot be handled manually and must

be pumped into the vessels through sanitary piping that is rinsed and sterilized after each use. The level control will measure the volume of water or media in the tank and adjust a valve located on the inlet flow stream. The liquid volumes must be measured with precision for two important reasons: maintain the proper ratio of media to water and provide enough void space in the fermentors. In order to provide enough room for mixing and air circulation, the three fermentors are only filled with 90% of their capacity with cell broth. Additionally, the media is purchased in sterile packages with a pre-measured volume equaling the amount for one batch; hence the volume of water in the tank must correspond to the volume of media added to the tank.

## Temperature and Pressure Controls

There are two steam spargers used to increase the temperature of warm water streams for use in a heating jacket and a heat exchanger. Due to the sensitivity of the organic media and the CHO cells, the temperature gradients of the heating streams must be very small. The 130 °C steam produced in the plant is too hot to be used as the heating media in the heat exchanger or the half-pipe heating jacket around the 5000L fermentor. As an alternative, steam is sparged into a water stream to produce temperatures of 55 °C and 38 °C for the Heat Exchanger and Heating Jacket respectively. A temperature control is placed on the outlet stream of the sparger for the heating jacket to ensure that the water flowing around the fermentor is an unvarying temperature of 38 °C. The temperature control on the heat exchanger sparger measures the temperature of the media outlet stream to adjust the valve on the steam pipeline. Any deviance from the setpoint will send a signal to adjust the appropriate valve on each stream.

The volume of steam sparged into the water stream is extremely small in comparison to the volumetric flow of the water, but over time the volume would build in the closed loop system. Since the streams never come into contact with the cells or the cell media, unsterile recycle streams can be used for this process. In addition, recycle streams cut down on the utility requirement for the plant. Pressure controls are placed on the streams in to purge off the additional volume added by the steam. The pressure control sends a signal to adjust a valve placed on the purge stream. The flow rate of the purge stream is very small but it is necessary to maintain a steady flow through the units.

The flow rate of the heating jacket water stream is regulated with a temperature control that measures the temperature of the cell broth in 5000 L fermentor. The interior environment of the fermentor must be maintained at a temperature of 37 °C. If the cell broth fluctuates from this temperature, the flow of 38 °C water in the jacket is increased or decreased stabilize the internal environment of the vessel.

## **Ratio Flow Control**

Only one control unit is needed in the separation and purification section of the tPA process. A ratio flow control is used to specify the % volume of one holding tank that is recovered in another holding tank, after it has been passed through an ultra filter. The ultra filter reduces the volume of a liquid flow by removing most of the water in the stream. The retentate is recycled through the filter to remove as much water as possible. The tpa product remains in the retentate flowing off the top of the filter and is collected in the ultra filter 2 holding tank. A meter that sends a signal to a flow controller, attached to the retentate stream, measures the ratio of the retentate flow rate and the water removal flow rate. The flow controller adjusts the valve on the retentate stream so that the flow is

equal to 0.06 times the flow the water removal. The function of this control system is to remove 94% of the liquid entering the ultra filter and forming a concentrated retentate flow stream containing all of the tPA product.

# ENERGY BALANCE & UTILITIES

TABLE 4: UTILITY COSTS FOR PROCESS UNITS

Equipment	Total # of Units (in Plant)	Utility	Usage (per year)		Total Cost per year 1 kW*hr = \$ 0.04
BR-1	2	Electricity	17718	kW*hr	\$1,417.44
BR-2	2	Electricity	59060	kW*hr	\$4,724.80
BR-3	2	Electricity	177180	kW*hr	\$14,174.40
T-1	2	Electricity	118120	kW*hr	\$9,449.60
RFU	2	Electricity	126720	kW*hr	\$10,137.60
P1	2	Electricity	2952	kW*hr	\$236.16
P2	2	Electricity	2952	kW*hr	\$236.16
P3	2	Electricity	2952	kW*hr	\$236.16
P4	2	Electricity	2952	kW*hr	\$236.16
P5	2	Electricity	2952	kW*hr	\$236.16
P6	2	Electricity	2952	kW*hr	\$236.16
P7	2	Electricity	23620	kW*hr	\$1,889.60
Centrifuge	1	Electricity	9000	kW*hr	\$360.00
Refrigerator	1	Electricity	87600	kW*hr	\$3,504.00
Sep-Pumps	1	Electricity	1461.37	kW*hr	\$58.45
Sep-Tank Impellers	1	Electricity	383900	kW*hr	\$15,356.00
Bottler	2	Electricity	8176	kW*hr	\$654.08
Freeze Dryers	2	Electricity	336530	kW*hr	\$26,922.40
WP-1	1	Electricity	399	kW*hr	\$15.96
WP-2	1	Electricity	260.98	kW*hr	\$10.44
WP-3	1	Electricity	870	kW*hr	\$34.80
WP-4	1	Electricity	130.5	kW*hr	\$5.22
WP-5	1	Electricity	348	kW*hr	\$13.92
WP-6	1	Electricity	435	kW*hr	\$17.40
WP-7	1	Electricity	2610	kW*hr	\$104.40
UV-1	1	Electricity	168	kW*hr	\$6.72
RO SYSTEM	1	Electricity	8948	kW*hr	\$357.92
<b>Total</b>			1380966.85	kW*hr	\$90,632.11
Boiler	1	Natural Gas	1053.6	MMBTU	\$2,739.36
<b>Total</b>			1053.6	MMBTU	\$2,739.36
Water Production	1	City Water	3000000	L	\$1,500.00
<b>Total</b>			3000000	L	\$1,500.00

Total Yearly Cost = \$95,000.00

## Utility Summary

### **Electricity**

The electricity for the plant comes from the local power plant. The electricity is used primarily to drive the pump motors, the motors for the impellers, and the refrigerators to cool the tanks and the separation room. The tank impellers and the freeze dyers account for half of the electricity usage in the plant. The high energy costs for these pieces of equipment are caused by the large amount of energy needed to run a condenser in the freeze dyer at  $-55\text{ }^{\circ}\text{C}$  and the energy needed to rotate large impellers at high rpms in a viscous solution. All of the pump motors are run on electricity but the yearly requirement is so small is it negligible. The electricity is purchased at a price of \$0.04 per kW\*hr (Seider p. 376).

### **City Water**

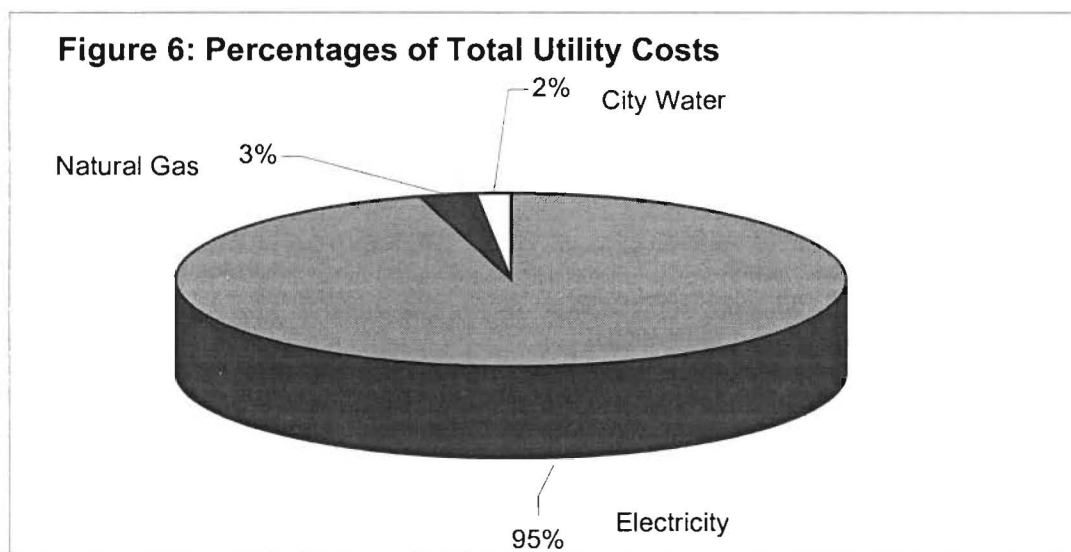
Water is purchased from a local water supply and sterilized in house to meet the standards required for a bioprocess production line. The HX-1 and the heating jacket on 5000L fermentor are the only two unit operations that use impure water. The remainder of the water used in the plant is sterilized in a reverse osmosis purification system.

Approximately 3,000,000 liters of water are purified each year for use in the process streams. These large volumes of purified water are needed to make the liquid media for cell growth, make up the basis of the elution buffers for the separation processed, and rinse all of the process units to remove debris before they are steam sterilized. Due to the location of the plant, the water is available at a relatively moderate temperature of  $20\text{ }^{\circ}\text{C}$ . The water is purchased at a cost of \$0.0005 per gal (Seider p.376).



## Steam

Low-pressure steam is used through out the plant as a sterilization medium and source of energy to heat water streams that supply feed for the HX-1 and the warm water-heating jacket around BR-1. Because the steam is used to sterilize the process equipment, it must be made from the sterile water produced in the plant. A boiler heats up the water to a temperature of 130 °C and a pressure of 39.16 psi. The boiler uses natural gas as fuel to produce 500 L steam per hour. The natural gas contains 92.6% CH<sub>4</sub> and can be purchased at a price of \$2.60 per MMBTU of heating value. (Seider p. 376)



## UNIT DESCRIPTIONS

**TABLE 5: Major Equipment Specifications**

Summary of Major Equipment		
Name	Description	Important Specifications
BR-1	40 L Fermentor	Capacity: 40 L Uptime per batch: 7 days Quantity required: 2 Purchase Cost: \$85,000
BR-2	400 L Fermentor	Capacity: 400 L Uptime per batch: 10 days Quantity required: 2 Purchase Cost: \$125,000
BR-3	5000 L Fermentor	Capacity: 5000 L Uptime per batch: 14 days Quantity required: 2 Purchase Cost: \$252,000
Centrifuge	Separates CHO cells from media	Throughput rate: 400 L/hr Uptime per batch: ~12 hr Quantity required: 1 Purchase Cost: \$355,000
Affinity Column & Skid/Pump	Purify tPA with using anti-tPA antibody	Bed volume: 58 L Resin: CNBr-activated Sepharose FF Superficial velocity: 100 cm/hr Uptime per batch: 48 hr Quantity required: 2 Purchase Cost: \$200,000
ER Column & Skid/Pump	Remove endotoxins from tPA solution	Bed volume: 8.2 L Resin: Acticlean Etox Superficial velocity: 100 cm/hr Uptime per batch: 36 hr Quantity required: 2 Purchase Cost: \$106,190
Bottler	Load tPA-solution into vials and load Freeze Dryer trays	Bottling/Loading time: 2 hr Electrical requirements: 1.5 hp Uptime per batch: 146 hrs Quantity required: 2 Purchase Cost: \$865,000
Freeze Dryer	Lyophilize tPA solution in 100-mL vials	Capacity in vials: 3500 Condenser capacity: 200 L water in 24 hrs Electrical requirements: 46.1 kW max Uptime per batch: 146 hrs Quantity required: 2 Purchase Cost: \$640,000
B-1	Create steam for sterilization and spargers	Capacity: 500 L/hr Quantity required: 1 Purchase Cost: \$112,000
RO-1	Reverse osmosis system	Volume of water per year: 3,000,000 L Quantity required: 1 Purchase Cost: \$7,500

## **Fermentors**

The Chinese Hamster Ovary (CHO) cell line, which is genetically altered to excrete the tissue plasminogen activator (tPA) product protein, is grown up in batches through a scale-up process containing a series of three fermentors of varying sizes. A small culture of cells is grown in a 40L bioreactor until a cell density of approximately  $3.0 \times 10^6$  cells/mL is reached. The cells from the 40L fermentor are transferred to the 400L where new media is added and the cells are allowed to grow up to the same density in the larger bioreactor. The final growth and production stage occurs in the 5000L fermentor after it has been inoculated with the cell broth from the 400L fermentor. A scale up process of this magnitude is required in order to produce enough cells to inoculate the largest fermentor with a cell density of  $0.25 \times 10^6$  cells/mL. Other options were investigated, but was determined unreasonable and not cost efficient. Two such options that were explored were initially growing up large volumes of cells and freezing them, or growing up cells in a laboratory, but it was concluded that a laboratory would not have the capacity to produce enough cellular mass to inoculate the 5000 L fermentors 50 times a year.

### **40 Liter Fermentor BR-1**

The function of BR-1 is to grow a culture CHO cells up to a density of  $3 \times 10^6$  cells/mL to be used to inoculate BR-2. The fermentor operates at an optimum growth temperature of 37 °C and a pressure of 14.7 psi. The fermentor temperature must be regulated with a conventional heating jacket to sustain an internal temperature of 37 °C.

The interior environment of the fermentor must remain constant because the CHO cell line is extremely sensitive to any changes. A temperature variance of  $\pm 2^{\circ}\text{C}$  can kill an entire cell culture.

In addition to the temperature and pressure, the pH of the cell broth is maintained at 7.3. A continuous flow of compressed air and  $\text{CO}_2$  are pumped into the vessel through a sparger on the bottom of the tank. This stream of air supplies the oxygen for cellular metabolic consumption and carbon dioxide needed to regulate the pH. The fermentors are all filled with a complete CHO media that is designed to provide all the essential nutrients and proteins for CHO cell growth. The media also contains a sodium bicarbonate buffer that functions to maintain the pH at 7.3. The pH is adjusted by the percent concentration of  $\text{CO}_2$  in the air stream pumped through the bottom (see Dynamic Control page 44). The average flow of  $\text{CO}_2$  is 5% of the inlet air stream, but a pH control unit continuously measures the pH inside of the tank and adjusts the  $\text{CO}_2$  flow accordingly (Goswami p. 633). The compressed air and the  $\text{CO}_2$  are supplied to the fermentor from tanks purchased from BOC gases. BR-1 requires approximately 0.03 tanks of compressed air and 0.001 tanks of  $\text{CO}_2$  per batch (see calculation page 244). These growth conditions are deemed optimal by the Bioprocess Engineering book by Schuler and Kargi

The fermentor is manufactured and designed by New Brunswick Scientific for the growth of animal cells. This fermentor is a BIOFLO 5000 Fermentor with a total volume of 40 L (see appendix page 225). In order to preserve an absolute sterile environment for the cells to grow, each vessel is made of 316 Stainless Steel with sanitary piping and couplings. A six blade standard marine impeller installed in the center of each bioreactor

disperses the air stream and mixes the cell culture. The agitation speed of the culture is only 30 rpm due to the sensitivity of CHO cells and the very low shear level the cells can endure (Shuler p.451). The agitator is run by an electric motor that requires 1.5 hp of energy utility. The reactors are sterilized in place by a process of flushing with water to remove any excess solid waste and automatically steam sterilized at 130 °C for approximately 3-4 hours. The reactor must reach a temperature of 130 °C and remain at that temperature for an extended period of time in order to kill or denature anything that remains inside the growing environment.

Attached to the fermentor is a ML-6100 controller system is that is capable of operating up to six process loops (see appendix page 229). The system also controls the sterilization and internal environment preservation. The menu-driven system is designed for simple control and minimal operator labor hours. The computer control system can be programmed to run a batch for any given amount of time. A vessel view window is installed into the wall of the fermentor in order to allow an operator monitor the process inside. The cell growth rate and the quality of the automatic sterilization are observed repeatedly through out the life of the batch. If a vessel is not completely clean after the water flush it must be manually scrubbed before steam sterilization.

The batch length for the BR-1 is 7 days. The batch schedule for the fermentor is the following:

- 0.5 Days Fill and Inoculate
- 5 Days of Cell Growth
- 0.5 Days Harvest
- 1 Day to Clean and Sterilize

The times allotted for each of these processes are slightly over estimated to ensure enough time, in the event of a disturbance in the system, to provide enough time for the

next fermentor in the process train to be prepared to receive the cell broth. The tPA production plant contains a total of 2, BR-1 fermentors; one for each of the two fermentation trains. The quoted price from Ted Shields of New Brunswick Scientific for one unit with a complete control system is \$85,000. (*see specification page 96 and appendix page 225*)

## **400 Liter Fermentor BR-2**

The function of BR-2 is to grow a culture CHO cells up to a density of  $3 * 10^6$  cells/mL to be used to inoculate the 5000 Liter Fermentor BR-3. The bioreactor is initially inoculated with a density of  $0.25 * 10^6$  cells/mL from the cell broth of the 40 L fermentor BR-1. The fermentor operates at an optimum growth temperature of 37 °C and a pressure of 14.7 psi. The fermentor temperature must be regulated with a conventional heating jacket to sustain the 37 °C internal temperature.

In addition to the temperature and pressure, the pH of the cell broth is maintained at 7.3. A continuous flow of compressed air and CO<sub>2</sub> are pumped into the vessel through a sparger on the bottom of the tank. This stream of air supplies the oxygen for cellular metabolic consumption and carbon dioxide needed to regulate the pH. The fermentor is filled with a complete CHO media that is designed to provide all the essential nutrients and proteins for CHO cell growth. The media also contains a sodium bicarbonate buffer to maintain the pH at 7.3. The pH is adjusted by the percent concentration of CO<sub>2</sub> in the air stream pumped through the bottom. The average flow of CO<sub>2</sub> is 5% of the inlet air stream, but a pH control unit continuously measures the pH inside of the tank and adjusts the CO<sub>2</sub> flow accordingly (*see Dynamic Control page 44*). The compressed air and the

CO<sub>2</sub> are supplied to the fermentor from tanks purchased from BOC gases. BR-2 requires approximately 0.35 tanks of compressed air and 0.1 tanks of CO<sub>2</sub> per batch (see calculations page 242). These growth conditions are deemed optimal by the Bioprocess Engineering book by Schuler and Kargi

The fermentor is manufactured and designed by New Brunswick Scientific for the growth of animal cells. A fermentor this size is custom ordered and designed for the specific purpose of growing animal cells. New Brunswick Scientific provides the engineering support to aid in the design of a reactor as large as 400L (see appendix page 231). In order to preserve an absolute sterile environment for the cells to grow, each vessel is made of 316 Stainless Steel with sanitary piping and couplings. A six blade standard marine impeller installed in the center of each bioreactor disperses the air stream and mixes the cell culture. The agitation speed of the culture is only 30 rpm due to the sensitivity of CHO cells and the very low shear level the cells can endure (Shuler p. 451). The agitator is run by an electric motor that requires 5 hp of energy utility. The reactors are sterilized in place by a process of flushing with water to remove any excess solid waste and automatically steam sterilized at 130 °C for approximately 3-4 hours. The reactor must reach a temperature of 130 °C and remain at that temperature for a period of time in order to kill or denature anything that remains inside the growing environment.

Attached to the fermentor is a ML-6100 controller system that is capable of operating up to sixteen process loops controls (see appendix page 229). The system also controls the sterilization and internal environment preservation. The menu-driven system is designed for simple control and minimal operator labor hours. The computer control system can be programmed to run a batch for any amount of time. A vessel view window



is installed into the wall of the fermentor in order to allow an operator monitor the process inside. The cell growth rate and the quality of the automatic sterilization are observed repeatedly through out the life of the batch. If a vessel is not completely clean after the water flush it must be manually scrubbed before steam sterilization.

The batch length of BR-2 is 9.5 days. The batch schedule for the fermentor is the following:

- 1 Days Fill and Inoculate
- 7 Days of Cell Growth
- 0.5 Days Harvest
- 1 Day to Sterilize

The times allotted for each of these processes are over estimated to ensure enough time, in the event of a disturbance in the system, to provide enough time for the next fermentor in the process train to be prepared to receive the cell broth. The tPA production plant contains a total 2, BR-2 fermentors, one for each of the two trains. The quoted price from Ted Shields of New Brunswick Scientific for one unit with a complete control system is \$125,000. (*see specification page 97 and appendix page 231*)

### **5000 Liter Fermentor BR-3**

The function of the BR-3 is to grow a culture CHO cells up to a density of  $3 * 10^6$  cells/mL and provide an environment for optimum tPA production. The fermentor operates at an optimum growth temperature of 37 °C and a pressure of 14.7 psi. A 4-inch half-pipe heating jacket located around the outside of the vessel and covered in a 2-inch layer of fiberglass insulation is used to maintain the internal temperature at 37 °C. The tubing is filled with flowing warm water at an average flow rate range of 0.09-0.2 kg/sec

(see calculations page 233). The range of flow rates is calculated using various environmental conditions. A recycled water stream is heated with sparged steam to maintain a temperature of 38 °C is circulated through the jacket. Ted Shields of New Brunswick Scientific suggested a half pipe jacket over a dimple jacket because a dimple jacket is more likely to corrode under to operating conditions.

In addition to the temperature and pressure, the pH of the cell broth is maintained at 7.3. A continuous flow of compressed air and CO<sub>2</sub> are pumped into the vessel through a sparger on the bottom of the tank. This stream of air supplies the oxygen for cellular metabolic consumption and carbon dioxide needed to regulate the pH. The fermentors are all filled with a complete CHO media that is designed to provide all the essential nutrients and proteins for CHO cell growth. The media also contains a sodium bicarbonate buffer to maintain the pH at 7.3. The pH is adjusted by the percent concentration of CO<sub>2</sub> in the air stream pumped through the bottom. The average flow of CO<sub>2</sub> is 5% of the inlet air stream, but a pH control unit continuously measures the pH inside of the tank and adjusts the CO<sub>2</sub> flow accordingly. The compressed air and the CO<sub>2</sub> are supplied to the fermentor from tanks purchased from BOC gases. BR-3 requires approximately 7 tanks of compressed air and 0.3 tanks of CO<sub>2</sub> per batch (see calculations page 240). These growth conditions are deemed optimal by the Bioprocess Engineering book by Schuler and Kargi

The fermentor is manufactured and designed by New Brunswick Scientific for the growth of animal cells. A fermentor this size is custom ordered and designed for the specific purpose of culturing animal cells. New Brunswick Scientific provides the engineering support to aid in the design of a reactor a large as 5000L (see appendix page

231). In order to preserve an absolute sterile environment for the cells to grow, each vessel is made of 316 Stainless Steel with sanitary piping and couplings. A six blade standard marine impeller installed in the center of each bioreactor disperses the air stream and mixes the cell culture. The agitation speed of the culture is only 30 rpm due to the sensitivity of CHO cells and the very low shear level the cells can endure. The agitator is run by an electric motor that requires 10 hp of energy utility. The reactors are sterilized in place by a process of flushing with water to remove any excess solid waste and automatically steam sterilized at 130 °C for approximately 5 hours. The reactor must reach a temperature of 130 °C and remain at that temperature for an extended period of time in order to kill or denature anything that remains inside the growing environment.

Attached to the fermentor is a ML-6100 controller system is that is capable of operating up to sixteen process loops (see appendix page 229). The system also controls the sterilization and internal environment preservation. The menu-driven system is designed for simple control and minimal operator labor hours. The computer control system can be programmed to run a batch for any amount of time. A vessel view window is installed into the wall of the fermentor in order to allow an operator monitor the process inside. The cell growth rate and the quality of the automatic sterilization are observed repeatedly through out the life of the batch. If a vessel is not completely clean after the water flush it must be manually scrubbed before steam sterilization.

The batch length of BR-3 is 14 days. The batch schedule for the fermentor is the following:

- 2 Days Fill and Inoculate
- 8 Days of Cell Growth (and lag time)
- 1 Day Harvest
- 3 Days to Sterilize

The times allotted for each of these processes are over estimated to ensure enough time for adequate cell growth and production and provide enough time to stagger 50 batches between 2 fermentor trains in the course of one year. The tPA production plant contains a total 2, BR-3 fermentors, one for each of the two trains. The quoted price from Ted Shields of New Brunswick Scientific for one unit with a complete control system is \$250,000. (*see specification page 98 and appendix page 231*)

### **Heat Exchanger (HX-1)**

HX-1 is a shell and tube heat exchanger, which heats the complete media from 4 °C to the optimal cell growth temperature of 37 °C. It is 4 ft. long with 1 tube pass and one shell pass. The unit had 18 tubes with a nominal outside diameter of 0.75 in. and a 1.25 in. square pitch. The shell has a 6.6 in. inner diameter. The heat duty for this heat exchanger is  $6.100 \times 10^4$  BTU/hr, with an overall heat transfer coefficient of 70 BTU/F\*ft<sup>2</sup>\*hr. Based on these calculations, the heat transfer area of this heat exchanger is 12 ft<sup>2</sup> (*see calculations page 245*).

The media is passed through the tube side at a flow rate of 2 gal/min counter-currently to a warm water stream flowing at an approximate flow rate of 9.7 gal/min (*see calculations p 245*) that enters the heat exchanger at a temperature of 55 °C and exits the unit at 48 °C. The warm water stream cannot enter HX-1 at a temperature greater than 55 °C because the organic materials in the media will be damaged. The warm water stream is made up of a recycled flow of city water that is temperature controlled with a steam

sparger. The additional water accumulated in the stream from the addition of steam is regulated by a pressure control and purged off to the waste treatment facility.

The HX-1 is made from 316 Stainless Steel with sanitary piping and couplings to ensure sterile conditions. After each batch the unit is flushed out with water twice its volume and steam sterilized in place with 130 °C steam for 2 hours. The tPA production plant contains a total 2, Heat Exchangers, one for each of the two fermentation trains.

The total purchase cost of one exchanger is estimated using cost table in the Process Design book by Dr. Warren Seider to be \$6500.00. (*see specification page 99 and appendix page 245*)

## **Blending Tank (T-1)**

The media blending tank is used to combine the dry powder HyQ PF-CHO media with 4500 L of sterile water in order to produce the complete media for use in BR-1, BR-2, and BR-3. The tank is first filled with 90% of the total volume of water desired. While the water is gently stirred at 50-60 rpm, the powder media is added and mixed until it is dissolved. The agitation motor that drives the impeller requires 10 hp and uses 59000 kW\*hr per year energy (see calculations page 255). The water must stay at room temperature, 20 °C while the powder is mixed into the water in order to ensure complete dissolution. The volume is then brought up to 100% and the media is cooled to a temperature of 4 °C. Due to staggered scheduling of each batch, the three fermentors are filled at different times over the course of 7 days. The media remains in the tank at 4 °C until it is needed in the fermentors. As suggested by Dr. Kivnick the T-1 is equipped with a refrigeration unit and a jacket of copper tubing that is filled with flowing

refrigerant (freon) in order to cool the liquid inside the tank. The outside of the tank is covered with a 2 in. layer of fiberglass insulation to decrease the refrigeration requirements. The refrigerator requires 65000 kW\*hr per year to cool the liquid.

The media is prepared 2 days before its first scheduled use in order to allow adequate time for quality testing of the mixed media. A sample of media extracted and used to grow up a cell culture in the quality assurance laboratory. The results of the culture are analyzed for the presence contamination and the average cellular growth rate for the batch of media. The tank is manufactured using pharmaceutical standards of materials and sterile quality. The tank and internal impeller are made from 316 stainless steel and the piping associated with the vessel is of sanitary grade. The unit is designed to be steam sterilize in place after each batch. The blending tank is flushed out with water twice its volume to remove any lingering debris and steam sterilized at 130 °C for 3-4 hours. A sight glass is installed on the top of the tank so operators can view the process inside and watch for any build up of material on the walls and impellor inside the vessel that would have to be manually washed before steam sterilization.

The tPA production plant contains a total 2, T-1 tanks, one for each of the two fermentation trains. Walker Stainless Steel Equipment approximates the cost of this blending tank at \$28,658.00 (see appendix page 250). Under the advice of Dr. Kivnick, an additional \$2,000 is added to the purchase cost for the copper tubing that makes up the cooling jacket and the insulation. The small refrigerator attached to the tank is quoted at \$20,000 and 8 tons of refrigerant is purchased at a price of \$250.00/ton to provide the fuel for the refrigerator. (see specification page 100 and appendix page 250)

## **Pumps**

### **Filter Pump P-1 and P-2**

Filter Pumps P-1 and P-2 increases the pressure of the 2 gal/min liquid media stream from 14.7 psi up to 29.7 psi and sustains the volumetric flow rate. P-1 controls the steam leaving T-1 and pushes the 4 °C stream through a 2 µm filter F-1. P-2 increases the pressure of the same stream to overcome the pressure drop from F-1 and push the stream through the next filter F-2. The pumps are Randolph Austin Series 610 vari-flow speed control peristaltic pumps that are equipped to handle a pharmaceutical grade product streams and maintain their sterile quality (see appendix page 256). The pumps are made out of stainless steel, which is sterilized in place by flushing water through the pump until all residues are removed and steam sterilized at 130 °C for 30 min. The power requirement for this peristaltic pump is specified by Randolph Austin Company at 0.25 hp. There are a total of four filter pumps in the tPA production plan, two for each of the two fermentation trains. The cost of one pump is listed as \$1595.00. *(see specification page 101 and appendix page 253)*

### **Heat Exchanger Pump P-3**

Heat Exchanger Pump P-3 increases the pressure of the 2 gal/min liquid media stream leaving F-2 at 14.7 psi to 29.7 psi and sustains the volumetric flow rate. The pressure increase of the stream is used to flow the 4 °C stream through HX-1. The pump is a Randolph Austin Series 610 vari-flow speed control peristaltic pump that is equipped to handle a pharmaceutical grade product stream and maintain its sterile quality (see

appendix page 256). The pump is made out of stainless steel, which is sterilized in place by flushing water through the pump until all residues are removed and steam sterilized at 130 °C for 30 min. The power requirement for this peristaltic pump is specified by Randolph Austin Company at 0.25 hp. There are a total of 2 Heat Exchanger pumps (P-3) in the tPA production plan, one for each of the two fermentation trains. The cost of one pump is listed as \$1595.00. (*see specification page 103 and appendix page 253*)

### **Cell Broth Pumps P-4 and P-5**

The Cell Broth pumps function to move the cell broth from one fermentor to the next one in the process train. The streams flow at a volumetric flow rate of 2 gal/min at 38 °C and a pressure of 29.7 psi. P-4 moves the cell broth from the BR-1 to BR-2 and P-5 moves the cell broth from BR-2 to the last unit of the train, BR-3. The pump increases the pressure of the stream from 14.7 psi to 29.7 psi to overcome any pressure drop that occurs over the piping between the bioreactors. The pumps are Randolph Austin Series 610 vari-flow speed control peristaltic pumps that are equipped to handle a pharmaceutical grade product stream and maintain its sterile quality (*see appendix page 256*). The pump is made out of stainless steel, which is sterilized in place by flushing water through the pump until all residues are removed and steam sterilized at 130 °C for 30 min. The power requirement for these peristaltic pumps is specified by Randolph Austin Company at 0.25 hp for each. There are a total of four cell broth pumps in the tPA production plan, two for each of the two fermentation trains. The cost of one pump is listed as \$1595.00. (*see specification page 104 and appendix page 253*)



## Heating Jacket Water Pump P-6

The function of the Heating Jacket Water Pump P-6 is to flow water through the half-pipe heating jacket that surrounds BR-3. The water is used to maintain an internal fermentor temperature of 37 °C. The flow rate of water is dependent upon the heat loss from the reactor to the environment. The flow rates are calculated to range from 0.2 kg/hr to 0.09 kg/hr for different environmental conditions (see calculations page 233). The pump also increases the pressure of the stream from 14.7 psi to 29.7 psi in order to overcome the pressure drop that occurs along the tubing. The pump is a Randolph Austin Series 610 vari-flow speed control peristaltic pump that is equipped to handle a pharmaceutical grade product stream and maintain its sterile quality (see appendix page 256). The pump is made out of stainless steel, which is sterilized in place by flushing water through the pump until all residues are removed and steam sterilized at 130 °C for 30 min. The power requirement for this peristaltic pump is specified by Randolph Austin Company at 0.25 hp. There are a total of two heating jacket water pumps in the tPA production plan, one for each of the two fermentation trains. The cost of one pump is listed as \$1595.00. (*see specification page 106 and appendix page 253*)

## Heat Exchanger Water Pump P-7

The function of the Heat Exchanger Water Pump is to flow warm water through the shell side of HX-1. The water is used to heat up a stream of media from 4 °C to 38 °C. The calculated flow rate of water of the water is approximately 9.6 gal/min (see calculations page 245). The pump also increases the pressure of the stream from 14.7 psi

to 29.7 psi in order to overcome the pressure drop that occurs over the heat exchanger and piping. The pump is a Corcoran Model 3000D-HD1 centrifugal pump that is equipped for small flow rates (see appendix page 260). The pump is made out of carbon steel and has a power requirement of 2.0 hp. There are a total of two heat exchanger water pumps in the tPA production plan, one for each of the two fermentation trains. The cost of one pump is listed as \$3250.00. *(see specification page 107 and appendix page 253)*

## **FILTERS**

### **Fermentor Air Filters (F-3, F-4, F-5, F-6 F-7, and F-8)**

The air filters attached to the air inlet streams and the air purge streams of the 6 bioreactors function to sterilize the air that is fed to the cell culture and purify the exhaust air from the fermentors of any biomaterial before it is released to the environment. The Osmonics Memtrex-FE Filters are dead-end filters that are made from PTFE membrane with an absolute filtration rating of 0.2  $\mu\text{m}$ . The filters are designed to handle inlet flows up to 15 cc/min per 10 inches of filtration area. Each cartridge is 2.75 inches in diameter and 10 inches in length. Multiple cartridges can be placed in a row to handle larger flow rates, but only one 10 in cartridge is needed to handle the air flows for the process that range from 0.03-0.35 kg/hr for the three different size fermentors. The pressure drop over the filter is calculated by the equations provided by Osmonics to be than one bar for each of the filters because of the small volumetric flow rate and working pressure of 29.7 psi. *(see appendix page 267).*

The air filters are replaced approximately every 3<sup>rd</sup> batch, but the outlet flows are still monitored by operators by gages on the pipes, in case a filter must be replaced before the end of the third batch. In between batches, the filters are flushed with water and sterilized with 130 °C steam for 30 min. There are a total of 12 air filters in the tPA production plan, six for each of the two fermentation trains. The cost of each filter is quoted by Osmonics to be \$44.00 per filter. Installation costs and piping are factored in to the overall construction cost of the plant in the economics flow sheet. (*see specification page 110 and appendix page 267*)

### **Media Filter- 2 $\mu$ m (F-1)**

The media filter F-1 function to sterilize the prepared media in the blending tank, before it is pumped into the fermentors. The 2  $\mu$ m filter is used primarily to protect the 0.2  $\mu$ m filter F-2 from clogging to easily. The Osmonics Flotrex-AP Filters are dead-end filters that are made from polypropylene fiber media with an absolute filtration rating of 2  $\mu$ m (see appendix page 263). Each cartridge is 2.75 inches in diameter and 10 inches in length with effective filtration area of 5.2 ft<sup>2</sup>. The filter operates with a flow of 2 gal/min at a temperature of 4 °C and a pressure of 29.7 psi. The pressure drop calculated using the equations provided by Osmonics is extremely small; therefore an estimated pressure drop of 15 psi was used for all design calculations.

F-1 is replaced every batch, but the outlet flows are still monitored by operators through the aid of gages on the pipes, in case a filter must be replaced before the end of the batch. During the course of one batch the filter is used three times: once to fill each of

the three fermentors. The filter is sterilized during down time between each media load with sterile water that is flushed through the filter to remove any debris and steam sterilized at 130 °C for 30 min. There are a total of 2, F-2 filters in the tPA production plan, one for each of the two fermentation trains. The cost of each filter is quoted by Osmonics to be \$32.00 per filter. Installation costs and piping are factored in to the overall construction cost of the plant in the economics flow sheet. (*see specification page 108 and appendix page 263*)

## **Media Filter- 0.2 $\mu\text{m}$ (F-2)**

The media filter F-2 function to sterilize the prepared media in the blending tank, before it is pumped into the fermentors. The 0.2  $\mu\text{m}$  filter removes 99.9% of all contaminants found in the media. The Osmonics Memtrex-FE Filters are dead-end filters that are made from PTFE membrane with an absolute filtration rating of 0.2  $\mu\text{m}$ . Each cartridge is 2.75 inches in diameter and 10 inches in length that can handle a maximum flow of 15 cc/min. The filter operates with a flow of 2 gal/min at temperature of 4 °C and a pressure of 29.7 psi. The pressure drop calculated using the equations provided by Osmonics is extremely small; therefore an estimated pressure drop of 15 psi was used for all design calculations (*see appendix page 267*).

F-2 is replaced every batch, but the outlet flows are monitored by operators by gages on the pipes, in case a filter must be replaced before the end of the batch. During the course of one batch the filter is used three times: once to fill each of the three fermentors. F-2 is sterilized in down time between each media load with sterile water

that is flushed through the filter to remove any debris and steam sterilized at 130 °C for 30 min. There are a total of 2 media filters in the tPA production plan, one for each of the two fermentation trains. The cost of each filter is quoted by Osmonics to be \$44.00 per filter. Installation costs and piping are factored in to the overall construction cost of the plant in the economics flow sheet. (*see specification page 109 and appendix page 267*)

## **Boiler**

A small single boiler generates all of the plant's sterile steam. The steam is used to sterilize all process equipment and piping, as well as a source of energy to increase the temperature of four water streams. The boiler produces approximately 500 lb/hr of 39.16 absolute pressure, 130 °C steam. The water used to make the steam enters the boiler from an in-house water purification system and is pumped in at 20 °C. The fuel for the boiler, 54.54 lb/hr of natural gas, which is 94.2% methane, is fed to the boiler. The heating values of the natural gas stream are 23,000 BTU/lb.

The boiler efficiency is predicted to be 85%. The plant sterilization requirement and the sparged streams are small compared to the capacity of the boiler, but an over estimate was assumed in order to account for an increased load on the system and provided enough capacity if the plant was expanded. The boiler is equipped with sanitary piping and stainless steel components in order to maintain the highest levels of sterility. Boiler controls include a feed-water flow control, fuel flow control, and the emission monitoring equipment. The Icarus Process Evaluator and Aspen Steady State Simulator aided in the boiler design. The approximate cost quoted by the IPE program for a boiler of this size is \$112,000. (*see specification page 111 and appendix page 279*)

## **Blending Tanks**

Blending tanks are used throughout the Separation section to ease batch scheduling and adjust the chemical composition of our product solution. The Separation section, including all blending tanks, is held at 4°C. Low temperatures prevent loss of protein activity over time, a primary concern during storage. Solutions are constantly being agitated during storage or blending. Electrical requirements were calculated assuming constant agitation over 7,920 hrs/year (large overestimate) and power needs of 7 hp for 300-500 L tanks and 10 hp for 4000-5000 L tanks. Yearly impeller electrical needs for Holdup and UF1 Tanks are 59,000 kW\*hr. The remaining smaller tanks each required 29,500 kW\*hr/year (see appendix page 362). All tanks are cleaned-in-place (CIP) after each use by flushing with two tank volumes of sterile water and sterilized-in-place (SIP) with steam for 30 minutes. Tanks are made with 316 Stainless steel and pricing was completed with information from Walker Stainless Equipment. *(see appendix page 250)*

## **Holdup Tank**

The function of this blending tank is to provide storage space for our product after removal from BR1. The capacity of this tank is 5000 L, but its use will only be necessary if the Centrifuge is being repaired. The cost of Holdup Tank is estimated at \$19,000 based on information provided by Walker Stainless Equipment. *(see specification page 112 and appendix page 250)*

## UF1 Tank

The function of this tank is to blend arginine to 1M (~850 kg) in the product stream taken from the Centrifuge. tPA will be concentrated in the next step, so protein aggregation will be a concern; the arginine is added to prevent this aggregation. The product solution is then stored overnight until it is pumped to UF1 the next morning. UF1 Tank also acts as a holding tank for the retentate recycled from UF1 during the first ultra filtration step. Its capacity is 4000 L, and its cost was quoted at \$17,000 by Walker Stainless Equipment. (*see specification page 113 and appendix page 250*)

## UF2 Tank

The function of this 300 L tank is to blend arginine to 2M (~67 kg) in the retentate collected from UF1. All of the retentate from UF2 is recycled to UF2 Tank during the second ultra filtration step. The cost of UF2 Tank was quoted at \$9,000 by Walker Stainless Equipment. (*see specification page 114 and appendix page 250*)

## Affinity Tank

The function of this tank is to store UF2 filtrate at 4°C before it is applied to Affinity Column. One quarter of its initial contents (~80 L) will be applied to Affinity Column in each of four cycles. The cost of this 400 L capacity tank is estimated at \$10,000 based on information provided by Walker Stainless Equipment. (*see specification page 115 and appendix page 250*)

## ER Tank

The function of this 400 L tank is to store the elution from Affinity Column at 4°C, increase its contents' pH by addition of 1M NaOH, and add the required amount of sucrose. Sucrose will also be added in this tank so any endotoxins introduced with it will be removed in the ER Column. Approximately 80 mL of 1M NaOH is added to ER Tank after each Affinity Column elution is collected. This is sufficient to neutralize the solution (see appendix page 345). Sucrose is then added in the amount of 0.03 kg. After water-for-injection (WFI) is added to MF Tank the concentration of sucrose will be 0.2 mM and the salt concentration will be well within the injection standards given by Prof. Diamond (see appendix page 289). Its cost is estimated at \$10,000. (*see specification page 116 and appendix page 250*)

## MF Tank

Final preparation of the product solution takes place in MF Tank. WFI is added to increase the volume to 400 L. This sets the volume loaded into each 100 mL vial at approximately 25 mL. The capacity of this tank is 500 L and its cost is estimated at \$11,000. (*see specification page 117 and appendix page 250*)

## FD Tank

The function of this tank is to store and agitate the MF filtrate at a temperature of 4°C until it is bottled (about 6 days). A small sample of FD Tank contents is taken to the control laboratory to determine the tPA concentration, which is required to set the amount



of solution that will be loaded in each 100-mL vial. The absorbance of light at 260 nm provides us with the protein concentration in our solution (the tryptophan in the protein absorbs light at this wavelength). The volume of solution per vial is such that 100 mg of tPA will be present after lyophilization (freeze drying). Bottling and freeze drying occur in five cycles, requiring one-fifth of the initial FD Tank (~80 L) contents to be transferred out every 26 hrs. FD Tank capacity is 500 L and its price is estimated at \$11,000. (*see specification page 118 and appendix page 250*)

## **Centrifuge**

The Centrifuge is used to remove the CHO cells from the media harvested from BR1. tPA is secreted from the cells after it is produced, so we can separate the intact cells while losing minimal protein. I assumed that 5% of the tPA present in the harvest media was lost during centrifugation, a large estimate. For the removal of mammalian cells, the CSC 4 Clarifier by Westfalia Separator has a capacity of 400-500 L/hr. A built-in control system measures the turbidity of the clarified liquid and ejects solids when necessary. Cleaning and sterilization are accomplished by washing with NaOH for 30 minutes and SIP for 30 minutes at 130°C. Given that processing time is less than 10 hrs, one centrifuge is capable of handling batches from both Bioreactor trains. Even if three 5000 L Bioreactor trains were present there would be sufficient downtime for regular maintenance of the centrifuge. The Westfalia representative assured me that the clarifier is extremely reliable and breakdowns are rare. Power requirements are 9,000 kW\*hr/year. The price of the CSC 4 Clarifier with controls, CIP, SIP, and a three

channel centripetal pump was quoted as \$355,000. *(see specification page 119 and appendix page 296)*

## **Pumps**

Since our product is produced batch-wise in relatively small volumes, peristaltic pumps are sufficient for us to use throughout our process. Maximum flow rates and an efficiency of 80% were used in calculating the power requirements and costs for each pump. All pumps operate at 4°C within the air-conditioned Separation section. *(see appendix page 362)*

### **UF1 Pump**

UF1 Pump is required to increase the pressure of stream P-6 solution to 40 psi before it is ultra filtered in UF1. The Series 750-362 Vari-flow pump from Randolph Austin Company can bring 830 L/hr of solution up to 40 psi from a 14.7 psi tank. Electrical requirements for this operations were calculated as 390 kW\*hr/year. The price for this pump was quoted by a Randolph Austin representative as \$2,656. *(see specification page 120 and appendix page 256)*

### **UF2 Pump**

UF2 Pump increases the pressure of stream P-11 from 14.7 psi to 40 psi at a flow rate of 1350 L/hr. Again, the Series 750-362 Vari-flow pump from Randolph Austin Company will be used. Electrical requirements are 625 kW\*hr/year, though it should be

much less given that the pump is used for less than an hour each batch. The price for UF2 Pump is also \$2,656. (*see specification page 121 and appendix page 256*)

## **MF Pump**

MF Pump produces a pressure of 34.7 psi in stream P-30, the inlet stream to MF. The pressure drop across MF is assumed to be 20 psi, though it is certainly much lower than that value. Increasing the pressure of MF Tank contents from 14.7 psi to 34.7 psi at a flow rate of 1135.6 L/hr can be accomplished by the Series 750-362 Vari-flow pump from Randolph Austin Company. Electrical requirements for a year of use are 425 kW\*hr. The price is again \$2,656. (*see specification page 122 and appendix page 256*)

## **Affinity Skid/Pump**

The Affinity Skid/Pump increases the pressure at the inlet of the Affinity Column. Its computer controls the volumes of the Load, Equilibration, Elution, and Wash steps (*see Affinity Column description page 82*). Given a superficial velocity of 100 cm/hr, the volumetric flow rate applied to this column is 135 L/hr. The pressure drop across the bed was calculated as 1.2 psi using the Ergun equation. This number was increased to 10 psi in determining the 25 kW\*hr of electricity required each year to run the pump. Due to the fact that items such as this are custom-made by Amersham Pharmacia Biotech, the representative was unable to quote an accurate price. Prof. Diamond recommended \$100,000, which includes all of the pumping, computer, and control equipment. (*see specification page 123 and appendix page 256*)

## **ER Skid/Pump**

The ER Skid/Pump controls flow through the ER Column and increases the inlet pressure to make up for the pressure drop across the packed bed. Product, Cleaning, and Regeneration solutions are applied to the column at 100 cm/hr or 11 L/hr (see ER Column description page 84). The pressure drop across the bed is 1.9 psi as calculated by the Ergun equation. Again, the value was increased to 10 psi in determining the yearly electrical requirements of 2 kW\*hr. Amersham Pharmacia Biotech could not provide a quote for ER Skid/Pump, so Prof. Diamond's recommendation of \$100,000 will be used again. (*see specification page 124 and appendix page 256*)

## **Tangential Flow Filtration**

Tangential flow filtration involves the flow of a feed stream through a hollow fiber filter in order to concentrate a product or remove solids from a stream. It is ideal for use in the separation of small biological molecules and organisms.

## Cross Flow Membrane Filtration

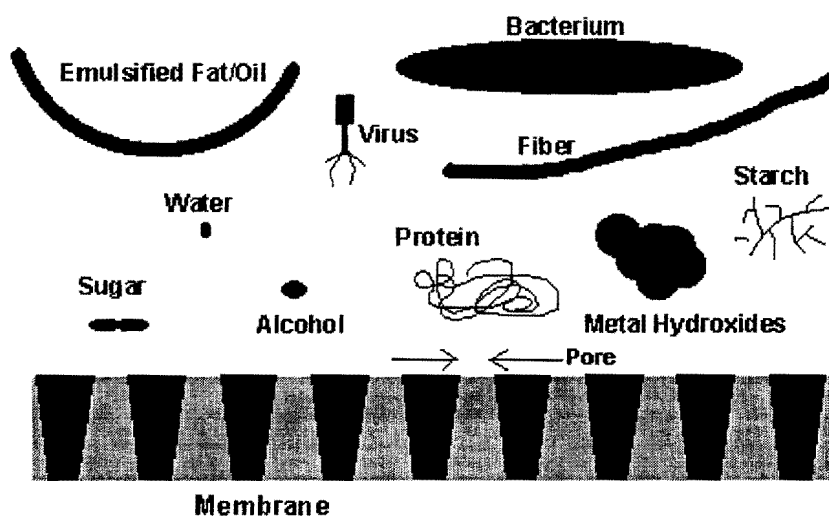


Figure: 6

Ultrafilters are used early in the process to concentrate the tPA solution after centrifugation. Following the first ultrafilter, a second is used to “clean up” the solution before the chromatography steps.

## Basic Membrane Concepts

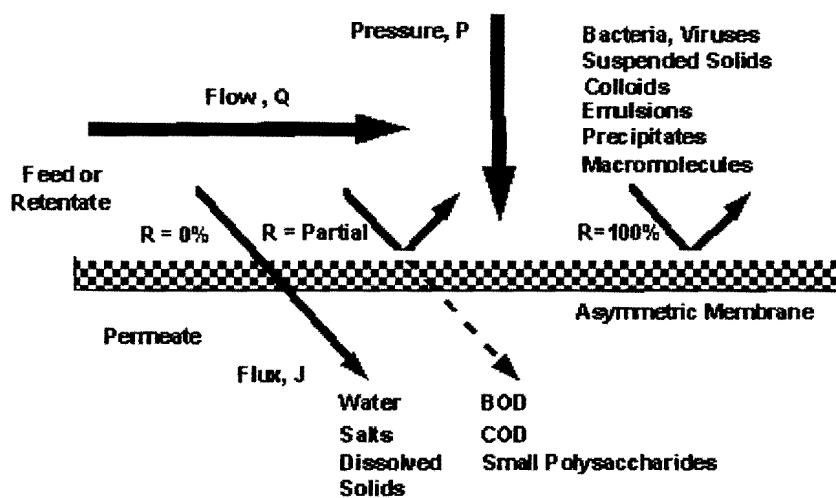


Figure: 7

The product stream flows along the filter, and osmotic pressure is used to push water or dissolved solids through the filter. Proteins will either be rejected by the membrane or flow through it, depending on the filter's specified Molecular Weight Cut-Off (MWCO). If the protein's size is larger than the MWCO it remains in the retentate; if the MWCO is larger than the protein it will flow through as permeate. (*see appendix page 303*)

## **UF1**

UF1 is used to concentrate tPA from the Centrifuge supernatant. UF1 Tank contains approximately 2 kg of tPA in about 3500 L of liquid media. This volume will be reduced to about 250 L. The MWCO of the Koch Membrane HF 14-43-PM50 is 50 kDa (a Dalton is equivalent to one gram per mole and is commonly used in the description of protein sizes). The molecular weight of tPA is 66 kDa, which causes it to be rejected from the membrane remain in the retentate. The filtrate flux is specified for the PM50 as 550 L/hr; given this flow rate and starting volume, 33 L/hr (or 6% of 550 L/hr) needs to be taken off as product retentate. The remaining retentate is recycled back to UF1 Tank as shown in Figure 8 on page 81. A ratio-flow controller is used to control the product retentate flow in stream P-8. An inlet pressure of 40 psi is required, and the pressure drop was quoted as 10 psi. The membrane is made of polysulfone and has an area of 14 ft<sup>2</sup>. The Koch Membrane representative assured me that fouling would not be a problem and that the PM50 filter would last for at least a year. The amount of tPA lost to denaturing is assumed to be only 2%. This is a conservative estimate given that the pores are designed to prevent clogging and aggregation at the openings. Cleaning is accomplished by recirculation through the membrane of 10 gal diluted surfactant

solution, priced at \$75 per concentrated bottle. The pump and blending tanks are priced separately, and the PM50 cartridge costs \$785. Piping and equipment setup costs are factored in during our economic analysis. *(see specification page 125 and appendix page 303)*

## **UF2**

UF2 removes larger components that were not separated in the Centrifuge. The MWCO of the Koch Membrane HF 14-43-PM500 is 500 kDa and will remove larger proteins and cell debris that can potentially foul the resin in Affinity Column or ER Column. tPA flows through the membrane and is collected along with the entire volume of UF2 Tank as filtrate. The inlet pressure is 40 psi and there is a pressure drop of 10 psi across the membrane. The filtrate flux is specified as 880 L/hr over the 14 ft<sup>2</sup> polysulfone filter, and all of the retentate is recycled to UF2 Tank. Cleaning is again done by recirculating the surfactant fluid through the membrane. Because the tPA flows through the membrane I increased the tPA loss to 5%, though the Koch representative stated that minimal protein would get caught. The price of the PM500 cartridge was quoted at \$950 and it will be replaced yearly. *(see specification page 126 and appendix page 303)*

## **MF (Microfiltration)**

MF performs a final sterilization of the product stream before it is bottled and freeze dried. The 9 ft<sup>2</sup> PTEF membrane has a pore size of 0.2 microns. Unlike the UF1 and UF2 above, MF is a dead-end filter, where all of the fluid passes through the membrane and is collected as filtrate. The pressure drop over MF is generously assumed

to be 20 psi, and the maximum flow rate is 1140 L/hr. Filter cartridges will be replaced after each batch and they are quoted at \$44 each by Osmonics. (*see specification page 127 and appendix page 267*)

## **Affinity Column and Resin**

Affinity Column is the most important separation unit in our process. It utilizes affinity chromatography to select for tPA. Affinity chromatography involves the binding of a protein or other biologically active molecule to a resin or other biologically active molecule. This process utilizes the binding of a monoclonal antibody to tPA. A monoclonal antibody simply means that will only bind with our protein. The anti-tPA antibody is bound to the Affinity resin where it remains indefinitely. A protein solution is loaded onto the column and as it flows through, the active protein (tPA in our case) binds to the antibody that is attached to the resin. After the Loading is complete, the column is washed or equilibrated with Equilibration buffer. Finally, the column is eluted. Elution takes place in a high salt or acidic solution; these conditions cause the protein to break its bond with the antibody. The Elution only contains the active protein and any salts or amino acids present in the Elution buffer. The column is then washed with Equilibration buffer and can be reused (the antibody remains attached to the resin throughout the process).

Our 58 L 316 Stainless steel column is completely filled with CNBr-activated Sepharose 4 Fast Flow resin. The resin is made up of agarose beads with an average diameter of 90 microns, and our anti-tPA antibody forms a bond with the CNBr that is attached to the resin. The contents of Affinity Tank are run through the Affinity Column



in four cycles. After the first Load is applied to Affinity Column (through the Affinity Skid/Pump), 2.5 bed volumes of Equilibration buffer is applied to the column (a bed volume is the volume of resin in the column, or 58 L). 1.3 bed volumes of Elution buffer then flows through the column, and this tPA-containing solution is collected and sent to ER Tank. The column is then washed with 5 bed volumes of Equilibration buffer and cycle repeats itself three more times until the entire batch has been eluted. After each batch the resin will be stored in 20% ethanol.

Amersham Pharmacia Biotech recommends flow through the column at 100 cm/hr superficial velocity or 135 L/hr in our 70 cm diameter column (15 cm height). The pressure drop across the packed bed was calculated at 1.2 psi using the Ergun equation. The buffer solutions used were the same as those used by Reagan et al. Equilibration buffer contains is 0.65M NaCl in PBS at a pH of 6.8, and an acidic elution buffer is used, which contains 0.5M glycine and 2.4M arginine at a pH of 2.85. In calculating the resin requirements I used the value of 12.3 mg antibody/mL resin from Reagan et al. I assumed that only 85% of the resin would be utilized and then provided enough resin to bind all of the protein applied to the column (0.5 kg per cycle). Even with the increased amount of resin I am assuming a loss of 15% of the tPA applied to the column based on the recommendations by Prof. Diamond.

Bioprocess Stainless steel chromatography columns are custom-made by Amersham Pharmacia Biotech, so we are using Prof. Diamond's recommendation of \$100,000. The anti-tPA antibody will be produced in our facility, but no data is available on its production so Prof. Diamond advised us to use an estimate of \$2,000/g antibody. We require 710 grams each the column so we accounted for 750 grams of antibody in our

economic evaluation. The Sepharose resin can be bought in bulk from Amersham Pharmacia Biotech at \$2,810/L. Both the resin and antibody will need to be replaced each year. (*see specification page 128 and appendix page 314*)

## **ER Column and Resin**

The ER Column is used to remove endotoxins from the tPA solution. Endotoxins, or pyrogens, are biochemicals that come from the cell wall of bacteria. If an endotoxin containing solution is injected into a human, it will cause a feverish reaction and make the person feel sick. Our process does not utilize bacteria in any way, but endotoxins will still be present in low, but significant amounts. Endotoxins may enter our process in the chemicals or materials we use, such as the media, PBS, sucrose, or NaCl (other chemicals are bought endotoxin free). Endotoxins cannot be removed or “killed” with steam sterilization, so they may remain on equipment even after a SIP cleaning. NaOH application can remove pyrogens, but this would destroy our protein.

For the removal of endotoxins in our process we will use the Acticlean Etox resin from Sterogene. This resin binds endotoxins while allowing a protein to flow through unaffected. The binding capacity of this resin ranges from 200 EU(endotoxin units)/mL resin to 20000 EU/mL resin. Prof. Diamond advised us to assume our product solution had 5 EU/mL. Our final endotoxin level must be below the detectable level of 0.05 EU/mL. Given these specifications, 8.2 L of the Acticlean Etox resin will suffice in rendering our product safe for injection. The 200/500 Bioprocess glass column from Amersham Pharmacia Biotech can hold 8.2 L of resin and provides a large bed height to increase contact time. The bed height is 26 cm (column height is 50 cm) and the column

diameter is 20 cm. Flow through the column takes place at a neutral pH and 100 cm/hr (11 L/hr). I am assuming a 5% loss of tPA, though Sterogene references experiments where 99% of the protein is recovered. After our product solution is loaded and collected, the resin needs to be washed with 1M NaOH for two hours. The resin is then regenerated with WFI and stored at a neutral pH of 7.0. Amersham Pharmacia Biotech quoted the column at \$6,190, and the resin was quoted at a bulk price of \$4,320/L by Sterogene. The resin is replaced each year, and costing is completed assuming a purchase of 10L/year. *(see specification page 130 and appendix page 329)*

## **Bottler**

The bottler loads a designated amount of the final tPA solution in 100 mL vials, stoppers them, and loads them into the freeze dryer. The Cozzoli Machine Company provides machines that interact with one another to complete these tasks. The VR 840S can load approximately 3200 100-mL vials with 25 mL of fluid in about an hour. A concentration meter is required to set the amount of tPA solution that will be loaded in each vial. The BT15 Tray Loader can load these vials into the freeze dryer in one hour, and the AW160 Automatic Washer can clean and sterilize the VR 840S and BT15 after each cycle. The freeze dryer needs to be loaded and unloaded five times per batch, so the total uptime for the Bottler is about 146 hrs, though it is idle for most of that time. Each machine requires 0.5 hp, and the total yearly electrical requirements are 8,200 kW\*hr. The total cost quoted by Cozzoli Machine Company is \$1,265,000. *(see specification page 132 and appendix page 346)*

## **Freeze Dryer**

The freeze dryer removes WFI from the final tPA solution, creating a stable solid product. The final product is lyophilized (freeze dried) because it can remain active indefinitely; the solid simply needs to be redissolved in WFI. The exact time it takes to lyophilize a protein can only be determined experimentally. Since we are unable to perform these tests we will be assuming a drying time of 24 hrs. The condenser capacity of the Virtis Benchmark 6000 SQ Stoppering Lyophilizer is 220 L of water in 24 hours. Each cycle will only contain about 80 L of solution, making our estimate of 24 hrs very reasonable. The freeze dryer first freezes the solution in a vacuum, causing the solid solution to sublime (water vapor can escape the partially stoppered vials). After this primary drying is complete, the remaining water molecules are removed by slowly introducing heat. This must be done gradually because any extreme temperature changes can cause the protein to lose activity. All heating and cooling is done by way of a silicon fluid. The silicon heat transfer medium flows through the baffled trays and back to the condenser, where it is again heated or cooled and returns to the vials. I chose the SQ (square) version as opposed to the Plus (circular) on a recommendation from the Virtis representative. He claimed there is a theory that a square lyophilizer is better for freeze drying (it is also \$100,000 more expensive). The freeze dryer requires 6 gal/min of cooling water, and the maximum power requirement is 46.1 kW, quoted in a phone conversation with a Virtis representative. The equipment uptime for each batch is about 146 hours. The yearly electrical usage is 340,000 kW\*hr. Virtis quoted the Benchmark 6000 SQ, SIP and CIP capable with full controls at \$640,000. The final rate of

production of tPA is about 1.6 kg/batch or 16,000 doses. (*see specification page 133 and appendix page 350*)

## **Refrigerator**

A refrigerator or air-conditioning system is required to keep the Separation trains at 4°C year-round. Prof. Diamond helped up estimate the total area of the Separation sections as 4,000 ft<sup>2</sup>. Rich Metallo at Baltimore AirCoil provided “rules of thumb” for the air-conditioning requirements. It is assumed that 1.25 tons of refrigerant are required per 300 ft<sup>2</sup> of space to be cooled. The power requirement for one ton of refrigerant is 1 kW, but a load factor of 0.6 is included in the calculation to account for the fact that outside temperature varies throughout the year (not as much cooling is necessary during the winter). Given these estimates, 17 tons of refrigerant are required for the Separation refrigeration, and the cost of refrigerant is \$250/ton. The yearly electrical consumption of this system is 87,600 kW\*hr. Prof. Diamond recommended we estimate the purchase cost of such a refrigeration system as \$10,000. (*see specification page 134*)

## **Initial Pump (PW-1)**

City water from stream W101 is brought into the water purification system through pump PW-1. PW-1 increases the pressure of stream W101 from 14.7 psi to 34.7 psi in order to compensate the pressure drop in filter FW-1. PW-1 is a peristaltic pump that is constructed from 316 stainless steel and has speed and flow controls. The pump is capable of producing a flow rate of 35 lit/min at 25 °C. According to the data in the Randolph Austin Company website, the power requirement is 1 hp. The actual cost of the

pump is \$5,250 quoted from Mr. Mike Milson, a representative from Randolph Austin Company. *(see specification page 135 and appendix page 387)*

### **10 $\mu$ m Microfilter (FW-1)**

The purpose of FW-1 is to remove large particles from original city water of stream W101 before it enters the water purification process. The city water is brought into the process at 25 °C and will flow through FW-1, which will remove dissolved particles larger than 10  $\mu$ m. The flow rate of water and the pressure drop across the filter are 18 L/min, and 10 psi, respectively. The membranes are constructed from high-purity polypropylene, which is an FDA-acceptable material as are all other parts of the filter. The FW-1 cartridge will be replaced after each batch to ensure the sterilization. The actual cost of the filter is \$44 per cartridge according to Mr. James Lewis, the application engineer at Osmonics. *(see specification page 136 and appendix page 389)*

### **Carbon Filter Pump (PW-2)**

The function of pump PW-2 is to increase the pressure in stream W103 from 14.7 psi to 26.7 psi in order to compensate the pressure drop in filter FW-2. PW-2 is a peristaltic pump constructed from 316 stainless steel with speed and flow control. The pump is capable of producing the flow rate of 18 L/min at 25 °C. According to the data in the Randolph Austin Company's website, the power requirement is 0.25 hp. The actual cost of the pump is \$2,656 quoted from Mr. Mike Milson, a representative at Randolph Austin Company. *(see specification page 137 and appendix page 387)*

### **Carbon Filter (FW-2)**

The function of filter (FW-2) is to remove chlorine and organic molecules from stream W104 since chlorine can degrade the membranes of the reverse osmosis system (RO-1) and filter (FW-4). The filter can produce 76 L/min (20 gal/min) at 25 °C, and is able to work at a maximum temperature of 50 °C. The maximum pressure drop across the filter is 12 psi recommended by the manufacturer, Ameriwater Company. The carbon filter utilizes 1000 lbs of bituminous coal as the absorbent. In addition, the manufacturer is also recommends a minimum contact time of 5.3 min based on a bed density of 28 lb/ft<sup>3</sup>. The actual cost of the filter according to Ms. Diane Dolan, a sale manager at Ameriwater Company, is \$1515. *(see specification page 138 and appendix page 389)*

### **Water Softener Pump (PW-3)**

After FW-2, water in stream W105 will proceed directly to pump PW-3, which will increase pressure in stream W105 from 14.7 psi to 24.7 psi to compensate the pressure drop in filter FW-3. PW-3 is a peristaltic pump is constructed from 316 stainless steel with speed and flow controls. The pump is capable of producing the flow rate of 76 L/min of water at 25 °C. According to the data in the Randolph Austin Company's website, the power requirement is 1 hp. The actual cost of the pump is \$5,250 quoted from Mr. Mike Milson, a representative Randolph Austin Company. *(see specification page 140 and appendix page 387)*

### **Water Softener (FW-3)**

The water softener (FW-3) is used to remove the cations such a calcium and magnesium salts from the city water. Stream W106 flows through the FW-3 at a rate of 76 L/min, with a pressure drop of 10 psi. The inner shell of FW-3 is constructed from polyethylene, while the outer surface is made from fiberglass to provide strength of up to 150 psi. The amount of resin required is approximately 7 ft<sup>3</sup>, according to from Ms. Diane Dolan, the sale manager at Ameriwater Company. The resin used for this particular softener is polystyrene sulfonate cation, which can operate at a maximum temperature of 140 °C and has a standard flow rate of 4 gal/(min ft<sup>3</sup>). The life of the resin is 10 years, while the price is \$60/ft<sup>3</sup>, according to Mr. Dally, a representative of Sybron Chemical, Inc, the manufacturer of the resin. Ms. Diane Dolan quotes the total cost of the FW-3 including resin at \$4537. (see specification page 139 and appendix page 377)

### **Reverse Osmosis Pump (PW-7)**

The water from stream W107 is drawn into the reverse osmosis system through the reverse osmosis pump (PW-7). PW-7 increases the pressure of stream W107 from 14.7 psi to 34.7 psi in order to compensate the pressure drop in reverse osmosis system. PW-7 is a Randolph Austin Model 880 Vari-Flow peristaltic pump that is constructed from 316 stainless steel with speed and flow controls. The pump produces a flow rate of 76 L/min at 25 °C, and has a power requirement of 1 hp. The purchase cost of the pump is \$5250 quoted by Mr. Mike Milson, a representative from Randolph Austin Company. (see specification page 141 and appendix page 387)

### **Reverse Osmosis System (RO-1)**



The reverse osmosis system RO-1 is a high-pressure membrane filter that is used to separate low molecular weight molecules out of the feed stream. Salts, bacteria, and other organic molecules are removed from the water stream W108 that enters RO-1 system at 25 °C. The water is produced at a rate of 13800 gal/day. The reverse osmosis system is capable of working in the pH range from 4-11 and a temperature range of 1-45 °C. The pressure drop across the system is 20 psi. In addition, the system is also equipped with a 1.5 hp pump and a 5 µm filter – to facilitate the flow within the system and to protect it from large particles that could damage the membrane. RO-1 is constructed from thin film composite. In the model we are using, there are 6 membranes that are each 4 x 40 inches. The electricity requirements of the system are 220 V, and 7.5 maximum Amps. The total power requirements per year for the system is 4474 kW\*hr. According to Ms. Diane Dolan, the sales manager at Ameriwater Company the purchase cost is approximately \$22500. (see specification page 142 and appendix page 374)

### **Storage Tank (TW-1)**

Water from RO-1 is stored in the Storage tank (TW-1) for future use in the 2 process trains. The horizontal tank is constructed from 4000 polyethylene, and has the capacity of 50000 L. The purchase cost for TW-1 is approximately \$3554 as quoted by Ms. Diane Dolan, the sales manager at Ameriwater. Note that this price does not include freight. (see specification page 143)

### **Ultraviolet Light System Pump (PW-4)**

The function of ultraviolet light system pump PW-4 is to increase the pressure in stream W110 from 14.7 psi to 19.7 psi in order to compensate the pressure drop in UV-1. PW-4 is a Randolph Austin Model 780 Vari-flow peristaltic pump that is constructed from 316 stainless steel and has speed and flow controls. The pump is capable of producing a flow rate of 23 L/min (6 gal/min) at 25 °C and has a power requirement of 0.25 hp. The purchase cost of the pump is \$2656 as quoted by Mr. Mike Milson, the salesperson at Randolph Austin. (*see specification page 144 and appendix page 387*)

### **Ultraviolet Light System (UV-1)**

In order to prevent contamination of any of the process trains, the water in TW-1 is recirculated through an ultraviolet light system that continuously purifies the water. Water from stream W111 enters the purifier and flows into the annular space between the quartz sleeve and the outside chamber wall. Within the chamber, water is exposed to intense germicidal UV radiation. UV-1 can produce 23 L/min (6 gal/min) of purified water with a pressure drop at this flow rate of 5 psi. The Atlantic Ultraviolet Corporation recommends that the maximum operating pressure of the lamp should not exceed 100 psi. The power consumption of the UV lamp is 21 W. It also has the effective life of 10000 hours, and maximum rated output at 254 nanometers. The purchase cost of UV-1 as quoted by Ms. Diane Dolan of Ameriwater Company is \$1567. (*see specification page 145 and appendix page 384*)

### **0.2 µm Microfilter Pump (PW-5)**

The function of 0.2  $\mu\text{m}$  microfilter pump PW-5 is to increase the pressure in stream W113 from 14.7 psi to 24.7 psi in order to compensate the pressure drop in FW-4. PW-5 is a Randolph Austin model 780 Vari flow peristaltic pump is constructed from 316 stainless steel with speed and flow controls. The pump is capable of producing 30 L/min (8 gal/min) flow rate at 25 °C and has a power requirement of 0.25 hp. The purchase cost of the pump is \$2656 as quoted by Mr. Mike Milson, the salesperson of Randolph Austin Company. (*see specification page 147 and appendix page 387*)

### **0.2 $\mu\text{m}$ Microfilter (FW-4)**

The 0.2  $\mu\text{m}$  Microfilter FW-4 is used to remove particles that may be left in water of stream W114 before it enters the fermentors. Water in storage tank can settle out particles and ions that must be removed before it enters the process. Therefore, FW-4 acts as the last line of protection to ensure that the water produced is uncontaminated. The size of the filter pore is 0.2  $\mu\text{m}$ . The flow rate through the filter is 18 L/min (5 gal/min) and the pressure drop is 10 psi. The filter is constructed from high-purity polypropylene. The filter will be replaced after every batch and costs \$44 per filter cartridge. (*see specification page 147 and appendix page 389*)

### **Final Pump (PW-6)**

After FW-4, purified water in stream W115 will flow through final pump (PW-6) to increase the pressure from 14.7 psi to 34.7 psi in order to compensate the pressure drop along the pipe. PW-6 is a Randolph Austin Model 880 Vari flow peristaltic pump that is constructed from 316 stainless steel with speed and flow controls. The pump is capable of

producing a flow rate of 18 L/min (5 gal/min) flow rate at 25 °C and has a power requirement of 1 hp. The purchase cost of the pump is \$5250 as quoted by Mr. Mike Milson, the salesperson at Randolph Austin. (*see specification page 148 and appendix page 387*)

# UNIT SPECIFICATION SHEETS

## **40L FERMENTOR (BR-1)**

**FUNCTION:** Batch reactor used to grow CHO Cells up to a density of  $3.0 \times 10^6$  cells/mL to be used to inoculate BR-2.

**TYPE:** New Brunswick Scientific BioFlo 5000 Fermentor with Conventional Heating Jacket

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per batch</u></b>
Complete Media	30 L
Cellular Material	3.5 kg
Compressed Air	0.3 kg/batch (3% of one tank)
CO <sub>2</sub>	0.025 kg/batch (0.1% of one tank)

**CHARACTERISTICS:**

Diameter:	0.3 m
Height:	0.6 m
Volume:	40 L
Working Capacity:	30 L
Material:	316 Stainless Steel
Agitator:	6-Blade Stainless Steel standard Marine Blade
Insulation:	2 in of Fiberglass Insulation

**OPERATING CONDITIONS:**

Temperature:	37 °C
Pressure:	14.7 psi
pH:	7.3
Compressed Air Flow Rate:	0.003 kg/hr
CO <sub>2</sub> Flow Rate (% of total air flow):	0.0002 kg/hr (5%)
Agitator Speed:	10-30 rpm
Power Requirements:	1.5 hp
Utility Cost/year	\$355.00
Sterilization:	Flush with water and Steam in Place at 130 °C for 2 hours

**PURCHASE COST:** \$85,000.00

**REFERENCE PAGES:** 54, 225-230, 232, 244, 254

## **400L FERMENTOR (BR-2)**

**FUNCTION:** Batch reactor used to grow CHO Cells up to a density of  $3.0 \times 10^6$  cells/mL to be used to inoculate BR-3.

**TYPE:** New Brunswick Scientific custom-built 400 L bioreactor with Conventional Heating Jacket

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per batch</u></b>
Complete Media	300 L
Cellular Mass	35.0 kg
Compressed Air	4.5 kg/batch (50% of one tank)
CO <sub>2</sub>	0.33 kg/batch (2% of one tank)

**CHARACTERISTICS:**

Diameter:	0.6 m
Height:	1.2 m
Volume:	400 L
Working Capacity:	300 L
Material:	316 Stainless Steel
Agitator:	6-Blade Stainless Steel Marine Blade
Insulation:	2 inch Fiberglass Insulation

**OPERATING CONDITIONS:**

Temperature:	37 °C
Pressure:	14.7 psi
pH:	7.3
Compressed Air Flow Rate:	0.3 kg /hr
CO <sub>2</sub> Flow Rate (% of total):	0.02 kg/hr (5%)
Agitator Speed:	10-30 rpm
Power Requirements:	5 hp
Utility Cost/year	\$1180.00
Sterilization:	Flush with water and Steam in Place at 130 °C for 5 hours

**PURCHASE COST:** \$125,000.00

**REFERENCE PAGES:** 57, 231, 232, 243, 254

---

## **5000 L FERMENTOR (BR-3)**

**FUNCTION:** Batch reactor used to grow a CHO Cell-Line that produces the product protein tPA.

---

**TYPE:** New Brunswick Scientific custom-built 5000 L bioreactor with 4" half pipe Heating Jacket

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per batch</u></b>
Complete Media	4000 L
Cellular Mass	460 kg
Compressed Air	69.5 kg/batch (7 tanks)
CO <sub>2</sub>	5.5 kg/batch (0.25% of one tank)

---

**CHARACTERISTICS:**

Diameter:	1.5 m
Height:	3.0 m
Volume:	5000 L
Working Capacity:	4000 L
Material:	316 Stainless Steel
Harvest Pump:	Peristaltic Harvest Pump
Agitator:	6-Blade Stainless Steel Marine Blade
Insulation:	2 inch Fiberglass Insulation

---

**OPERATING CONDITIONS:**

Temperature:	37 °C
Pressure:	14.7 psi
pH:	7.3
Compressed Air Flow Rate:	0.3 kg /hr
CO <sub>2</sub> Flow Rate (% of total air flow):	0.025 kg/hr (5%)
Agitator Speed:	10-30 rpm
Agitator Power Requirements:	10 hp
Pump Power Requirements:	5 hp
Utility Cost/year	\$3543.00
Sterilization:	Flush with water and Steam in Place at 130 °C for 5 hours

---

**PURCHASE COST:** \$225,000.00

**REFERENCE PAGES:** 59, 231, 232, 233-242, 254

---



---

## **HEAT EXCHANGER (HX-1)**

**FUNCTION:** Bring the temperature of the Media from 4 °C up to 38 °C using warm water on the shell side.

---

**TYPE:** Shell and Tube

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Flow Rates</u></b>
Water	2.2*10 <sup>3</sup> kg/hr
Complete Media	455 kg/hr

---

**CHARACTERISTICS:**

Heat Transfer Coefficient:	75 BTU/hr*ft <sup>2</sup> *F
Heat Transfer Area:	11.5 ft <sup>2</sup>
No. of Tube Passes:	1
No. of Shell Passes:	1
Number of Tubes:	18
Nominal Outside Diameter:	0.75 in
Pitch:	1.25 in, Square
Flow Direction:	Countercurrent
Material:	316 Stainless Steel

---

**OPERATING CONDITIONS:**

**SHELL SIDE:**

Pressure:	29.7 psi
Temperature In:	55 °C
Temperature Out:	48 °C

**TUBE SIDE:**

Pressure:	29.7 psi
Temperature In:	4 °C
Temperature Out:	38 °C

Heat Duty: 6.2\*10<sup>4</sup> BTU/hr

Sterilization: Flush with water and Steam in Place at 130 °C for 2 hrs.

---

**PURCHASE COST:** \$6500.00

**REFERENCE PAGES:** 62, 245-249

---

## **BLENDING TANK (T-1)**

**FUNCTION:** Mix Powdered Media and Sterile water for use in BR-1, BR-2, and BR-3.

---

**TYPE:** Walker Stationary Blending Tank with 6-blade Rushton agitator

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per batch</u></b>
Sterile Water	4500 L
Media (powder)	Powder equivalent to make 4500L of liquid media

---

**CHARACTERISTICS:**

Diameter:	1.5 m
Height:	3.0 m
Volume:	5000 L
Capacity:	4500 L
Retention Time:	7 Days
Material:	316 Stainless Steel
Cooling Coils:	2 in Copper Tubing
Refrigerator:	Standard freon refrigeration unit

---

**OPERATING CONDITIONS:**

Inlet Water Temperature:	20 °C
Outlet Water Temperature	4 °C
Pressure	14.7 psi
Outlet Flow:	2 gal/min
Agitator Speed:	50 rpm
Cooling duty:	8 tons (95386 BTU/hr)
Agitation Motor Power Requirements:	10 hp
Refrigerator Energy Requirements:	63360 kW*hr
Utility Cost per year (total):	\$4896.00
Sterilization:	Flush with water and Steam in Place at 130 °C for 2 hrs.

---

**PURCHASE COST:** \$40,658.00

**REFERENCE PAGES:** 62, 250-252, 254-255

---

## **FILTER PUMP (P-1)**

**FUNCTION:** To pump the media from the blending tank through the 2.0 $\mu$  filter.

**TYPE:** Peristaltic

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per batch</u>
Complete Media	4500 L

**CHARACTERISTICS:**

Material:	316 Stainless Steel
Length:	17 ¼ in.
Width:	13 3/8 in.
Height:	7 3/4 in.

**OPERATING CONDITIONS:**

Temperature:	4°C
Pressure In:	14.7 psi
Pressure Out:	29.7 psi
Volumetric Flow Rate:	2 gal/min
Pump Efficiency:	80%
Power Requirement:	0.25 hp
Utility Cost/yr:	\$59.00
Sterilization:	Flush with water and Steam in Place at 130 °C for 30 min

**PURCHASE COST:** \$1595.00

**REFERENCE PAGES:** 65, 253, 256-259

## **FILTER PUMP (P-2)**

**FUNCTION:** To pump the media from F-1 through F-2.

---

**TYPE:** Peristaltic

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per batch</u></b>
Complete Media	4500 L

---

**CHARACTERISTICS:**

Material:	316 Stainless Steel
Length:	17 ¼ in.
Width:	13 3/8 in.
Height:	7 3/4 in.

---

**OPERATING CONDITIONS:**

Temperature:	4°C
Pressure In:	14.7 psi
Pressure Out:	29.7 psi
Volumetric Flow Rate:	2 gal/min
Pump Efficiency:	80%
Power Requirement:	0.25 hp
Utility Cost/yr:	\$59.00
Sterilization:	Flush with water and Steam in Place at 130 °C for 30 min

---

**PURCHASE COST:** \$1595.00

**REFERENCE PAGES:** 65, 253, 256-259

---

## **HEAT EXCHANGER PUMP (P-3)**

**FUNCTION:** To pump the media through the heat exchanger and into the three bioreactors.

---

**TYPE:** Peristaltic

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per batch</u></b>
Complete Media	4500 L

---

**CHARACTERISTICS:**

Material:	316 Stainless Steel
Length:	17 ¼ in.
Width:	13 3/8 in.
Height:	7 ¾ in.

---

**OPERATING CONDITIONS:**

Temperature:	37 °C
Pressure In:	14.7 psi
Pressure Out:	29.7 psi
Volumetric Flow Rate:	2 gal/min
Pump Efficiency:	80%
Power Requirement:	0.25 hp
Utility Cost/yr:	\$59.00
Sterilization:	Flush with water and Steam in Place at 130 °C for 30 min

---

**PURCHASE COST:** \$1595.00

**REFERENCE PAGES:** 65, 253, 256-259

---

---

## **CELL BROTH PUMP - 1 (P-4)**

**FUNCTION:** To pump the cell broth from BR-1 to BR-2.

---

**TYPE:** Peristaltic

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per batch</u></b>
Cell Broth	30 L

---

**CHARACTERISTICS:**

Material:	316 Stainless Steel
Length:	17 ¼ in.
Width:	13 3/8 in.
Height:	7 3/4 in.

---

**OPERATING CONDITIONS:**

Temperature:	37 °C
Pressure In:	14.7 psi
Pressure Out:	29.7 psi
Volumetric Flow Rate:	2 gal/min
Pump Efficiency:	80%
Power Requirement:	0.25 hp
Utility Cost/yr:	\$59.00
Sterilization:	Flush with water and Steam in Place at 130 °C for 30 min

---

**PURCHASE COST:** \$1595.00

**REFERENCE PAGES:** 66, 253, 256-259

---

## **CELL BROTH PUMP - 2 (P-5)**

**FUNCTION:** To pump cell broth from BR-2 to BR-3.

---

**TYPE:** Peristaltic

---

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per batch</u>
Cell Broth	300 L

---

**CHARACTERISTICS:**

Material:	316 Stainless Steel
Length:	17 ¼ in.
Width:	13 3/8 in.
Height:	7 3/4 in.

---

**OPERATING CONDITIONS:**

Temperature:	37 °C
Pressure In:	14.7 psi
Pressure Out:	29.7 psi
Volumetric Flow Rate:	2 gal/min
Pump Efficiency:	80%
Power Requirement:	0.25 hp
Utility Cost/yr:	\$59.00
Sterilization:	Flush with water Steam in Place at 130 °C for 30 min

---

**PURCHASE COST:** \$1595.00

**REFERENCE PAGES:** 65, 253, 256-259

---

## WATER JACKET PUMP (P-6)

**FUNCTION:** To pump water through a half pipe heating jacket surrounding BR-3.

---

**TYPE:** Peristaltic

---

**MATERIALS HANDLED:**

<u>Component</u>	<u>Flow Rate</u>
Water	0.2 - 0.09 kg/sec

---

**CHARACTERISTICS:**

Material:	316 Stainless Steel
Length:	17 ¼ in.
Width:	13 3/8 in.
Height:	7 3/4 in.

---

**OPERATING CONDITIONS:**

Temperature:	38 °C
Pressure In:	14.7 psi
Pressure Out:	29.7 psi
Volumetric Flow Rate:	0.2 - 0.09 kg/sec
Pump Efficiency:	80%
Power Requirement:	0.25 hp
Utility Cost/yr:	\$59.00
Sterilization:	Flush with water and Steam in Place at 130 °C for 30 min

---

**PURCHASE COST:** \$1595.00

**REFERENCE PAGES:** 67, 253, 256-259

---



## **HEAT EXCHANGER WATER PUMP (P-7)**

**FUNCTION:** To pump water through a recycle stream that passes through the shell side of the HX-1.

---

**TYPE:** Corcoran Company model 3000D-HD1 centrifugal pump

---

**MATERIALS HANDLED:**

<u>Component</u>	<u>Flow Rate</u>
Water	17 gal/min

---

**CHARACTERISTICS:**

Material:	316 Stainless Steel
Length:	1.5 feet
Height:	1 foot

---

**OPERATING CONDITIONS:**

Temperature:	40 °C
Pressure In:	14.7 psi
Pressure Out:	29.7 psi
Volumetric Flow Rate:	17 gal/min
Pump Efficiency:	80%
Power Requirement:	2 hp
Utility Cost/yr:	\$59.00

---

**PURCHASE COST:** \$3250.00

**REFERENCE PAGES:** 67, 253, 260-261

---

---

## **MEDIA FILTER (2 $\mu$ ) F-1**

**FUNCTION:** Filter out particles larger than 2  $\mu$  in the prepared media and prevent clogging of the 0.2  $\mu$  filter F-2.

---

**TYPE:** Osmonics Flotrex-AP Filter with 2  $\mu$  absolute rating

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per batch</u></b>
Complete Media	4500L

---

**CHARACTERISTICS:**

Material:	Polypropylene Fiber
Pore Size:	2.0 $\mu$
Diameter:	2.75 in
Length:	10 in
Filtration Area:	5.2 m <sup>2</sup>

---

**OPERATING CONDITIONS:**

Temperature:	4°C
Pressure In:	29.7 psi
Pressure Out:	14.7 psi
Pressure Drop:	15 psi
Volumetric Flow Rate:	2 gal/min
Filters per Batch	1
Sterilization:	Flush with water and Steam in Place at 130 °C for 30 min

---

**PURCHASE COST:** \$32.00/carteriage

**REFERENCE PAGES:** 69, 262, 263-266

---

---

## **MEDIA FILTER (0.2 $\mu$ ) F-2**

**FUNCTION:** Filter out particles larger than 0.2  $\mu$  in the prepared media.

---

**TYPE:** Osmonics Memtrex-FE Filter with 0.2  $\mu$  absolute rating

---

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per batch</u>
Complete Media	4500L

---

**CHARACTERISTICS:**

Material:	PTFE Membrane
Pore Size:	0.2 $\mu$
Diameter:	2.75 in
Length:	10 in

---

**OPERATING CONDITIONS:**

Temperature:	4°C
Pressure In:	29.7 psi
Pressure Out:	14.7 psi
Pressure Drop:	15 psi
Volumetric Flow Rate:	2 gal/min
Filter Cartridge Life:	1 batch
Sterilization:	Flush with water and Steam in Place at 130 °C for 30 min

---

**PURCHASE COST:** \$44.00/carteriage

**REFERENCE PAGES:** 70, 262, 267-270

---

## **FERMENTOR AIR FILTERS (0.2 $\mu$ ) F-3 - F-8**

**FUNCTION:** Filter out particles larger than 0.2  $\mu$  in the compressed air and CO<sub>2</sub> that is sparged into the base of the fermentors and filter the exhaust gasses from the bioreactors..

**TYPE:** Osmonics Memtrex-FE Filters with 2  $\mu$  absolute rating

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per batch</u>
Compressed Air	0.03 – 6.65 tanks
CO <sub>2</sub>	0.001 – 0.015 tanks

**CHARACTERISTICS:**

Material:	PTFE Membrane
Pore Size:	0.2 $\mu$
Diameter:	2.75 in
Length:	10 in

**OPERATING CONDITIONS:**

Temperature:	4°C
Pressure Drop	2 psi
Flow Rate:	0.025 – 0.35 kg/hr
Life Span of Filter	1/3 batch
Sterilization:	Flush with water and Steam in Place at 130 °C for 30 min

**PURCHASE COST:** \$44.00 per filter

**REFERENCE PAGES:** 68, 262, 267-270

---

## **STEAM BOILER (B-1)**

**FUNCTION:** Produce the Steam from sterile water for use in the entire plant.

---

**TYPE:** Standard Natural Gas fueled boiler with sanitary piping and fixtures

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Amount Heated per Hour</u></b>
Sterile Water	500 L/hr

---

**CHARACTERISTICS:**

Capacity:	500 L/hr
Material:	316 Stainless Steel

---

**OPERATING CONDITIONS:**

Inlet Water Temperature:	20 °C
Outlet Steam Temperature:	130 °C
Pressure	39.16 psi
Natural Gas Consumption:	54.5 lb/hr
Boiler Efficiency:	85%

---

**PURCHASE COST:** \$112,000

**REFERENCE PAGES:** 71, 279-281

---

---

## HOLDUP TANK

**FUNCTION:** Provide a place to keep bioreactor effluent if Centrifuge is down.

---

**TYPE:** Vertical blending tank, CIP, SIP; Walker Stainless Equipment

---

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per batch</u>
CHO cells	457 kg
Media	3565 L
TPA	2.16 kg

---

**CHARACTERISTICS:**

Capacity:	5000 L
Height:	3 m
Diameter:	1.5 m
Material:	316 Stainless Steel

---

**OPERATING CONDITIONS:**

Pressure:	14.7 psi
Temperature:	22 °C
Storage time:	Only when Centrifuge is down.

---

CIP by flushing with a vessel volume of water, followed by a SIP cycle. This unit will probably never be used because the centrifuge was quoted as very reliable. We included this tank because it is important to remove 4000L Bioreactor contents promptly to eliminate process down time if something happens to go wrong with the Centrifuge.

---

**PURCHASE COST:** \$19,000

**REFERENCE PAGES:** 72, 365

---

## UF1 TANK

**FUNCTION:** Blend arginine to a concentration of 1M and store at 4°C until needed. Act as a recycling tank for UF1.

**TYPE:** Vertical blending tank, CIP, SIP; Walker Stainless Equipment

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per batch</u>
Media	3565 L
Arginine	850 kg
Tpa	2.05 kg

**CHARACTERISTICS:**

Capacity:	4000 L
Height:	2.7 m
Diameter:	1.4 m
Material:	316 Stainless Steel

**OPERATING CONDITIONS:**

Pressure:	14.7 psi
Temperature:	4 °C
Arginine input:	850 kg
Storage time:	Approx. 14 hrs. (overnight)

CIP by flushing with a vessel volume of water, followed by a SIP cycle.

**PURCHASE COST:** \$17,000

**REFERENCE PAGES:** 73, 365

---

## UF2 TANK

**FUNCTION:** Blend arginine to a concentration of 2M and store at 4°C until needed. Act as a recycling tank for UF2.

---

**TYPE:** Vertical blending tank, CIP, SIP; Walker Stainless Equipment

---

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per batch</u>
Media	200 L
Arginine	115 kg
Tpa	1.94 kg

---

**CHARACTERISTICS:**

Capacity:	400 L
Height:	1.3 m
Diameter:	0.6 m
Material:	316 Stainless steel

---

**OPERATING CONDITIONS:**

Pressure:	14.7 psi
Temperature:	4 °C
Arginine input:	67.4 kg
Storage time:	Negligible unless UF2 is down

CIP by flushing with a vessel volume of water, followed by a SIP cycle.

---

**PURCHASE COST:** \$10,000

**REFERENCE PAGES:** 73, 365

---



## **AFFINITY TANK**

**FUNCTION:** Store UF2 filtrate at 4 °C before it is run through Affinity column. Each batch is split into four equal volumes and applied to the column.

---

**TYPE:** Vertical blending tank, CIP, SIP; Walker Stainless Equipment

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per batch</u></b>
Media	196 L
Arginine	112.9 kg
tPA	1.89 kg

---

**CHARACTERISTICS:**

Capacity:	400 L
Height:	1.3 m
Diameter:	0.6 m
Material:	316 Stainless steel

---

**OPERATING CONDITIONS:**

Pressure:	14.7 psi
Temperature:	4 °C
Storage time:	48 hrs

---

CIP by flushing with a vessel volume of water, followed by a SIP cycle.

---

**PURCHASE COST:** \$10,000

**REFERENCE PAGES:** 73, 365

---

---

## ER TANK

**FUNCTION:** Store Affinity column elution at 4 °C, increase pH to 7.0 with NaOH, and input 0.0258 kg sucrose.

---

**TYPE:** Vertical blending tank, CIP, SIP; Walker Stainless Equipment

---

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per batch</u>
WFI	219 L
Arginine	175 kg
Glycine	8.7 kg
Sucrose	0.026 kg
1M NaOH	0.33 L
tPA	1.6 kg

---

**CHARACTERISTICS:**

Capacity:	400 L
Height:	1.2 m
Diameter:	0.6 m
Material:	316 Stainless steel

---

**OPERATING CONDITIONS:**

Pressure:	14.7 psi
Temperature:	4 °C
NaOH input:	0.33 L
Sucrose input:	0.026 kg
Storage time:	Negligible unless UF2 is down

CIP by flushing with a vessel volume of water, followed by a SIP cycle.

---

**PURCHASE COST:** \$10,000

**REFERENCE PAGES:** 74, 365

---

---

## MF TANK

**FUNCTION:** Input 59 L WFI and store contents at 4 °C.

---

**TYPE:** Vertical blending tank, CIP, SIP; Walker Stainless Equipment

---

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per batch</u>
WFI	278 L
Arginine	175 kg
tPA	1.6 kg
NaOH	0.013 kg
Glycine	8.7 kg
Sucrose	0.026 kg

---

**CHARACTERISTICS:**

Capacity:	500 L
Height:	1.4 m
Diameter:	0.7 m
Material:	316 Stainless steel

---

**OPERATING CONDITIONS:**

Pressure:	14.7 psi
Temperature:	4 °C
WFI input:	59 L
Storage time:	Negligible unless UF2 is down

CIP by flushing with a vessel volume of water, followed by a SIP cycle.

---

**PURCHASE COST:** \$11,000

**REFERENCE PAGES:** 74, 365

---

---

## **FD TANK**

**FUNCTION:** Store MF filtrate at 4 °C before bottling. Each batch is split and bottled in five equal volumes and freeze dried in the bottles in five batches.

---

**TYPE:** Vertical blending tank, CIP, SIP; Walker Stainless Equipment

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per batch</u></b>
WFI	278 L
Arginine	175 kg
tPA	1.6 kg
NaOH	0.013 kg
Glycine	8.7 kg
Sucrose	0.026 kg

---

**CHARACTERISTICS:**

Capacity:	500 L
Height:	1.4 m
Diameter:	0.7 m
Material:	316 Stainless steel

---

**OPERATING CONDITIONS:**

Pressure:	14.7 psi
Temperature:	4 °C
Storage time:	146 hrs

CIP by flushing with a vessel volume of water, followed by a SIP cycle.

---

**PURCHASE COST:** \$11,000

**REFERENCE PAGES:** 74, 365

---

---

## **CENTRIFUGE**

**FUNCTION:** Separate CHO cells from harvest media.

---

**TYPE:** CSC 4 Clarifier, In-steam sterilizable from Westfalia Separator

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per batch</u></b>
CHO cells	457 kg
Media	3565 L
TPA	2.16 kg

---

**CHARACTERISTICS:**

Bowl Volume:	1.8 L
Bowl Speed:	approx. 10,000 rev/min
Weight:	approx. 230 kg
Material:	Cr-Ni-Mo stainless steel

---

**OPERATING CONDITIONS:**

Rated capacity:	400 L/hr
Maximum pressure:	approx. 73.5 psi
Entering cell broth temperature:	37 °C
Centrifuge temperature:	4 °C (room is at 4 °C)
Cell removal:	100%
Equipment uptime per batch:	<10 hr
tPA lost per run	5%

---

To be cleaned after each run with NaOH solution for 20-30 minutes. Followed by SIP.

---

**PURCHASING COST:** \$355,000

**REFERENCE PAGES:** 75, 297-302, 367

---

---

## UF1 PUMP

**FUNCTION:** Increase pressure of UF1 Tank contents to 40 psi.

---

**TYPE:** Peristaltic, Series 750-362; Randolph Austin Company

---

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per batch</u>
Media	3565 L
Arginine	850 kg
tPA	2.05 kg

---

**CHARACTERISTICS:**

Material:	316 Stainless steel
-----------	---------------------

---

**OPERATING CONDITIONS:**

Pressure in:	14.7 psi
Pressure out:	40 psi
Temperature:	4 °C
Volumetric flow rate:	828 L/hr
Efficiency:	80%
Power Requirement:	49 W
Equipment uptime per batch	6.09 hr
Utility Cost/yr	\$16

---

**PURCHASE COST:** \$2,656

**REFERENCE PAGES:** 76, 256-259, 366

---

---

## **UF2 PUMP**

**FUNCTION:** Increase pressure of UF2 Tank contents to 40 psi.

---

**TYPE:** Peristaltic, Series 750-362; Randolph Austin Company

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per batch</u></b>
Media	200 L
Arginine	115 kg
tPA	1.94 kg

---

**CHARACTERISTICS:**

Material: 316 Stainless steel

---

**OPERATING CONDITIONS:**

Pressure in:	14.7 psi
Pressure out:	40 psi
Temperature:	4 °C
Volumetric flow rate:	1325 L/hr
Efficiency:	80%
Power Requirement:	79 W
Equipment uptime per batch:	<1 hr
Utility Cost/yr	\$25

---

**PURCHASE COST:** \$2,656

**REFERENCE PAGES:** 77, 256-259, 366

---

---

## **MF PUMP**

**FUNCTION:** Increase pressure of MF Tank contents to 34.7 psi.

---

**TYPE:** Peristaltic, Series 610-362; Randolph Austin Company

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per batch</u></b>
WFI	278 L
Arginine	175 kg
tPA	1.6 kg
NaOH	0.013 kg
Glycine	8.7 kg
Sucrose	0.026 kg

---

**CHARACTERISTICS:**

Material: 316 Stainless steel

---

**OPERATING CONDITIONS:**

Pressure in:	14.7 psi
Pressure out:	34.7 psi
Temperature:	4 °C
Volumetric flow rate:	1140 L/hr
Efficiency:	80%
Power Requirement:	53.27 W
Equipment uptime per batch:	0.25 hr
Utility Cost/yr	\$17

---

**PURCHASE COST:** \$2,656

**REFERENCE PAGES:** 77, 256-259, 366

---



## AFFINITY SKID/PUMP

**FUNCTION:** Increase pressure of Affinity column inputs to 15.9 psi and control the volume of equilibration and elution buffers used.

---

**TYPE:** Peristaltic pump with computer

---

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per batch</u>
Media	196 L
WFI	285 L
Arginine	126 kg
Glycine	11.3 kg
PBS	1710 L
NaCl	66 kg
tPA	1.89 kg

---

**CHARACTERISTICS:**

Material: 316 Stainless steel

---

**OPERATING CONDITIONS:**

Pressure in:	15.9 psi
Pressure out:	14.7 psi
Temperature:	4 °C
Volumetric flow rate:	135 L/hr
Efficiency:	80%
Power Requirement:	0.4 W
Equipment uptime per batch:	12 hr
Utility Cost/yr	\$1

---

**PURCHASE COST:** \$100,000\*

**REFERENCE PAGES:** 77, 256-259, 366

---

---

## ER SKID/PUMP

**FUNCTION:** Increase pressure of ER Tank contents to 16.6 psi.

---

**TYPE:** Peristaltic pump with computer

---

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per batch</u>
WFI	266 L
Arginine	9.96 kg
Glycine	8.7 kg
1M NaOH	193 L
tPA	1.6 kg

---

**CHARACTERISTICS:**

Material:	316 Stainless steel
-----------	---------------------

---

**OPERATING CONDITIONS:**

Pressure in:	14.7 psi
Pressure out:	16.6 psi
Temperature:	4 °C
Volumetric flow rate:	11 L/hr
Efficiency:	80%
Power Requirement:	0.3 W
Equipment uptime per batch:	32 hr
Utility Cost/yr	negligible

---

**PURCHASE COST:** \$100,000\*

**REFERENCE PAGES:** 77, 256-259, 366

---

## UF1

**FUNCTION:** Concentrate tPA to a volume of approximately 200 L.

---

**TYPE:** Tangential flow filter, PM50 Hollow fiber cartridge; Koch Membrane Systems

---

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per batch</u>
Media	3565 L
Arginine	850 kg
tPA	2.05 kg

---

**CHARACTERISTICS:**

Diameter:	3 in.
Length:	25 in.
Membrane area:	14 ft <sup>2</sup>
MWCO:	50 kDa
Material:	Polysulfone

---

**OPERATING CONDITIONS:**

Pressure in:	40 psi
Pressure out:	30 psi
Pressure drop:	10 psi
Temperature:	4 °C
Mean flux (filtrate flow rate):	550 L/hr
Split ratio (retentate/filtrate):	0.06
Filter cartridge life:	One year
tPA lost per run	2%

---

Retentate stream is split and recycled to UF1 Tank where it again flows through the filter. Flow is controlled using a ratio-flow controller.

---

**PURCHASE COST:** \$785/cartridge

**REFERENCE PAGES:** 79, 303-313

---

---

## UF2

**FUNCTION:** Remove remaining components larger than 500 kDa that could damage or inhibit flow through the affinity column.

---

**TYPE:** Tangential flow filter, PM500 Hollow fiber cartridge; Koch Membrane Systems

---

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per batch</u>
Media	200 L
Arginine	115 kg
tPA	1.94 kg

---

**CHARACTERISTICS:**

Diameter:	3 in.
Length:	25 in.
Membrane area:	14 ft <sup>2</sup>
MWCO:	500 kDa
Material:	Polysulfone

---

**OPERATING CONDITIONS:**

Pressure in:	40 psi
Pressure out:	30 psi
Pressure drop:	10 psi
Temperature:	4 °C
Mean flux (filtrate flow rate):	880 L/hr
Filter cartridge life:	One year
tPA lost per run	5%

---

Retentate stream is recycled to UF2 Tank where it again flows through the filter. Entire volume of UF2 Tank is collected as filtrate.

---

**PURCHASE COST:** \$950/cartridge

**REFERENCE PAGES:** 81, 303-313

---

---

**MF**

**FUNCTION:** Filter and sterilize final product stream before freeze drying.

---

**TYPE:** Tangential flow filter, 0.2 µm filter; Osmonics

---

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per batch</u>
WFI	278 L
Arginine	175 kg
tPA	1.6 kg
NaOH	0.013 kg
Glycine	8.7 kg
Sucrose	0.026 kg

---

**CHARACTERISTICS:**

Diameter:	2.75 in.
Length:	10 in.
Membrane area:	9 ft <sup>2</sup>
Pore size:	0.2 µm
Material:	PTEF Membrane

---

**OPERATING CONDITIONS:**

Pressure in:	40 psi
Pressure out:	20 psi
Pressure drop:	20 psi
Temperature:	4 °C
Max flow rate:	1140 L/hr
Filter cartridge life:	One batch
tPA lost per run	1%

---

**PURCHASE COST:** \$44/cartridge

**REFERENCE PAGES:** 81, 267-270

---

## AFFINITY COLUMN

**FUNCTION:** Separate tPA from Affinity tank contents.

---

**TYPE:** Bioprocess stainless steel column; Amersham Pharmacia Biotech

---

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per batch</u>
Media	196 L
WFI	285 L
Arginine	340 kg
Glycine	11.3 kg
PBS	1709 L
NaCl	66.2 kg
tPA	1.89 kg

---

**CHARACTERISTICS:**

Column volume:	58 L
Bed volume:	58 L
Bed Height:	15 cm
Diameter:	70 cm
Resin:	CNBr-activated Sepharose 4 Fast Flow
Void volume:	20.2 L
Materials:	316 Stainless steel

---

**OPERATING CONDITIONS:**

Superficial velocity:	100 cm/hr
Volumetric flow rate:	135 L/hr
Pressure drop:	1.2 psi
Temperature:	4 °C
Load volume:	One quarter of Affinity tank contents
Equilibration volume:	2.5 bed volumes (145 L)
Elution volume:	1.3 bed volumes (75 L)
Wash volume:	5 bed volumes (289 L)
tPA lost per run:	15%
Equipment uptime per batch:	48 hrs

---

**PURCHASE COST:** \$100,000\*

**REFERENCE PAGES:** 82, 314-328

---

## AFFINITY RESIN

**FUNCTION:** Provide matrix to hold anti-tPA antibody.

---

**TYPE:** CNBr-activated Sepharose 4 Fast Flow; Amersham Pharmacia Biotech

---

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per batch</u>
Media	196 L
WFI	285 L
Arginine	340 kg
Glycine	11.3 kg
PBS	1709 L
NaCl	66.2 kg
tPA	1.89 kg

---

**CHARACTERISTICS:**

Bed volume:	58 L
Height:	15 cm
Diameter:	70 cm
Bead size:	90 µm
Void volume:	20.2 L
Materials:	Highly cross-linked 4% agarose, spherical

---

**OPERATING CONDITIONS:**

Superficial velocity:	100 cm/hr
Volumetric flow rate:	134.6 L/hr
Pressure drop:	1.2 psi
Temperature:	4 °C
Antibody binding:	12.3mg AB/mL resin
Resin utilization:	85%
Equipment uptime per batch:	48 hrs

Store in 20% ethanol between batches.

---

**PURCHASE COST:** \$350,000/year

**REFERENCE PAGES:** 82, 314-328

---

---

## **ER COLUMN**

**FUNCTION:** Remove endotoxins from Affinity column elution.

---

**TYPE:** Bioprocess glass column 200/500; Amersham Pharmacia Biotech

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per batch</u></b>
WFI	266 L
Arginine	175 kg
Glycine	8.7 kg
Sucrose	0.026 kg
1M NaOH	193 L
tPA	1.6 kg

---

**CHARACTERISTICS:**

Column volume:	15.7 L
Bed volume:	8.2 L
Bed Height:	26 cm
Diameter:	20 cm
Resin:	Acticlean Etox
Void volume:	2.9 L
Materials:	316 Stainless steel

---

**OPERATING CONDITIONS:**

Superficial velocity:	100 cm/hr
Volumetric flow rate:	11.0 L/hr
Pressure drop:	1.9 psi
Temperature:	4 °C
Load volume:	Entire volume of ER Tank contents
Cleaning:	1M NaOH for 2 hrs
Regeneration volume:	2 bed volumes (75 L)
tPA lost per run:	5%
Equipment uptime per batch:	36 hrs

---

**PURCHASE COST:** \$6,190

**REFERENCE PAGES:** 84, 329-345

---



## **ER RESIN**

**FUNCTION:** Bind endotoxins while allowing tPA to flow through undamaged.

---

**TYPE:** Acticlean Etox; Sterogene

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per batch</u></b>
WFI	266 L
Arginine	175 kg
Glycine	8.7 kg
Sucrose	0.026 kg
1M NaOH	193 L
tPA	1.6 kg

---

**CHARACTERISTICS:**

Bed volume:	8.2 L
Height:	26 cm
Diameter:	20 cm
Bead size:	110 $\mu$ m
Void volume:	2.9 L
Materials:	Actigel ALD; Sterogene

---

**OPERATING CONDITIONS:**

Superficial velocity:	100 cm/hr
Volumetric flow rate:	11.0 L/hr
Pressure drop:	1.9 psi
Temperature:	4 °C
Endotoxin binding:	200 – 20,000 EU/mL resin
Cleaning:	1M NaOH for 2 hrs
Equipment uptime per batch:	36 hrs

Store in WFI between batches.

---

**PURCHASE COST:** \$87,000/year

**REFERENCE PAGES:** 84, 329-345

---

---

## **BOTTLER**

**FUNCTION:** Load approximately 25 mL final product solution into 100 mL vials and partially stopper them. Load vials onto freeze dryer trays.

---

**TYPE:** Model VR 840S, BT15, AW160; Cozzoli Machine Company

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per batch</u></b>
WFI	278 L
Arginine	175 kg
tPA	1.6 kg
NaOH	0.013 kg
Glycine	8.7 kg
Sucrose	0.026 kg

---

**CHARACTERISTICS:**

Vials volume:	100 mL
Materials:	316 Stainless steel

CIP and SIP capability.

---

**OPERATING CONDITIONS:**

Pressure:	14.7 psi
Temperature:	4 °C
Bottling time/3200 100 mL vials:	1 hr
Volume loaded per vial:	~25 mL (depends on tPA conc. in FD Tank)
Tray loading time:	1 hr
Power requirements:	1.5 hp total
Equipment uptime per batch:	146 hrs
Utility cost/yr:	\$330

---

**PURCHASE COST:** \$1,265,000

**REFERENCE PAGES:** 85, 346-349, 367

---

## **FREEZE DRYER**

**FUNCTION:** Remove WFI from tPA and form a stable, solid product that can be dissolved and injected.

---

**TYPE:** Virtis Benchmark 6000 SQ Stoppering Lyophilizer

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per batch</u></b>
WFI	278 L
Arginine	175 kg
tPA	1.6 kg
NaOH	0.013 kg
Glycine	8.7 kg
Sucrose	0.026 kg

---

**CHARACTERISTICS:**

Shelves:	9
100 mL vials per shelf:	396
Heat transfer medium:	Silicon fluid
Condenser Capacity:	220 liters H <sub>2</sub> O in 24 hrs
Material:	316 Stainless steel

---

**OPERATING CONDITIONS:**

Liters per cycle:	~80 L
100 mL vials per batch:	~3200 (depends on tPA conc. in FD tank)
Cycle time:	24 hrs
Standard shelf temperature:	-55°C
Cooling water requirements:	6 gal/min
Power requirements:	Max 46.1 kW
Equipment uptime per batch:	146 hrs
Utility cost/yr	\$13,500

---

SIP for 30 minutes after each cycle.

---

**PURCHASE COST:** \$640,000

**REFERENCE PAGES:** 85, 350-361, 367

---

## REFRIGERATOR

**FUNCTION:** Keep entire Separation Process at 4°C at all times.

---

**TYPE:** Air conditioning system

---

**MATERIALS HANDLED:**

**Component**  
Refrigerant

**Quantity required:**  
16.67 tons

---

**CHARACTERISTICS:**

Refrigerant requirements:	1.25 tons refrigerant/300 ft <sup>2</sup> of cooled space
Area to be cooled:	4,000 ft <sup>2</sup> for two separation trains
Power rating:	1 kW/ton refrigerant for low temperature cooling*
Load factor:	0.6*

---

**OPERATING CONDITIONS:**

Cooling time/yr	8760 hrs
Power requirements:	10 kW
Utility cost/yr:	\$3,504

---

**PURCHASE COST:** \$10,000\*

**REFERENCE PAGES:** 87, 356

---

---

## **INITIAL PUMP (PW-1)**

**FUNCTION:** To bring city water into the water purification process

---

**TYPE:** Peristaltic Pump model No. 880-300 varied flow, speed control from Randolph Austin Company

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per Day</u></b>
City Water:	50000 L

---

**CHARACTERISTICS:**

Dimension L x W x H:	29 x 17 x 21 (inch)
Material:	316 Stainless Steel
Motor Horse Power:	1 hp
Pump Speed:	190 RPM

---

**OPERATING CONDITIONS:**

Pressure in:	14.7 psi
Pressure out:	34.7 psi
Temperature:	25 °C
Flow Rate:	35 L/min

---

**PURCHASE COST:** \$5,250

**REFERENCE PAGES:** 87, 369, 387-388

---

## 10 µM MICROFILTER (FW-1)

**FUNCTION:** To separate particles in feed water before it enters the reverse osmosis system, and to prevent unwanted particles from damaging the reverse osmosis system membrane.

**TYPE:** Tangential flow filter model Flotrex™-AP Filters Polypropylene Microfiber from Osmonics.

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per Day</u>
City Water:	50000 L

**CHARACTERISTICS:**

Cartridge Dimension:	2.75 inch (outside diameter)
Cartridge Dimension:	1.25 inch (inside diameter)
Length:	10 inches
Membrane Area:	6.7 ft <sup>2</sup>
Pore Size:	10 µm
Membrane Material:	Polypropylene

**OPERATING CONDITIONS:**

Temperature:	25 °C
Water Flow Rate:	18 L/min
Filter cartridge life:	One Batch
Pressure in:	34.7 psi
Pressure out:	14.7 psi

**PURCHASE COST:** \$44 per cartridge per 10 inches equivalent

**REFERENCE PAGES:** 88, 376, 389-390

---

## **CARBON FILTER PUMP (PW-2)**

**FUNCTION:** To compensate pressure drop in the carbon filter.

---

**TYPE:** Peristaltic pump model No. 750-362 varied flow, speed control from Randolph Austin Company

---

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per Day</u>
City Water:	50000 L

---

**CHARACTERISTICS:**

Material:	316 Stainless steel
Pump Horse Power:	0.25 hp
Pump Speed:	12-500 RPM

---

**OPERATING CONDITIONS:**

Pressure in:	14.7 psi
Pressure out:	26.7 psi
Temperature:	25 °C
Volumetric Flow Rate:	18 L/min
Efficiency:	80%

---

**PURCHASE COST:** \$2,656

**REFERENCE PAGES:** 88, 369-370, 387-388

---

## **CARBON FILTER (FW-2)**

**FUNCTION:** To remove chlorine and organic particles from feed water stream and to protect water softener's resin, microfilter, and reverse osmosis system from degradation.

---

**TYPE:** Model WTTS16C Carbon Filter from Ameriwater.

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per Day</u></b>
Municipal City Water:	50000 L/day

---

**CHARACTERISTICS:**

Material:	Polyethylene
Diameter:	42 inches
Height:	87 inches
Absorbent:	Bituminous Coal
Absorbent Weight:	1000 lbs

---

**OPERATING CONDITIONS:**

Flow Rate:	76 L/min
Water Temperature:	25 °C
Pressure in:	26.7 psi
Pressure out:	14.7 psi
Max Contact Time:	5.3 min

Empty bed contact time at design flow and a bed density of 28 lb/ft<sup>3</sup>.

---

**PURCHASE COST:** \$1,515

**REFERENCE PAGES:** 88

---



## **WATER SOFTENER (FW-3)**

**FUNCTION:** To remove to unwanted ions from the feed water before it enters the reverse osmosis system.

---

**TYPE:** Model WTMD16 meter initiated system from Ameriwater

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per Day</u></b>
City Water:	50000 L

---

**CHARACTERISTICS:**

Resin Type:	Polystyrene Sulfonate Cation
Life of Resin:	10 years
Flow Rate:	4 gallon /(min ft <sup>3</sup> )
Amount of Resin filled:	7 ft <sup>3</sup>
Outer Tank Shell Material:	Fiberglass
Inner Tank Shell Material:	Polyethylene

---

**OPERATING CONDITIONS:**

Pressure in:	24.7 psi
Pressure out:	14.7 psi
Temperature:	25 °C
Flow Rate:	76 L/min

---

**PURCHASE COST:** \$4,537

**REFERENCE PAGES:** 89, 377-383

---

---

## **WATER SOFTENER PUMP (PW-3)**

**FUNCTION:** To compensate pressure drop in the water softener

---

**TYPE:** Peristaltic Pump model No. 880-300 varied flow, speed control from Randolph Austin Company

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per Day</u></b>
City Water:	50000 L

---

**CHARACTERISTICS:**

Material:	316 Stainless steel
Dimension L x W X H:	29 x 17 x 21 (inch)
Pump Speed:	190 RPM
Pump Horse Power:	1 hp

---

**OPERATING CONDITIONS:**

Pressure in:	14.7 psi
Pressure out:	24.7 psi
Temperature:	25 °C
Flow Rate:	76 L/min
Efficiency:	80%

---

**PURCHASE COST:** \$5,250

**REFERENCE PAGES:** 89, 370, 377-378

---

## **REVERSE OSMOSIS PUMP (PW-7)**

**FUNCTION:** To compensate the pressure drop across the reverse osmosis system.

---

**TYPE:** Peristaltic Pump model No. 880-300 varied flow, speed control from Randolph Austin Company

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per Day</u></b>
City Water:	50000 L

---

**CHARACTERISTICS:**

Material:	316 Stainless steel
Pump Speed:	190 RPM
Pump Horse Power:	1 hp

---

**OPERATING CONDITIONS:**

Pressure in:	14.7 psi
Pressure out:	34.7 psi
Temperature:	25 °C
Flow Rate:	76 L/min
Efficiency:	80%

---

**PURCHASE COST:** \$5,250

**REFERENCE PAGES:** 90, 373, 387-388

---

## **REVERSE OSMOSIS SYSTEM (RO-1)**

**FUNCTION:** The reverse osmosis system separates salts, organic molecules, and bacteria from feed water stream.

**TYPE:** Model IRO6 Open Skid Style System from Ameriwater.

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per day</u></b>
City Water:	50000 L

**CHARACTERISTICS:**

Membrane Size:	4 x 40 (inch)
Membrane Type:	Thin Film Composite
Number of Membranes:	6
Pump Horse Power:	1.5 hp
Electricity:	220 V 7.5 Maximum Amp
Dimension H x W x D:	72 x 24 x 35 (inch)

**OPERATING CONDITIONS:**

Rated Capacity:	36 L/min
Production per Day:	52000 L
Temperature:	25 °C
Operating pH Range:	4-11
Pressure in:	34.7 psi
Pressure out:	14.7 psi
Pressure Feed Range (psi):	20-80
Cleaning pH Range:	2-11.5
Prefiltration:	5 µm

**PURCHASE COST:** \$22,500

**REFERENCE PAGES:** 90, 371, 393-395

---

## **STORAGE TANK (TW-1)**

**FUNCTION:** To store excess purified water for various uses through out the plant.

---

**TYPE:** 4000 Polyethylene, horizontal holding tank.

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per Day</u></b>
Purified Water:	50000 L

---

**CHARACTERISTICS:**

Capacity:	50000 L
Height:	140 inches
Diameter:	94 inches
Material:	4000 Polyethylene

---

**OPERATING CONDITIONS:**

---

**PURCHASE COST:** \$3,554

---

**REFERENCE PAGES:** 91

---

---

## **UV SYSTEM PUMP (PW-4)**

**FUNCTION:** To compensate the pressure drop in the Ultraviolet Light system

---

**TYPE:** Peristaltic pump model 750-362 vari-flow speed control from Randolph Austin Company

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per Day</u></b>
Purified Water:	50000 L

---

**CHARACTERISTICS:**

Material:	316 Stainless steel
Pump Horse Power:	0.25 hp
Pump RPM:	12-500 RPM

---

**OPERATING CONDITIONS:**

Pressure in:	14.7 psi
Pressure out:	19.7 psi
Temperature:	25 °C
Volumetric flow rate:	23 L/min
Efficiency:	80%

---

**PURCHASE COST:** \$2,656

**REFERENCE PAGES:** 91, 372, 387-388

---

---

## ULTRAVIOLET LIGHT SYSTEM (UV-1)

**FUNCTION:** To prevent bacterial growth and to destroy particles formed in the Storage tank.

---

**TYPE:** UV Lamp Model Minipure-MIN6, and Lamp No. 05-1370 from Atlantic Ultraviolet Corporation.

---

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per Day</u>
Purified Water:	50000 L

---

**CHARACTERISTICS:**

Material for chamber and hardware:	304 Stainless steel
Lamp Power Consumption:	21 Watts
Rated Effective Life:	10000 Hours
Dimension L x W x H:	23 x 5 x 6 (inch)

---

Maximum rated output at 254 nanometers

---

**OPERATING CONDITIONS:**

Pressure Drop at Max Flow Rate:	5 psi
Pressure in:	19.7 psi
Pressure out:	14.7 psi
Max Operating Pressure:	100 psi
Flow Rate:	23 L/min

---

**PURCHASE COST:** \$1,567

**REFERENCE PAGES:** 92, 384-386

---

---

## **0.2 $\mu$ M MICROFILTER PUMP (PW-5)**

**FUNCTION:** To compensate the pressure drop in 0.2  $\mu$ m microfilter.

---

**TYPE:** Peristaltic pump model 750-362 vari-flow speed control from Randolph Austin Company

---

**MATERIALS HANDLED:**

<u>Component</u>	<u>Quantity per Day</u>
Purified Water:	50000 L/min

---

**CHARACTERISTICS:**

Material:	316 Stainless Steel
Pump Horse Power:	0.25 hp
Pump Speed:	12-500 RPM

---

**OPERATING CONDITIONS:**

Pressure in:	14.7 psi
Pressure out:	24.7 psi
Temperature:	25 °C
Flow Rate:	30 L/min
Efficiency:	80%

---

**PURCHASE COST:** \$2,656

**REFERENCE PAGES:** 92, 373, 387-388

---



## **0.2 MICRON FILTER (PW-4)**

**FUNCTION:** To separate newly formed particles before the purified water enters the process.

---

**TYPE:** Model Memtrex™-FE-S by Osmonics

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per Day</u></b>
Purified Water:	50000 L

---

**CHARACTERISTICS:**

Material:	Polypropylene Membrane
Cartridge Dimension:	2.75 inch (outside diameter)
Cartridge Dimension:	1.25 inch (inside diameter)
Length:	10 inch
Membrane Area:	9 ft <sup>2</sup>
Pore Size:	0.2 micron

---

**OPERATING CONDITIONS:**

Pressure in:	24.7 psi
Pressure out:	14.7 psi
Temperature:	25 °C
Flow Rate:	18 L/min

---

**PURCHASE COST:** \$44 per cartridge per 10 inches equivalent.

**REFERENCE PAGES:** 93, 391-392

---

---

## **FINAL PUMP (PW-6)**

**FUNCTION:** To adjust the pressure within pipes so that the desired flow rate can be achieved.

---

**TYPE:** Peristaltic Pump model No. 880-300 varied flow, speed control from Randolph Austin Company

---

**MATERIALS HANDLED:**

<b><u>Component</u></b>	<b><u>Quantity per Day</u></b>
Purified water:	50000 L

---

**CHARACTERISTICS:**

Material	316 Stainless Steel
Horse Power Required	1 hp
Pump Speed	190 RPM
Dimension L x W x H:	29 x 17 x 21 (inch)

---

**OPERATING CONDITIONS:**

Water Temperature:	25 °C
Flow Rate:	18 L/min
Pressure in:	14.7 psi
Pressure out:	34.7 psi
Efficiency:	80%

---

**PURCHASE COST:** \$5,250

**REFERENCE PAGES:** 93, 373, 387-388

---

# ECONOMIC SUMMARY

## **Economic Evaluation**

Generic tissue plasminogen activator (tPA) has the potential to take a large portion of the thrombolytic therapy market. Genentech's Activase<sup>TM</sup> and streptokinase are currently the primary treatment methods for heart disease and related illness. Genentech sells their product for about \$2,000 per dose, while streptokinase is priced at about \$200 per dose. We aim to enter the market at about \$500 per dose, and this will allow us to sell to consumers for whom cost is not an issue and also to those who cannot afford premium medications. Activase<sup>TM</sup> is so expensive because Genentech must make up for the billions of dollars spent on research and clinical trials for its products; we will not have to worry about those expenses in production of generic tPA.

Genentech currently sells about \$300 MM a year of tPA in the U.S. The rest of the world, primarily Europe and Japan, use much less tPA and relies upon the less expensive streptokinase. We are estimating that the total worldwide sales of tPA are \$400 MM/yr. When patents run out, pharmaceutical companies can lose half of their market share to generic versions of their drug. Given that we will be selling our drug for a quarter of the Activase<sup>TM</sup> price, we are estimating that we can acquire half of the tPA sales worldwide.

Many studies have been completed that compare the effectiveness of tPA and streptokinase for the treatment of heart-related illnesses. When taking into account quality of life, one-year mortality, and long-term medical costs tPA was concluded to be much a more cost-effective treatment compared to streptokinase, even with the large difference in price (Kalish et al, 1995). This study used data from a widely published study entitled the Global Utilization of Streptokinase and Tissue Plasminogen Activator

for Occluded Coronary Arteries (GUSTO). A questionnaire from the GUSTO-1 trial was also used in an analysis of patient preferences for thrombolytic therapy. When costing was not an issue, tPA was preferred overall due to its lower mortality and fewer occurrences of side effects. When costing was introduced to the decision, especially under self-pay circumstances streptokinase became more preferred (Stanek et al, 1997).

Obviously the high cost of tPA causes it to lose market share to streptokinase, so lowering the price would increase its use in hospitals in less wealthy areas. Streptokinase is used much more widely overseas, so we estimate U.S. sales to be approximately \$50 MM/year and worldwide sales to be \$150 MM/year, based on a discussion with Prof. Diamond. Cost and availability are the primary reasons streptokinase is used instead of tPA in the world; so selling it at the comparable price of \$500/dose would allow us to gain two-thirds of the streptokinase. Calculating half of the tPA market and two-thirds of the streptokinase market leads us to a production number of about 76,000 doses each year, or 76 kg/yr. This yields about \$380 MM/year in sales, and given our production and start-up costs, generic tPA can be very profitable. A detailed description of our costs is presented in the next sections.

### **Capital Costs**

Capital costs for our plant are just over \$100 MM. These include the purchase of equipment, installation and construction, working capital, and accounts receivable. The cost of purchasing all of our equipment is about \$5.4 MM; over 75% of this comes from the Separation Trains, in particular the Bottlers, Freeze Dryers, and chromatography columns (see Figure 9 on page 153). The remaining costs are derived from the Fermentors and Centrifuge. A list of equipment costs is presented in Table 6 on page

152. Installation costs are set at 200% of the total equipment costs (\$10.8 MM), a high estimate because we are using sanitary piping throughout the plant. Other capital costs are derived from cost factors recommended by Prof. Seider for the cost spreadsheet created by Holger Nickisch (see page 160). These factors can be viewed in the Input Summary on page 160. The final large portion of our capital costs comes from total Accounts Receivable. This cost results from the fact that it takes time to collect payments from customers, and this revenue is needed in advance to pay for yearly fixed and variable costs. The number is so large because our sales sum to \$360 MM/yr.

**TABLE 6: Unit Purchase Costs**

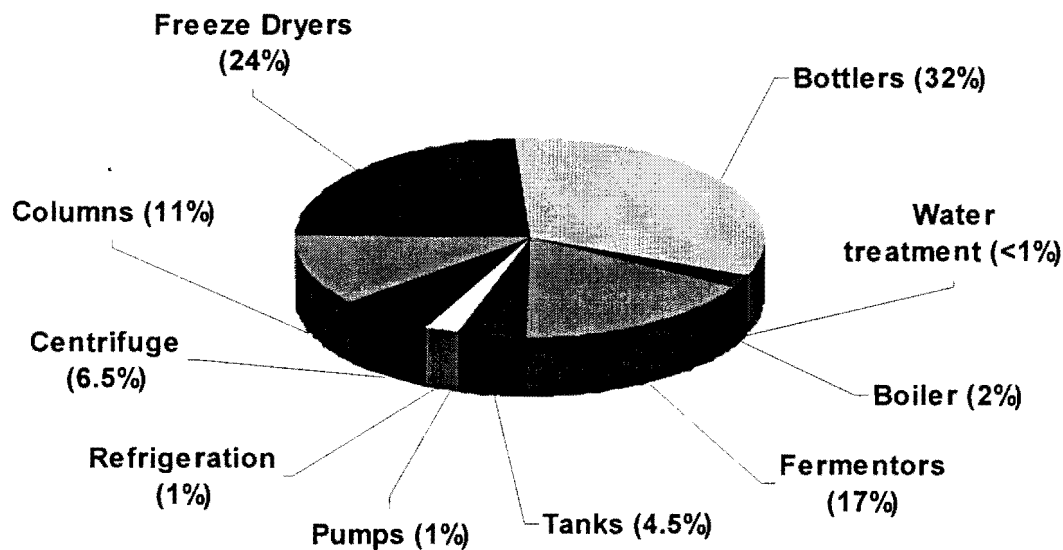
Unit Purchase Costs				
Name	Description	Purchase Cost	Quantity	Total
BR-1	40 L Fermentor	\$85,000*	2	\$170,000
BR-2	400 L Fermentor	\$125,000*	2	\$250,000
BR-3	5000 L Fermentor	\$250,000*	2	\$500,000
HX-1	Heat Exchanger	\$6,456†	2	\$12,911
T-1	Blending Tank	\$28,658*	2	\$57,316
CTI	Copper tubing and insulation	\$2,000*	2	\$4,000
RFU	Refrigeration Unit for media tank	\$20,000*	2	\$40,000
RF	Refrigerant for media	\$250*	16	\$4,000
P1	Peristaltic Pump	\$1,595*	2	\$3,190
P2	Peristaltic Pump	\$1,595*	2	\$3,190
P3	Peristaltic Pump	\$1,595*	2	\$3,190
P4	Peristaltic Pump	\$1,595*	2	\$3,190
P5	Peristaltic Pump	\$1,595*	2	\$3,190
P6	Peristaltic Pump	\$1,595*	2	\$3,190
P7	Centrifugal Pump	\$3,250*	2	\$6,500
Holdup Tank	Tank after BR3	\$19,000*	1	\$19,000
Centrifuge	Centrifuge	\$355,000*	1	\$355,000
UF1 Tank	Recycle tank for UF1	\$17,000*	1	\$17,000
UF1 Pump	Pump for UF1	\$2,656*	1	\$2,656
UF2 Tank	Recycle tank for UF2	\$9,000*	1	\$9,000
UF2 Pump	Pump for UF2	\$2,656*	1	\$2,656
UF Cleaning	Cleaning solution	\$75*	1	\$75
Affinity Tank	Tank for Affinity Column	\$10,000*	2	\$20,000
Affinity Column	Affinity chromatography column	\$100,000†	2	\$200,000
Affinity Skid/Pump	Controller/pump for Affinity Column	\$100,000†	2	\$200,000
ER Tank	Tank for ER Column	\$10,000*	2	\$20,000
ER Column	Endotoxin removal column	\$6,190*	2	\$12,380

Name	Description	Purchase Cost	Quantity	Total
ER Skid/Pump	Controller/pump for ER Column	\$100,000†	2	\$200,000
MF Tank	Tank for MF	\$11,000*	2	\$22,000
MF Pump	Pump for MF	\$2,656*	2	\$5,312
FD Tank	Tank before Bottler	\$11,000*	2	\$22,000
Bottler	Bottler/Tray Loader	\$865,000*	2	\$1,730,000
Freeze Dryer	Freeze Dryer	\$640,000*	2	\$1,280,000
Refrigerant	AC refrigerant	\$250*	17	\$4,250
Refrigerator	Air-conditioning system	\$10,000*	1	\$10,000
B-1	Boiler	\$112,000#	1	\$112,000
RO-1	Reverse osmosis system	\$7,500*	1	\$7,500
Water Softener	Ion removal	\$4,537*	1	\$4,537
WP-1	Initial pump	\$5,250*	1	\$5,250
WP-2	Pump for Carbon filter	\$2,656*	1	\$2,656
WP-3	Pump for Water Softener	\$5,250*	1	\$5,250
WP-4	Pump for UV lamp	\$2,656*	1	\$2,656
WP-5	Pump for microfilter	\$2,656*	1	\$2,656
WP-6	Final pump	\$5,250*	1	\$5,250
WP-7	Pump for RO-1	\$5,250*	1	\$5,250
WT-1	Holding tank for sterile water	\$15,000*	3	\$45,000
WT-2	Holding tank for waste	\$10,000*	2	\$20,000
				<b>Total: \$5,410,000</b>

### Key for the Table

Symbol	Price origin
*	Quote from vendor
#	IPE calculation
†	Quote from Prof. Diamond or Dr. Kivnick
‡	Estimation from cost charts

**FIGURE 9**



## **Variable Costs**

The variable costs for our process are made up of raw materials and utilities costs. The raw materials account for almost all of the variable costs, primarily because most of the materials we use are of the highest quality and purity. The powdered media is extremely expensive; for this is essentially the “feed,” and our reactors are the cells, which require the best ingredients to yield the highest “conversion.” Chemicals introduced in the Separation section are also of the highest purity because we cannot afford to introduce more contaminants or impurities (such as endotoxins). An enormous amount of arginine is also required for the process because this keeps our protein “intact” by preventing aggregation. More than half of the cost due to raw materials comes from the purchase of arginine.

Other variable costs included are filters, which can be very expensive and are replaced fairly often. The chromatography columns also increase our variable costs. The resins used in our columns are extremely expensive; though they last an entire year. The Affinity Resin costs \$2,810/L, and the ER Resin costs \$4,320/L. Finally, about 750 kg of anti-tPA antibody are required for our Affinity Columns. This antibody will actually be produced in our plant, but time constraints prevented us from designing the antibody production (a design project in and of itself). Prof. Diamond recommended we use a cost of \$2,000/g of antibody for our calculations.

The waste disposal is also considered a raw material; one that we are buying for a “negative price” in a sense. A phenomenal amount of waste is produced during production and cleaning of our plant. This disposal is contracted out to EarthCare for a price of \$0.21/gal, though we used \$1.00/gal in our calculations to provide for



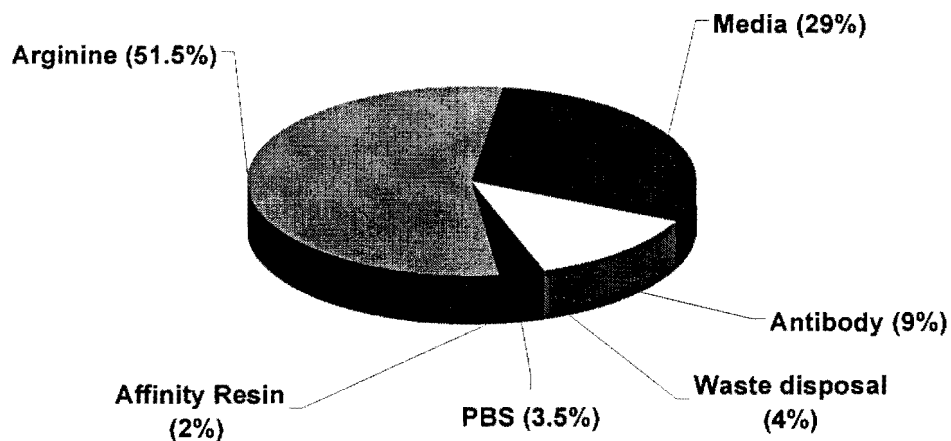
transportation and excess waste. A list of the raw materials used in our plant is provided below in Table 7.

The majority of our variable costs arise from expenses such as research, management compensation, and administrative expenses. Factors from Prof. Seider's *Process Design Principles* were used to determine these costs. Direct and allocated research factors were increased significantly to account for research and development for our process. Pharmaceutical and biotechnology companies invest a significantly larger portion of their profits toward research because it can be so costly and fruitless in some instances. These other variable costs account for almost 80% of the \$82 MM in variable costs (see page 166).

**TABLE 7: Raw Material Costs**

Raw Materials			
Item	Purchase Cost (1 unit)	Quantity/yr	Yearly Cost
Affinity Resin	\$2,910*	120L	\$349,200
ER Resin	\$4,320*	20L	\$86,400
UF2 filters	\$950*	1unit	\$950
UF1 filters	\$785*	1unit	\$785
Arginine	\$119*	74500kg	\$8,865,500
Glycine	\$43*	770kg	\$32,879
PBS	\$5*	117000L	\$566,982
NaCl	\$18*	4500kg	\$81,000
Sucrose	\$1*	3kg	\$2
NaOH	\$18*	550kg	\$9,625
Powdered Media	\$20*	250000L	\$5,000,000
anti-tPA antibody	\$2,000†	750g	\$1,500,000
Waste	\$1*	660400gal	\$660,400
Tank Compressed Air	\$172*	360tank	\$61,920
Carbon filter	\$1,515*	1unit	\$1,515
10 micron filter	\$32*	50unit	\$1,564
2 micron filter	\$32*	50unit	\$1,564
0.2 micron filter	\$44*	170unit	\$4,400
<b>Total:</b>			<b>\$16,500,000</b>

FIGURE 10



### Fixed Costs

Fixed costs for our plant were also calculated using the economic spreadsheet provided by Holger Nickisch (see page 165). Salaries and wages for operators and maintenance personnel contribute the majority of our fixed costs. These figures were again calculated using Prof. Seider's recommendations in *Process Design Principles*. Key differences lie in wages for our operators, which are significantly higher than those for chemical plants. According to Prof. Diamond, most operators in biotechnology plants have PhD's and require higher pay; for this reason our wages and benefits accounted for \$100,000 for 6 operators. There are many different unit operations that each requires significant expertise to master, for example the affinity chromatography steps.

In addition to these costs we included \$500,000/yr for a control laboratory. This molecular biology laboratory is responsible for completing quality control tests throughout the process (see Other Considerations page 174). This laboratory will require expensive instrumentation, though some of this will certainly be available from the

laboratory that derived our cell line. The \$500,000/yr estimate was recommended by Prof. Diamond in our design meeting, and the total fixed costs sum to \$11.4 MM/yr.

### **Profitability Analysis**

The production of generic tPA has the potential to be a very profitable venture. The capital investment required from our plant is just over \$104 MM, and the annual costs required to run the plant are over \$88 MM (see page 167). Fortunately, our product is expensive enough to place our annual sales at \$360 MM/yr. This value is derived from a production rate of 80 kg/yr. The table below demonstrates that the market share we predict will be available for generic tPA priced at \$500/dose. Our calculations were completed at a 90% production rate (72 kg/yr), so our production works well with the estimate market of 76 kg/yr.

**TABLE 8: Market Share Analysis**

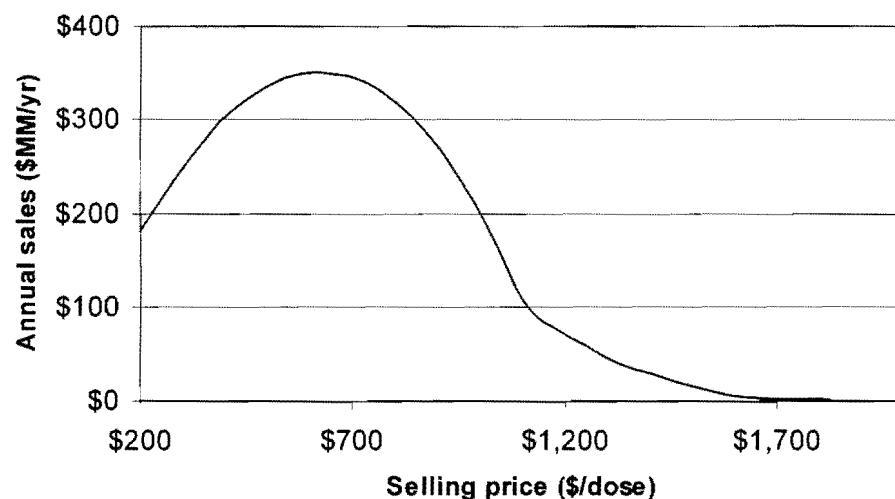
	tPA	SK
US market (\$/yr)	\$300,000,000	\$50,000,000
Europe/Japan market (\$/yr)	\$100,000,000	\$150,000,000
World market (\$/yr)	\$400,000,000	\$200,000,000
% of market share taken	50	66
Competitor price (\$/dose)	\$2,000	\$200
Our price (\$/dose)	\$500	
Yearly sales from tPA & SK (\$/yr)	\$50,000,000	\$330,000,000
Our total yearly sales (\$/yr)	\$380,000,000	
Production Rate	760,000 doses/year	
Production Rate (100mg/dose)	76kg/year	

Calculations obtained from the Nickisch spreadsheet provide and investor's rate of return (IRR) at 59.7% and a return on investment (ROI) of 145.8% (see page 167). These numbers make our plant extremely profitable. The payback period is less than two years for our company, given that we allowed for three years of construction. This was done to allow time for the production of a viable cell line by our research facility. After eight years of production the net present value (NPV) of our plant is approximately \$1

billion. While these numbers are high, we feel confident that our numbers are reasonable. If we were unsure of any estimated costs, we made certain that they were overstated. Our annual sales will be very sensitive to price, so a more detailed estimate on the effect of price is presented below.

As the selling price of our generic tPA is lowered we will acquire a greater percentage of both the SK and Activase<sup>TM</sup> markets. A decrease in price will also decrease annual sales and revenue, so there will be a maximum in annual sales that we can achieve for the range of selling prices from \$200/dose to \$2000/dose. The following plot is presented to demonstrate the effect of price on sales. The percentage of SK and Activase<sup>TM</sup> market share that we can attain was roughly estimated based on discussions with Prof. Diamond. In order to determine the ideal selling price a comprehensive marketing report would need to be completed, but Figure 11 on page 169 is shown to give a general idea of the strong effect that pricing can have on market share.

It is possible that there would not be such a large market for generic tPA; if so, our plant would not run at capacity. In addition to the calculations presented above, we have also performed calculation for our plant given an annual production of 40 kg/yr, or half the previous value of 80 kg/yr. This cut in production only affects the variable costs and annual sales: both of which are cut in half. Even with a \$180 MM decrease in annual sales our plant is still extremely profitable. The IRR is now 41% and the ROI is 80.25%, very good values for a chemical process. The NPV after ten years of production is \$782 MM (see page 169).

**Annual sales per Year vs. Selling Price****FIGURE 11**

Our calculations demonstrate that the production of generic recombinant tPA is a very profitable and worthwhile venture. All cost estimates are either direct quotes from company representatives or conservative estimates from Prof. Diamond and Dr. Kivnick. Given the final values in our profitability analysis, it would not be surprising to see generic tPA enter the market three or four years from now.

# Input Summary

April 21, 2000

## General Information

Title of Process: **Human Tissue Plasminogen Activator**  
 Plant Site: **U.S. Northeast**  
 Starting Year: **2000**  
 Years of Design: **1**  
 Years of Construction: **3**  
 Years of Plant Life: **12**  
 First Year of Production: **2004**

## Product Information

The process yields a single product: **tPA**

## Capacity

Operating Hours per Year: **8,400**  
 The Process will Yield: **0.01** kg per hour or **80** kg per year of tPA

## Market Price

The Price per kg of tPA is: **5000000.00**

## Equipment Costs

<u>Equipment Type</u>	<u>Cost</u>
Fermentation Section	\$1,075,000
Water/Waste Treatment	\$106,000
Separation Section	\$4,132,000
Steam Production	\$112,000
Cell Line Research	\$1,000,000

Percentage of Purchased Costs for Installation Materials: 200.00 %  
 Percentage of Purchased Costs for Labor : 150.00 %  
 Percentage of Purchased Costs for Freight, Insurance and Taxes : 15.00 %  
 Percentage of Purchased Costs for Construction Overhead: 150.00 %  
 Percentage of Purchased Costs for Contractor Engineering Expenses: 130.00 %

## Total Capital Investment

Percentage of Total Bare Module Costs for Site Preparation and Service Facilities: 10.00 %  
 Allocated Utility and Related Facility Costs (see Table 9.4): \$ -  
 Percentage of Direct Permanent Investment for Contingencies: 15.00 %  
 Land: Enter either a dollar value or a percentage of Total Depreciable Capital: 2.00 %  
 Percentage of Total Depreciable Capital for Royalties: - %  
 Percentage of Total Depreciable Capital for Start-Up: 20.00 %  
 Site Factor (see Table 9.5): 1.10

## Working Capital

Inventory will be kept of the following Materials:

Arginine:	14 days	3,160 kg
Glycine:	14 days	33 kg
Sucrose:	14 days	0 kg
PBS:	14 days	4,931 L
NaOH:	14 days	22 kg
NaCl:	14 days	191 kg
Powdered Media:	14 days	10,606 L
Compressed Air:	14 days	16 tank
10 micron filter:	14 days	2 unit
2 micron filter:	14 days	2 unit
0.2 micron filter:	14 days	11 unit

Accounts Receivable: 30 days

## Raw Materials

kg Arginine per kg tPA:	931.0430000	Cost (\$) per kg Arginine:	119.0000000
kg Glycine per kg tPA:	9.6158000	Cost (\$) per kg Glycine:	42.7100000
kg Sucrose per kg tPA:	0.0290000	Cost (\$) per kg Sucrose:	4.8500000
L PBS per kg tPA:	1452.9300000	Cost (\$) per L PBS:	0.7990000
kg NaOH per kg tPA:	6.5500000	Cost (\$) per kg NaOH:	17.5400000
kg NaCl per kg tPA:	56.3000000	Cost (\$) per kg NaCl:	18.0500000

L Powdered Media per kg tPA:	3125.000000	Cost (\$) per L Powdered Media:	20.000000
tank CO2 per kg tPA:	0.3750000	Cost (\$) per tank CO2:	142.800000
tank Compressed Air per kg tPA:	4.7500000	Cost (\$) per tank Compressed Air:	171.630000
g anti-tPA antibody per kg tPA:	9.3750000	Cost (\$) per g anti-tPA antibody:	2000.000000
unit 10 micron filter per kg tPA:	0.6250000	Cost (\$) per unit 10 micron filter:	32.000000
unit 2 micron filter per kg tPA:	0.6250000	Cost (\$) per unit 2 micron filter:	32.000000
unit 0.2 micron filter per kg tPA:	3.3750000	Cost (\$) per unit 0.2 micron filter:	44.000000
L Affinity Resin per kg tPA:	1.5000000	Cost (\$) per L Affinity Resin:	2809.600000
L ER Resin per kg tPA:	0.2500000	Cost (\$) per L ER Resin:	4320.000000
unit Carbon Filter per kg tPA:	0.0125000	Cost (\$) per unit Carbon Filter:	2000.000000
unit UF1 filter per kg tPA:	0.0125000	Cost (\$) per unit UF1 filter:	785.000000
unit UF2 filter per kg tPA:	0.0125000	Cost (\$) per unit UF2 filter:	950.000000
gal Waste per kg tPA:	8255.000000	Cost (\$) per gal Waste:	0.500000

**Utilities**

gal Process Water per kg tPA:	17,014.500000	Cost per gal Process Water:	0.000500
MMBTU Natural Gas per kg tPA:	13.1700000	Cost per MMBTU Natural Gas:	2.600000
kW*hr Electricity per kg tPA:	17,300.000000	Cost per kW*hr Electricity:	0.040000
yr Anti-body License per kg tPA:	0.0125000	Cost per yr Anti-body License:	120000.00

**Other Variable Costs**

Selling/Transfer Expense:	1.00 % of sales
Direct Research:	10.00 % of sales
Allocated Research:	2.00 % of sales
Administrative Expense:	2.00 % of sales
Management Incentive Compensation:	1.25 % of sales

**Packaging**

Labor:	0.00 per kg tPA
Materials:	2000.00 per kg tPA

**Fixed Costs****Operations**

Number of Operators per Shift:	6 (assuming 5 Shifts)
Annual Wages per Operator:	\$100,000 Including Benefits
Direct Salaries and Benefits:	15 % of wages
Operating Supplies and Services:	6 % of wages
Technical Assistance to Manuf.:	\$52,000 per labor year
Control Laboratory:	\$500,000 per labor year

**Maintenance**

Wages:	3.50 % of Total Depreciable Capital,	Including Benefits
Salaries and Benefits:	25.00 % of Maintenance Wages and Benefits	
Materials and Services:	100.00 % of Maintenance Wages and Benefits	
Maintenance Overhead:	5.00 % of Maintenance Wages and Benefits	

**Operating Overhead**

General Plant Overhead:	7.10 % of Maintenance and Operations Salaries, Wages and Benefits
Mechanical Department Services:	2.40 % of Maintenance and Operations Salaries, Wages and Benefits
Employee Relations Department:	5.90 % of Maintenance and Operations Salaries, Wages and Benefits
Business Services:	7.40 % of Maintenance and Operations Salaries, Wages and Benefits

**Property Taxes and Insurance**

Property Taxes and Insurance	1.50 % of Total Depreciable Capital
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**Depreciation**

Direct Plant	8.00 % of Total Depreciable Capital
Allocated Plant	6.00 % of Allocated Costs

**Catalyst Replacement**

Catalyst Replacement	- \$/year
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**Financial Information**

Cost of Capital:	1.00 %
General Inflation Rate:	- %
Income Tax Rate:	37.00 %

**MACRS Tax-Basis Depreciation Schedule**

YEAR 1	10.00%
YEAR 2	18.00%
YEAR 3	14.40%
YEAR 4	11.52%
YEAR 5	9.22%

**Distribution of Investment**

DESIGN 1	25%
CONSTRUCTION 1	25%
CONSTRUCTION 2	25%
CONSTRUCTION 3	25%

*Generic tPA**Economic Summary*

YEAR 6	7.37%
YEAR 7	6.55%
YEAR 8	6.55%
YEAR 9	6.56%
YEAR 10	6.55%
YEAR 11	3.28%



Human Tissue Plasminogen Activator

April 21, 2000

**Equipment**

		<b>TOTAL</b>
<b>I.</b>		
Fermentation Section	\$1,075,000	
Water/Waste Treatment	\$106,000	
Separation Section	\$4,132,000	
Steam Production	\$112,000	
Cell Line Research	\$1,000,000	
<b>Total Purchased Cost:</b>	<b>\$6,425,000</b>	
<b>Total Materials for Installation:</b>	<b>\$12,850,000</b>	
<b>Total Direct Materials:</b>	<b>\$19,275,000</b>	
<b>Total Direct Labor:</b>	<b>\$9,638,000</b>	
<b>Total Direct Project Expenses</b>	<b>\$28,913,000</b>	
Freight, Insurance, Taxes:	\$964,000	
Construction Overhead:	\$9,638,000	
Contractor Engineering Expenses:	\$8,353,000	
<b>Total Indirect Project Expenses:</b>	<b>\$18,955,000</b>	
<b>Total Bare Module Costs I :</b>	<b>\$47,868,000</b>	
<b>Total Bare Module Costs :</b>		<b>\$47,868,000</b>
Cost of Site Preparation and Service Facilities:	\$4,787,000	
Allocated Costs for Utilities and Related Facilities:	\$0	
<b>Direct Permanent Investment:</b>		<b>\$52,655,000</b>
Cost of Contingencies:	\$7,898,000	
<b>Total Depreciable Capital:</b>		<b>\$60,553,000</b>
Cost of Land:	\$0	
Cost of Royalties:	\$0	
Costs of Startup:	\$12,111,000	
<b>Total Permanent Investment:</b>		<b>\$79,930,400</b>

**Working Capital**Inventory

Arginine	2,850 kgs	\$339,000	
Glycine	30 kgs	\$1,000	
Sucrose	- kgs	\$0	
PBS	4,460 Ls	\$4,000	
NaOH	20 kgs	\$0	
NaCl	170 kgs	\$3,000	
Powdered Media	9,580 Ls	\$192,000	
Compressed Air	10 tanks	\$2,000	
10 micron filter	- units	\$0	
2 micron filter	- units	\$0	
0.2 micron filter	10 units	\$0	
<b>Total Inventory :</b>		<b>\$541,000</b>	
<b>Accounts Receivable:</b>	<b>30 days</b>	<b>\$36,364,000</b>	
<b>Total Working Capital</b>		<b>\$36,905,000</b>	
<b>Total Capital Investment:</b>		<b>\$116,835,400</b>	

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April 21, 2000

# Cash Flows

## Human Tissue Plasminogen Activator

Year	% of Capacity	Sales	Capital Costs	Working Capital	Variable Costs	Fixed Costs	Depreciation	Taxable Income	Income Tax	Net Earnings	Annual Cash	Cumulative PV @ 1 %
2000	0.0		(\$19,983,000)					(\$19,983,000)		(\$19,983,000)	(\$19,983,000)	(\$19,983,000)
2001	0.0	DESIGN	(\$19,983,000)					(\$19,983,000)		(\$19,983,000)	(\$19,983,000)	(\$39,768,000)
2002	0.0	CONSTRUCTION	(\$19,983,000)					(\$19,983,000)		(\$19,983,000)	(\$19,983,000)	(\$59,357,000)
2003	0.0	CONSTRUCTION	(\$19,983,000)					(\$56,888,000)		(\$56,888,000)	(\$56,888,000)	(\$114,572,000)
2004	45.0			(\$36,721,000)	(\$11,354,000)	(\$7,983,000)	\$123,932,000	(\$45,855,000)	\$78,077,000	\$86,070,000	\$86,070,000	(\$31,860,000)
2005	67.5			(\$55,081,000)	(\$11,354,000)	(\$14,388,000)	\$189,177,000	(\$69,995,000)	\$119,182,000	\$133,570,000	\$133,570,000	\$95,227,000
2006	90.0			(\$73,442,000)	(\$11,354,000)	(\$11,510,000)	\$263,694,000	(\$97,567,000)	\$166,127,000	\$177,637,000	\$177,637,000	\$262,569,000
2007	90.0			(\$73,442,000)	(\$11,354,000)	(\$9,208,000)	\$265,996,000	(\$98,419,000)	\$167,577,000	\$176,785,000	\$176,785,000	\$427,460,000
2008	90.0			(\$73,442,000)	(\$11,354,000)	(\$7,370,000)	\$267,834,000	(\$99,099,000)	\$168,735,000	\$176,105,000	\$176,105,000	\$590,090,000
2009	90.0			(\$73,442,000)	(\$11,354,000)	(\$5,891,000)	\$269,313,000	(\$99,646,000)	\$169,667,000	\$175,558,000	\$175,558,000	\$750,610,000
2010	90.0			(\$73,442,000)	(\$11,354,000)	(\$5,236,000)	\$269,968,000	(\$99,888,000)	\$170,080,000	\$175,316,000	\$175,316,000	\$909,321,000
2011	90.0			(\$73,442,000)	(\$11,354,000)	(\$5,236,000)	\$269,968,000	(\$99,888,000)	\$170,080,000	\$175,316,000	\$175,316,000	\$1,066,461,000
2012	90.0			(\$73,442,000)	(\$11,354,000)	(\$5,244,000)	\$269,960,000	(\$99,885,000)	\$170,075,000	\$175,319,000	\$175,319,000	\$1,222,048,000
2013	90.0			(\$73,442,000)	(\$11,354,000)	(\$5,236,000)	\$269,968,000	(\$99,888,000)	\$170,080,000	\$175,316,000	\$175,316,000	\$1,376,092,000
2014	90.0			(\$73,442,000)	(\$11,354,000)	(\$2,622,000)	\$272,562,000	(\$100,855,000)	\$171,727,000	\$174,349,000	\$174,349,000	\$1,527,769,000
2015	90.0			(\$73,442,000)	(\$11,354,000)		\$312,109,000	(\$115,480,000)	\$196,629,000	\$196,629,000	\$196,629,000	\$1,697,135,000

## Fixed Costs

### Human Tissue Plasminogen Activator

April 21, 2000

		TOTAL
<b><u>Operation</u></b>		
Wages and Benefits	\$3,000,000	
Direct Salaries and Benefits	\$450,000	
Operating Supplies and Services	\$180,000	
Technical Assistance to Manufacturing	\$52,000	
	\$500,000	
<b>Total Operations:</b>	<b>\$4,182,000</b>	<b>\$4,182,000</b>
<b><u>Maintenance</u></b>		
Wages and Benefits	\$2,119,000	
Salaries and Benefits	\$530,000	
Materials and Services	\$2,119,000	
	\$106,000	
<b>Total Maintenance:</b>	<b>\$4,874,000</b>	<b>\$9,056,000</b>
<b><u>Operating Overhead</u></b>		
General Plant Overhead	\$433,000	
Mechanical Department Services	\$146,000	
Employee Relations Department	\$360,000	
	\$451,000	
<b>Total Operating Overhead:</b>	<b>\$1,390,000</b>	<b>\$10,446,000</b>
<b>Property Taxes and Insurance:</b>	<b>\$908,000</b>	<b>\$11,354,000</b>
<b>Total Fixed Costs:</b>		<b>\$11,354,000</b>

# Variable Costs

## Human Tissue Plasminogen Activator

April 21, 2000

TOTAL

### Raw Materials

Arginine	11,079,411.70¢	per kg tPA
Glycine	41,069.08¢	per kg tPA
Sucrose	14.07¢	per kg tPA
PBS	116,089.11¢	per kg tPA
NaOH	11,488.70¢	per kg tPA
NaCl	101,621.50¢	per kg tPA
Powdered Media	6,250,000.00¢	per kg tPA
CO2	5,355.00¢	per kg tPA
Compressed Air	81,524.25¢	per kg tPA
anti-tPA antibody	1,875,000.00¢	per kg tPA
10 micron filter	2,000.00¢	per kg tPA
2 micron filter	2,000.00¢	per kg tPA
0.2 micron filter	14,850.00¢	per kg tPA
Affinity Resin	421,440.00¢	per kg tPA
ER Resin	108,000.00¢	per kg tPA
Carbon Filter	2,500.00¢	per kg tPA
UF1 filter	981.25¢	per kg tPA
UF2 filter	1,187.50¢	per kg tPA

<b>Total Raw Materials:</b>	<b>20,527,282.15¢ per kg tPA</b>	<b>→ \$16,422,000 →</b>	<b>\$16,422,000</b>
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### Utilities

Process Water	850.73¢	per kg tPA
Natural Gas	3,424.20¢	per kg tPA
Electricity	69,200.00¢	per kg tPA

<b>Total Utilities:</b>	<b>223,474.93¢ per kg tPA</b>	<b>→ \$179,000 →</b>	<b>\$16,601,000</b>
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### Byproducts

<b>Total Byproducts:</b>	<b>0.00¢ per kg tPA</b>	<b>→ \$0 →</b>	<b>\$16,601,000</b>
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### Other Variable Costs

Selling/Transfer Expense	5,000,000.00¢	per kg tPA
Direct Research	50,000,000.00¢	per kg tPA
Allocated Research	10,000,000.00¢	per kg tPA
Administrative Expense	10,000,000.00¢	per kg tPA
Management Compensation	6,250,000.00¢	per kg tPA
Packaging Materials	0.00¢	per kg tPA

<b>TOTAL</b>	<b>102,002,757.08¢ per kg tPA</b>	<b>→ \$81,602,000</b>	<b>\$81,602,000</b>
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IRR

59.12%

ROI (Third Year)

Annual Sales	\$360,000,000
Annual Costs	(\$89,640,000)
Income Tax	(\$100,033,000)
Net Income	\$170,327,000
Capital Investment	\$116,837,000

145.78%

NPV

\$1,697,135,000

# Input Summary

April 21, 2000

## General Information

Title of Process: **Human Tissue Plasminogen Activator**  
 Plant Site: **U.S. Northeast**  
 Starting Year: **2000**  
 Years of Design: **1**  
 Years of Construction: **3**  
 Years of Plant Life: **12**  
 First Year of Production: **2004**

## Product Information

The process yields a single product: **tPA**

## Capacity

Operating Hours per Year: **8,400**  
 The Process will Yield: **0.00** kg per hour or **40** kg per year of tPA

## Market Price

The Price per kg of tPA is: **5000000.00**

## Equipment Costs

Equipment Type	Cost
Fermentation Section	\$1,075,000
Water/Waste Treatment	\$106,000
Separation Section	\$4,132,000
Steam Production	\$112,000
Cell Line Research	\$1,000,000

Percentage of Purchased Costs for Installation Materials:	200.00 %
Percentage of Purchased Costs for Labor :	150.00 %
Percentage of Purchased Costs for Freight, Insurance and Taxes :	15.00 %
Percentage of Purchased Costs for Construction Overhead:	150.00 %
Percentage of Purchased Costs for Contractor Engineering Expenses:	130.00 %

## Total Capital Investment

Percentage of Total Bare Module Costs for Site Preparation and Service Facilities:	10.00 %
Allocated Utility and Related Facility Costs (see Table 9.4): \$	-
Percentage of Direct Permanent Investment for Contingencies:	15.00 %
Land: Enter either a dollar value or a percentage of Total Depreciable Capital:	2.00 %
Percentage of Total Depreciable Capital for Royalties:	- %
Percentage of Total Depreciable Capital for Start-Up:	20.00 %
Site Factor (see Table 9.5):	1.10

## Working Capital

Inventory will be kept of the following Materials:

Arginine:	14 days	1,580 kg
Glycine:	14 days	16 kg
Sucrose:	14 days	0 kg
PBS:	14 days	2,466 L
NaOH:	14 days	11 kg
NaCl:	14 days	95 kg
Powdered Media:	14 days	5,303 L
Compressed Air:	14 days	8 tank
10 micron filter:	14 days	2 unit
2 micron filter:	14 days	2 unit
0.2 micron filter:	14 days	11 unit

Accounts Receivable: 30 days

## Raw Materials

kg Arginine per kg tPA:	931.0430000	Cost (\$) per kg Arginine:	119.0000000
kg Glycine per kg tPA:	9.6158000	Cost (\$) per kg Glycine:	42.7100000
kg Sucrose per kg tPA:	0.0290000	Cost (\$) per kg Sucrose:	4.8500000
L PBS per kg tPA:	1452.9300000	Cost (\$) per L PBS:	0.7990000
kg NaOH per kg tPA:	6.5500000	Cost (\$) per kg NaOH:	17.5400000
kg NaCl per kg tPA:	56.3000000	Cost (\$) per kg NaCl:	18.0500000

IRR

41.00%
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ROI (Third Year)

Annual Sales	\$180,000,000
Annual Costs	(\$52,975,000)
Income Tax	(\$46,999,000)
Net Income	\$80,026,000
Capital Investment	\$99,718,000

80.25%
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NPV

\$782,585,000
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## OTHER CONSIDERATIONS



## **Scheduling**

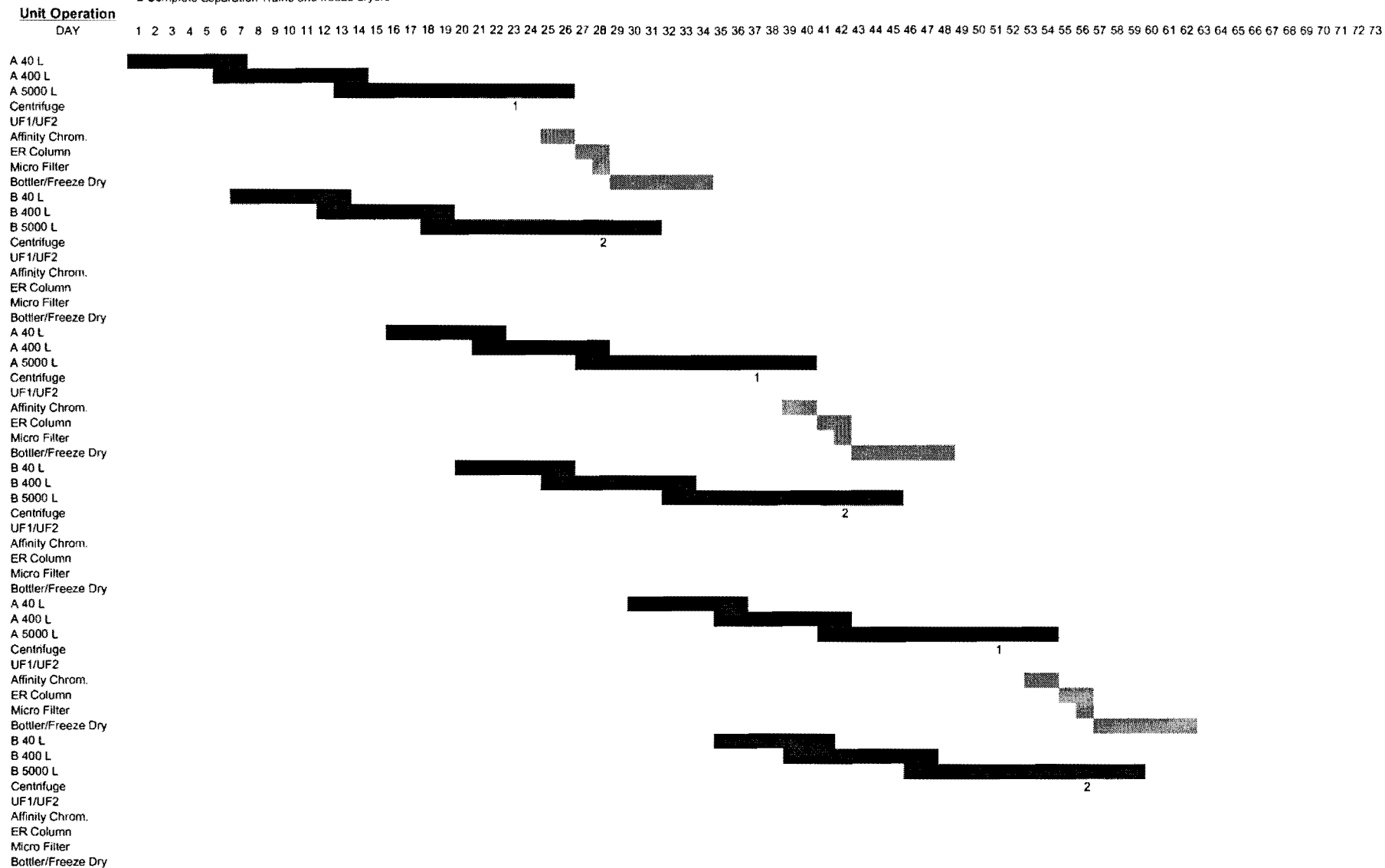
The most important consideration in running a batch process is proper scheduling of each batch. The tissue plasminogen activator (tPA) process consists of two individual fermentation trains and two separation trains that share a common centrifuge and set of ultra filters. Batch scheduling had to be designed to fit 50 batches into one year without any overlap at the common unit operations. As shown in Figure 12 on page 172, a new batch is begun every 7 days in the 40 L fermentors and remains in the fermentation train for a total of 27 days.

The batch schedule is designed to run the 5000 L continuously over the course of the year. In other words, once a batch is harvested from the vessel the bioreactor is sterilized and filled with media for the next cell culture. Extra time was added into the schedule during the cleaning and sterilization portion of the batch to ensure enough time is available for routine maintenance or repairs. Each batch is properly scheduled to stagger the inoculation of the 40 L and 400 L fermentors so they can be harvested when the next fermentor in line is ready to start a new batch. The 40 L and the 400 L fermentors have a down time of 7 and 4 days respectively.

Another important consideration of the batch schedule is common equipment used by each train. The centrifuge and the ultra filters begin the purification process of the product protein. Cell cultures are sent immediately to the centrifuge after they are harvested. Precise timing of the batches in each train, staggers the harvest of the two 5000 L fermentors every seven days. Subsequently, the centrifuge and the ultra filters are used only every seven days. The large down time of these two pieces of equipment

**Figure 12 - Plant Wide Scheduling for 3 Fermentation Trains and 2 Separation Trains.**

- 1 centrifuge and 1 Set of Ultra Filters for the whole plant
- 2 Complete Separation Trains and freeze dryers



prevents any bottlenecks from forming and allows enough time for maintenance and repairs if they are needed.

The centrifuge and the ultra filters is the only area that bottlenecking could occur, but the scheduling design prevents this from occurring. The rest of the fermentation trains and the separation trains do not contain any other problematic areas. In fact, the plant design contains many holding tanks in between unit operations to retain material in the event of a shut down some where on the process line. Once the cells have been harvested the tPA protein can be held at 4 °C for a long period of time.

The holding tanks are also part of a design for future expansion of the plant. In order to fulfill the entire problem statement, the plant was designed to handle a yearly production of 100 kg. The plant designed described in this report only has two fermentation trains, but a study of a three fermentation train plant was also explored. It was determined that the market was not large enough to sell 100 kg of tPA product, but the market may expand in the future. The plant would contain three fermentation trains and only 2 separation trains. Because of smaller batch lengths for the separation verse the fermentation processes, only two separation trains would be more than adequate.

## **Quality Testing**

Before, during, and after each batch begins, tests must be run in order to confirm that we are making functional tPA and using good materials. It is always possible that the media is no good or that a batch will be infected. Contamination is a great concern given that our batches are worth approximately \$15 MM each. In order to control for quality throughout the process we will need to perform tests on the media, take cell

samples from each reactor, and estimate tPA productivity at the beginning of the Separation train. This information is also invaluable to process development researchers who are working to optimize the production of tPA in our plant.

To prevent an entire batch from being lost because of low quality media, a sample will be taken from the packaging when it is opened. This small sample of media will be used to run a small, bottle-scale fermentation experiment to make sure the cells can grow in that particular batch of media. Ordinary non-recombinant CHO cells will be used for this so as to avoid wasting the precious stock of tPA producing cells. For quality testing purposes they will be sufficient.

Bacteria and viruses can contaminate mammalian cells very easily, so samples will be taken from each bioreactor to verify that the tPA-CHO cells are surviving and flourishing. In pharmaceutical plants such as this less than 1 percent of all batches are lost due to contamination. The loss of any batches is unacceptable in a process such as this given the sheer value of the product being produced. Again, process development researchers can use this growth information to improve the cells and techniques used to grow them.

Our plant's primary goal is to produce tPA, so testing for tPA production throughout the process is also very important. Unfortunately, it is quite difficult to measure the concentration of a particular protein when it is mixed with all of the other proteins produced in the cell's metabolism. The most effective test of protein presence or concentration is a Western blot, which can be run by our molecular biology control laboratory. The dead time of a Western blot is at least a day, so the results will serve as

research tools and to save money on the latter steps in the separation sequence if the batch is no good.

Finally the activity of the final product will need to be tested. Random doses are used to test for functional tPA, i.e., the ability to activate plasminogen (see Introduction page 14). At this point the batch is ready for shipment to hospitals throughout the world.

A relatively small molecular biology laboratory can easily complete all of the required testing. This lab will also be responsible for determining the final concentration of tPA in FD Tank and calculating the volume required per 100 mL vial. The price of such a lab, including salary and equipment, is estimated at \$500,000 based on recommendations by Prof. Diamond.

## **Sterilization**

Current Good Manufacturing Processes (cGMP's) require production of therapeutic medications to take place in a sterile environment. As a result, all equipment in our plant is capable of being cleaned-in-place (CIP) and sterilized-in-place (SIP). All vessel-like equipment (blending tanks and bioreactors) contains stainless steel pipes with nozzles that are lowered into the tank section. Sterile water is then sprayed out radially to wash away any remaining cell or media waste. Approximately two vessel volumes of water will be used during each cleaning. This step is then followed by SIP with 130°C steam for 30 minutes or longer. Other units have their own cleaning and sterilizing mechanisms and utilize caustic solution in some cases. The Centrifuge and ER Column require application of NaOH, and UF1 and UF2 are cleaned with a 0.01% caustic cleaning solution.

In addition, sanitary piping is used throughout our process. This requires all pipes to contain inlets and drains for sterile cleaning water. The sanitary pipes can also be easily disconnected and scrubbed manually if necessary. These sterilization procedures take place after each batch or after each cycle in the case of the Bottler and Freeze Dryer. In following cGMP's we will minimize batch losses and produce a clean, functional medication.

## **Start-Up**

There are many steps needed to take the plant from its completed construction to a full-scale operational plant. The steady-state operation of the plant, as designed in the project, can only be obtained through careful start-up procedure. All of the plant equipment and piping will have to be brought up to proper sterile conditions. The unit operations will be rinsed with water and steam sterilized before the process begins. The vessels can be pressurized through the use of pumps, while various utility streams can help to control the temperature of the units.

Before the plant can become fully operational, the water recycle streams that provide the warm water for used in HX-1 and BR-3 must be charged. These two streams are made up of city water that is heated with sparged steam. The initial load of water on the system is necessary to bring the recycle stream up to steady state before any of the process liquids are added to the system.

The final consideration for start-up is the purchase and application the monoclonal anti-tPA antibody for the affinity chromatography column. We have licensed a hybridoma cell line that produces this antibody for \$100,000 (see Problem Statement page 189). The antibody will be produced in our plant, using equipment

purchased for tPA production. A detailed process for the antibody production cannot be completed with the given information, so Prof. Diamond advised us to use a costing value of \$2,000/gram. Approximately 750 grams of antibody is necessary for each Affinity Column (see Appendix D page 314). The antibody will be bound to the Affinity Resin using the protocol provided by Amersham Pharmacia Biotech (see specification page 314) and will remain intact for a year; after which both the resin and antibody will be replaced.

### **Sanitization/Waste Removal**

The FDA has strict standards for the use and disposal of genetically modified organisms and cell waste in general. Any materials that come into contact with our microorganisms must be disposed of properly. Our process will be producing an enormous amount of this waste. Solid waste removed in the Centrifuge, water waste from UF1, and the volumes not collected from Affinity Column and ER Column are all process waste streams that need to disposal. In addition to process waste, all water that is used for sterilization and cleaning is also considered hazardous biological waste. Given these amounts it is estimated that 670,000 gal of waste will be produced each year. The number has been rounded up because extra waste is certain to arise during the course of a year. The simplest way of accomplishing this task is by contracting the disposal out to a waste disposal company. The EarthCare Company can eradicate this waste at a cost of \$0.21/gal (see Appendix E page 395). This price is rounded to \$1/gal to account for transportation and disposal charges.

## CONCLUSIONS & RECOMMENDATIONS



The analysis of our process indicates that production of generic human tissue plasminogen activator (tPA) can be an extremely profitable venture. Economic calculations yielded an investor's rate of return (IRR) of 59.7% and a net present value (NPV) of \$1.7 billion after ten years. This study was completed for the sale of approximately 80 kg/yr, which was based on marketing estimates provided Prof. Diamond. A more conservative economic analysis was also conducted, estimating sales of only 40 kg/yr while holding our capital costs constant. This venture is also extremely profitable, with an IRR of 41.0% and a NPV of almost \$800 MM.

This is the first time the patent for a recombinant protein produced in mammalian cells will be expiring. The price for the original product is extremely high due to the large amount of research costs accumulated during its discovery and development. In following the expired patent we will avoid these costs and produce an expensive medication at cost.

The introduction of a cheap, generic version of tPA should cause a tremendous increase in the market for recombinant thrombolytic agents. Streptokinase (SK), the only other major anti-clot drug on the market is only preferred for its cheaper price. In decreasing the price difference between tPA we will obtain a large share of the SK market, which is quite sizable overseas.

Our production methods are fairly straightforward. Research in the form of a pilot plant will be required in order to determine growth kinetics and optimal conditions for the separation processes. Genentech obtained a cell production rate of 50 pg/cell/day in patent 4,766,075. Molecular biology techniques have improved significantly in the

past fifteen years, so it is likely that our cells could attain higher production rates, further increasing our profits.

Biological processes can be very unpredictable and there is no data published on the downstream processing of tPA. A great deal of data would need to be obtained to determine exact purification protocols. In order to account for these research needs an extra year of construction (three years total) was included in our economic calculations.

The expiration of the patent for a recombinant tPA-producing cell line opens the door to an extremely profitable venture. This is one of the first opportunities for “horizontal growth” the biotechnology industry. We feel that generic tPA production provides a tremendous opportunity for a company to enter the pharmaceutical market. The profits gained from this venture could provide capital for research in other products. It would not be surprising for a large pharmaceutical company to undertake a venture such as this due to its growth potential and value. In conclusion, we strongly urge construction of a generic tPA production plant in the near future.



## **BIBLIOGRAPHY**

### **LITERATURE**

1. Asenjo, J.A., and Merchuck, J. C., *Bioreactor System Design*, Marcel Dekker Inc., New York, 1995.
2. Harrison, R. G., *Protein Purification Process Engineering*, Marcel Dekker Inc., New York, 1994.
3. Ladisch, M. R., Wilson, R. C., Painton, C., Builder, C., and Stuart E., *Protein Purification From Molecular Mechanisms to Large-Scale Processes*, ACS Symposium Series 427, American Chemical Society, Washington, D.C., 1990
4. Sourirajan, S., and Matsuurai, T., *Reverse Osmosis and Ultrafiltration*, ACS Symposium Series 281, American Chemical Society, Washington, D.C., 1985.
5. Merten, O. W., Perrin, P., and Griffiths, B., *New Developments and New Applications in Animal Cell Technology*, Kluwer Academic Publishers, Dordrecht, the Netherlands.
6. Zahid, A., *Reverse Osmosis: Membrane Technology, Water Chemistry, and Industrial Application*, Van Nostrand Reinhold, New York, 1993.
7. Todd, P., Sikdar, S. K., and Beir, M., *Frontiers in Bioprocessing II*, Conference Proceedings Series, American Chemical Society, Washington, D.C., 1992.
8. Twork, J. V., and Yacynych, A. M., *Sensors in Bioprocess Control*, Marcel Dekker Inc., New York, 1990.
9. Harris, T. J. R., *Protein Production by Biotechnology*, Elsevier Science Publishers Ltd., Essex, England, 1990.
10. Seider, W. D., Seader, J. D., and Lewin, W. R., *Process Design Principles: Synthesis, Analysis, and Evaluation*, John Wiley & Sons, Inc., New York, 1999.
11. Shuler, M. L., and Kargi, F., *Bioprocess Engineering: Basic Concepts*, Prentice Hall, Englewood Cliffs, NJ, 1992.
12. Wernicke, D., Will, H. (1992). Generation of Recombinant CHO(dhfr-) Cell Lines by Single Selection for dhfr+ Transformants, *Analytical Chemistry* **203**, 146-150.
13. Goswami, J., Sinskey, A. J., Steller, H., Stephanopoulos, G. N. (1999). Apoptosis in Batch Cultures of Chinese Hamster Ovary Cells, *Biotechnology and Bioengineering* **62**, 632-639.

14. Datar, R. J., Cartwright, T., Rosen, C. (1993). Process Economics of Animal Cell and Bacterial Fermentations: A case Study Analysis of Tissue Plasminogen Activator, *Biotechnology* **11**, 349-357.
15. Grammatikos, S. I., Tobien, K., Noe, W., Werner, R. G. (1999). Monitoring of Intracellular Ribonucleotide Pools is a Powerful Tool in the Development and Characterization of Mammalian Cell Culture Processes, *Biotechnology and Bioengineering* **64**, 357-363.
16. Reagan, M. E., Robb, M., Bornstein, I., and Niday, E. G. (1985). Immunoaffinity Purification of Tissue Plasminogen Activator from Serum-Supplemented Conditioned Media Using Monoclonal Antibody, *Thrombosis Research* **40**, 1-9.
17. Stanek, E. J., Cheng, J. W., Peeples, P. J., Simko, R. J., and Spinler, S. A. (1997). Patient preferences for thrombolytic therapy in acute myocardial infarction, *Medical Decision Making* **17**, 464-471
18. Kalish, S. C., Gurwitz, J. H., Krumholz, H. M., and Avorn, J. (1995). A cost effectiveness model of thrombolytic therapy for acute myocardial infarction, *Journal of General Internal Medicine* **10**, 321-330
19. U.S. Patent 4,766,075 – Goeddel et al. August 23, 1988.

**INTERNET**

<http://www.amerewater.com>

<http://www.atlanticuv.com>

<http://www.cocorampump.com>

<http://www.hyclone.com>

<http://www.kochmembrane.com>

<http://www.nbsc.com>

<http://www.osmonics.com>

<http://www.randolphaustin.com>

<http://www.sigma-aldrich.com>

<http://www.sterogene.com>

<http://www.thomasregister.com>

<http://www.virtis.com>

<http://www.walkerstainless.com>

<http://www.westfaliaseparatorus.com>

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Troy from Virtis  
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Finally, our design group would like to thank Dr. Arnold Kivnick and Professor Scott Diamond. We learned an enormous amount from Dr. Kivnick this semester; his experience in process design pointed us in the right direction time and time again. Professor Diamond wrote a very interesting and challenging design proposal, and his suggestions throughout the semester were crucial to the completion of the process. This was the first report of its kind, and we are sure that in the future Professor Diamond will be creating more projects for chemical engineering seniors to complete. This project forced us to draw on the knowledge we acquired in the past four years at Penn. We have learned and matured a great deal and owe this class and this department a debt of thanks in growing with us.





# APPENDIX

## **Generic Recombinant Human Tissue Plasminogen Activator (tPA)**

(Recommended by Dr. Scott L. Diamond, University of Pennsylvania)

### **Setting:**

Plasminogen activators are powerful enzymes that trigger the proteolytic degradation of blood clots that cause strokes and heart attacks. Genentech owns the patent for tPA, and currently sells 100 mg doses of recombinant tPA (activase) for about \$2000. The annual sales for tPA are about \$300MM/yr. However, the patent for tPA will be expiring soon. In response, Genentech has developed a next generation, FDA-approved, Plasminogen activator called “TNK-tPA”, which is slightly easier and safer for clinician to use.

While a generic form of tPA may not compete well against TNK-tPA in the U.S., there may exist the opportunity to market a low-cost generic tPA in foreign markets, where urokinase and streptokinase are low-cost (~\$200/dose) alternatives that are associated with increased bleeding risks. Additionally, reduced healthcare reimbursement to U.S. hospitals may allow a generic tPA to compete against TNK-tPA or activase.

### **Process:**

Produce recombinant tPA using CHO cells. Since Genentech will not license their CHO cell, your group will be responsible for cloning the human tPA gene and creating a stably expressing cell line for your process.

### **Constraints:**

1. The product must be sold as lyophilized, sterile powder (100 mg/bottle).
2. The product must be free of endotoxin contamination.
3. Affinity chromatography will be necessary.
4. Your separation system will operate as a batch system.
5. Your annual production will need to range from 30 to 100 kg/yr.

### **Determine:**

1. Compare the cost of batch and CSTR (4 month per run) bioreactor operations.
2. Design reverse osmosis/deionized water purification system to supply all process water.
3. Determine the steam requirements for sterilization of the bioreactor.
4. Does an economic opportunity exist for the production of generic tPA? Assume that Genentech is your only competitor.
5. Estimate the actual production cost per 100 mg/dose for Genentech to make tPA.

Assumptions:

1. Your reactor will use serum-free growth medium.
2. You have licensed the use of a hybridoma cell line that secretes tPA monoclonal antibody for the development of your affinity columns (life of column is 3 years). The license costs \$120000/yr.



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## United States Patent [19]

Goeddel et al.

[11] Patent Number: 4,766,075

[45] Date of Patent: Aug. 23, 1988

## [54] HUMAN TISSUE PLASMINOGEN ACTIVATOR

[75] Inventors: David V. Goeddel, Hillsborough;  
William J. Kohr, San Mateo; Diane  
Peanica, Foster City; Gordon A.  
Vehar, San Carlos, all of Calif.

[73] Assignee: Genentech, Inc., South San  
Francisco, Calif.

[21] Appl. No.: 483,052

[22] Filed: Apr. 7, 1983

## Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 398,003, Jul. 14, 1982,  
and a continuation-in-part of Ser. No. 374,860, May 3,  
1982.

[51] Int. Cl.<sup>2</sup> ..... C12N 5/00; C12N 15/00;  
C12N 9/48; C12N 9/72; C12N 1/20; C12N  
1/00; C07H 17/00

[52] U.S. Cl. .... 435/240.2; 435/172.3;  
435/212; 435/215; 435/253; 435/320; 536/27;  
935/14; 935/29; 935/32; 935/70; 935/73

[58] Field of Search ..... 435/172.3, 253, 317,  
435/240, 212, 215, 320, 240.2; 536/27; 935/14,  
27, 29, 32, 72, 73, 70

## [56] References Cited

## U.S. PATENT DOCUMENTS

3,904,480 9/1975 Hull et al. .... 435/212  
4,245,051 1/1981 Reich et al. .... 435/212  
4,259,447 3/1981 Hafeli ..... 435/215  
4,314,994 2/1982 d'Hinterland et al. .... 424/95  
4,317,882 3/1982 Horiguchi et al. .... 435/212  
4,370,417 1/1983 Hung et al. .... 935/18 X  
4,505,893 3/1985 Mori et al. .... 435/241 X

## FOREIGN PATENT DOCUMENTS

0005644 11/1979 European Pat. Off. .... 435/215  
0041766 12/1981 European Pat. Off. .... 435/212  
1492959 11/1977 United Kingdom ..... 424/94.64  
1551275 8/1979 United Kingdom ..... 424/94.64  
2025977 1/1980 United Kingdom ..... 435/215  
2092154 8/1982 United Kingdom ..... 435/215

## OTHER PUBLICATIONS

*Gene Expression*, vol. 2, Lewin, B. (Ed.), pp. 148-153,  
1974.  
Ordahl, C. et al., *Proc. Natl. Acad. Sci.*, vol. 77, No. 8,  
pp. 4519-4523, 1980.  
*Molecular Cloning* (Maniatis, Fritsch & Sambrook,  
Eds.), Cold Spring Harbor Laboratory, pp. 224-228,  
1982.  
Heyneker, H. et al., *Proc. of IVth Internat'l Symposium  
on Genetics of Industrial Microorganisms* (Eds. Ikeda &  
Beppu), pp. 214-221, 1982.  
Ratzkin, B. et al., *Proc. Natl. Acad. Sci.*, vol. 78, No. 6,  
pp. 3313-3317, 1981.  
*Genetic Engineering Letter*, vol. 2, No. 7, (Fishbeyn, G.,  
Publisher), Sep. 10, 1982.  
*Biotechnology News*, vol. 3, No. 5, Mar. 1, 1983.  
*Genetic Engineering News*, p. 24, Mar. 1985.  
Sargent, T. et al., *Proc. Natl. Acad. Sci.*, vol. 78, No. 1,  
pp. 243-246, Jan. 1981.  
Opdenakker, H. et al., *Eur. J. Biochem.*, vol. 121,  
269-274, 1982.  
Ringold, G. et al., *J. Mol. Appl. Genet.*, 1(3), 165-75,  
1982; *Chem. Abst.* 96:175313p, 1982.  
O'Hare et al., *Proc. Natl. Acad. Sci.*, vol. 78(3), 1527-31,  
1981.  
Martal, J. et al., *Science*, vol. 205, 602-607, 1979.  
Rijken, D. C. et al., *J. of Biol. Chem.*, vol. 256(13), pp.  
7035-7041 (1981).  
Rijken, D. C. et al., *Biochim Biophys. Acta.*, vol. 580, pp.  
140-153 (1979).

(List continued on next page.)

Primary Examiner—Thomas G. Wiseman  
Assistant Examiner—Jayme A. Huleatt

## [57] ABSTRACT

Human tissue plasminogen activator (t-PA) is pro-  
duced in useful quantities using recombinant DNA  
techniques. The invention disclosed thus enables the  
production of t-PA free of contaminants with which it  
is ordinarily associated in its native cellular environ-  
ment. Methods, expression vehicles and various host  
cells useful in its production are also disclosed.

11 Claims, 14 Drawing Sheets

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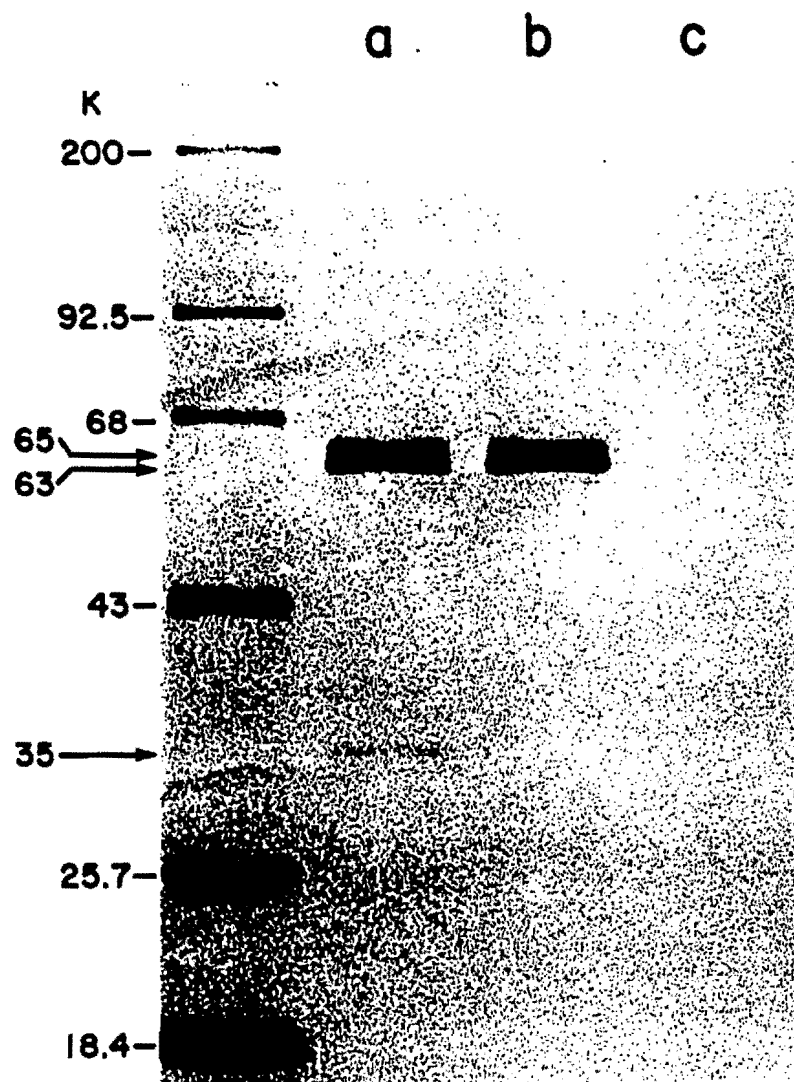


Fig.1.

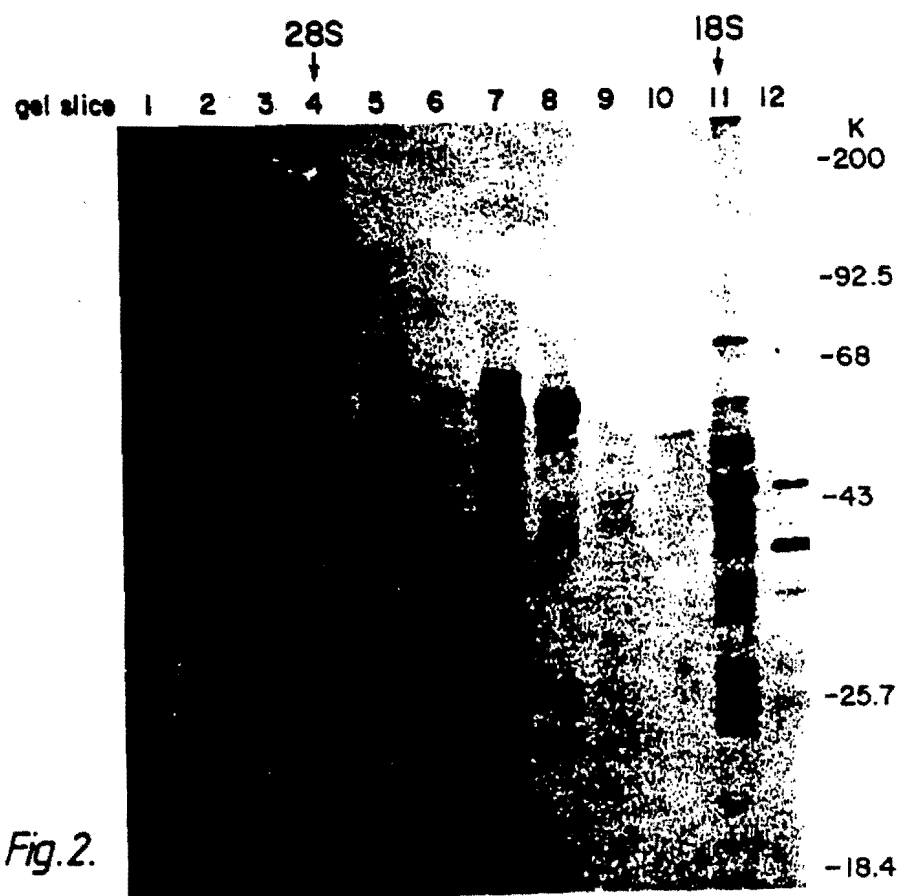
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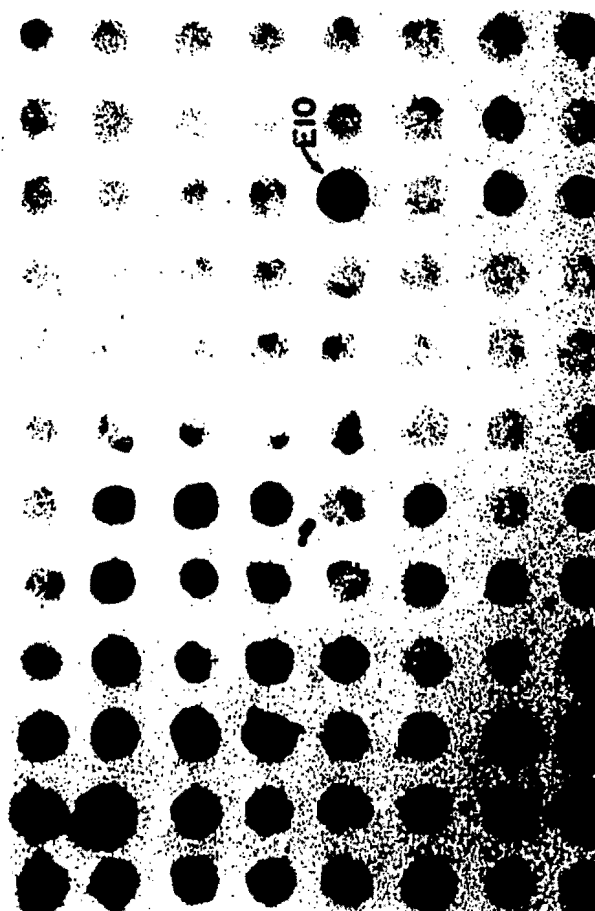
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COLONY HYBRIDIZATION

RNA Gel Slice 7 cDNA Clones

Fig. 3.

vs.

 $^{32}\text{P}$ -TC(A<sub>6</sub>)CA(A<sub>6</sub>)TA(G<sub>7</sub>)TCCCCA Probe

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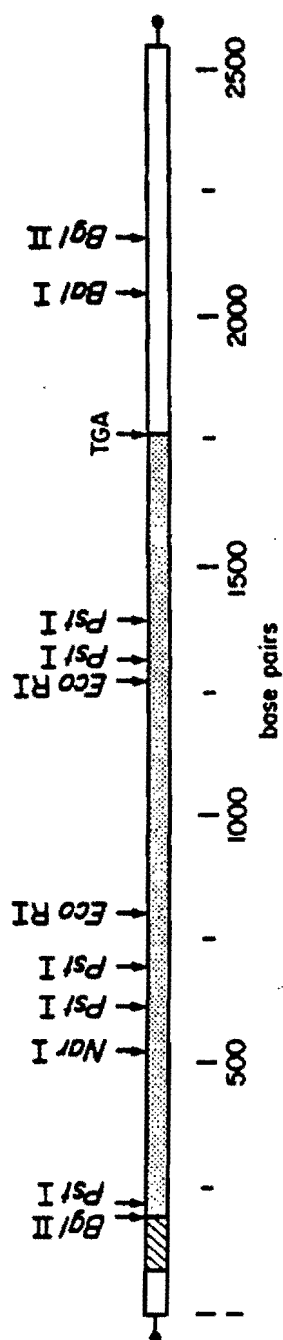


Fig. 4.

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GTTCTGAGCACAGGGCTGGAGAGAAAACCTCTGCGAGGAAAGGGAAGGAGCAAGCCGTGA

ATTTAAGGGACGCTGTGAAGCAATC      -35      met asp ala met lys arg gly leu      -30  
ATG GAT GCA ATG AAG AGA GGG CTC

cys cys val leu leu leu cys gly ala val phe val ser pro ser  
 TGC TGT GTG CTG CTG CTG TGT GGA GCA GTC TTC GTT TCG CCC AGC

gln glu ile his ala arg phe arg arg gly ala arg SER TYR GLN  
CAG GAA ATC CAT GCC CGA TTC AGA AGA GGA GCC AGA TCT TAC CAA

10  
VAL ILE CYS ARG ASP GLU LYS THR GLN MET ILE TYR GLN GLN HIS  
GTG ATC TGC AGA GAT GAA AAA ACG CAG ATG ATA TAC CAG CAA CAT

20 30  
GLN SER TRP LEU ARG PRO VAL LEU ARG SER ASN ARG VAL GLU TYR  
CAG TCA TGG CTG CGC CCT GTG CTC AGA AGC AAC CGG GTG GAA TAT

40  
CYS TRP CYS ASN SER GLY ARG ALA GLN CYS HIS SER VAL PRO VAL  
TGC TGG TGC AAC AGT GGC AGG GCA CAG TGC CAC TCA GTG CCT GTC

50 60  
LYS SER CYS SER GLU PRO ARG CYS PHE ASN GLY GLY THR CYS GLN  
AAA AGT TGC AGC GAG CCA AGG TGT TTC AAC GGG GGC ACC TGC CAG

70  
GLN ALA LEU TYR PHE SER ASP PHE VAL CYS GLN CYS PRO GLU GLY  
CAG GCC CTG TAC TTC TCA GAT TTC GTG TGC CAG TGC CCC GAA GGA

80 90  
PHE ALA GLY LYS CYS CYS GLU ILE ASP THR ARG ALA THR CYS TYR  
TTT GCT GGG AAG TGC TGT GAA ATA GAT ACC AGG GCC ACG TGC TAC

100  
GLU ASP GLN GLY ILE SER TYR ARG GLY THR TRP SER THR ALA GLU  
GAG GAC CAG GGC ATC AGC TAC AGG GGC ACG TGG AGC ACA GCG GAG

110 120  
SER GLY ALA GLU CYS THR ASN TRP ASN SER SER ALA LEU ALA GLN  
AGT GGC GCC GAG TGC ACC AAC TGG AAC AGC AGC GCG TTG GCC CAG

130  
 LYS PRO TYR SER GLY ARG ARG PRO ASP ALA ILE ARG LEU GLY LEU  
 AAG CCC TAC AGC GGG CGG AGG CCA GAC GCC ATC AGG CTG GGC CTG

140 150  
GLY ASN HIS ASN TYR CYS ARG ASN PRO ASP ARG ASP SER LYS PRO  
GGG AAC CAC AAC TAC TGC AGA AAC CCA GAT CGA GAC TCA AAG CCC

TRP CYS TYR VAL PHE LYS ALA GLY LYS TYR SER SER GLU PHE CYS  
TGG TGC TAC GTC TTT AAG GCG GGG AAG TAC AGC TCA GAG TTC TGC

170 180  
SER THR PRO ALA CYS SER GLU GLY ASN SER ASP CYS TYR PHE GLY  
AGC ACC CCT GCC TGC TCT GAG GGA AAC AGT GAC TGC TAC TTT GGG

*Fig. 5A.*

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190
ASN GLY SER ALA TYR ARG GLY THR HIS SER LEU THR GLU SER GLY
AAT GGG TCA GCC TAC CGT GGC ACG CAC AGC CTC ACC GAG TCG GGT

200
ALA SER CYS LEU PRO TRP ASN SER MET ILE LEU ILE GLY LYS VAL
GCC TCC TGC CTC CCG TGG AAT TCC ATG ATC CTG ATA GGC AAG GTT

210
TYR THR ALA GLN ASN PRO SER ALA GLN ALA LEU GLY LEU GLY LYS
TAC ACA GCA CAG AAC CCC AGT GCC CAG GCA CTG GGC CTG GGC AAA

220
HIS ASN TYR CYS ARG ASN PRO ASP GLY ASP ALA LYS PRO TRP CYS
CAT AAT TAC TGC CGG AAT CCT GAT GGG GAT GCC AAG CCC TGG TGC

230
HIS VAL LEU LYS ASN ARG ARG LEU THR TRP GLU TYR CYS ASP VAL
CAC GTG CTG AAG AAC CGC AGG CTG ACG TGG GAG TAC TGT GAT GTG

240
PRO SER CYS SER THR CYS GLY LEU ARG GLN TYR SER GLN PRO GLN
CCC TCC TGC TCC ACC TGC GGC CTG AGA CAG TAC AGC CAG CCT CAG

250
PHE ARG ILE LYS GLY GLY LEU PHE ALA ASP ILE ALA SER HIS PRO
TTT CGC ATC AAA GGA GGG CTC TTC GCC GAC ATC GCC TCC CAC CCC

260
TRP GLN ALA ALA ILE PHE ALA LYS HIS ARG ARG SER PRO GLY GLU
TGG CAG GCT GCC ATC TTT GCC AAG CAC AGG AGG TCG CCC GGA GAG

270
ARG PHE LEU CYS GLY GLY ILE LEU ILE SER SER CYS TRP ILE LEU
CGG TTC CTG TGC GGG GGC ATA CTC ATC AGC TCC TGC TGG ATT CTC

280
SER ALA ALA HIS CYS PHE GLN GLU ARG PHE PRO PRO HIS HIS LEU
TCT GCC GCC CAC TGC TTC CAG GAG AGG TTT CCG CCC CAC CAC CTG

290
THR VAL ILE LEU GLY ARG THR TYR ARG VAL VAL PRO GLY GLU GLU
ACG GTG ATC TTG GGC AGA ACA TAC CGG GTG GTC CCT GGC GAG GAG

300
GLU GLN LYS PHE GLU VAL GLU LYS TYR ILE VAL HIS LYS GLU PHE
GAG CAG AAA TTT GAA GTC GAA AAA TAC ATT GTC CAT AAG GAA TTC

310
ASP ASP ASP THR TYR ASP ASN ASP ILE ALA LEU LEU GLN LEU LYS
GAT GAT GAC ACT TAC GAC AAT GAC ATT GCG CTG CTG CAG CTG AAA

320
SER ASP SER SER ARG CYS ALA GLN GLU SER SER VAL VAL ARG THR
TCG GAT TCG TCC CGC TGT GCC CAG GAG AGC AGC GTG GTC CGC ACT

330
VAL CYS LEU PRO PRO ALA ASP LEU GLN LEU PRO ASP TRP THR GLU
GTG TGC CTT CCC CCG GCG GAC CTG CAG CTG CCG GAC TGG ACG GAG

340
CYS GLU LEU SER GLY TYR GLY LYS HIS GLU ALA LEU SER PRO PHE
TGT GAG CTC TCC GGC TAC GGC AAG CAT GAG GCC TTG TCT CCT TTC

350
360
370
380
390
400
410
420

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Fig. 5B.

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430  
TYR SER GLU ARG LEU LYS GLU ALA HIS VAL ARG LEU TYR PRO SER  
TAT TCG GAG CGG CTG AAG GAG GCT CAT GTC AGA CTG TAC CCA TCC

440 450  
SER ARG CYS THR SER GLN HIS LEU LEU ASN ARG THR VAL THR ASP  
AGC CGC TGC ACA TCA CAA CAT TTA CTT AAC AGA ACA GTC ACC GAC

460  
ASN MET LEU CYS ALA GLY ASP THR ARG SER GLY GLY PRO GLN ALA  
AAC ATG CTG TGT GCT GGA GAC ACT CGG AGC GGC GGG CCC CAG GCA

470 480  
ASN LEU HIS ASP ALA CYS GLN GLY ASP SER GLY GLY PRO LEU VAL  
AAC TTG CAC GAC GCC TGC CAG GGC GAT TCG GGA GGC CCC CTG GTG

490  
CYS LEU ASN ASP GLY ARG MET THR LEU VAL GLY ILE ILE SER TRP  
TGT CTG AAC GAT GGC CGC ATG ACT TTG GTG GGC ATC ATC AGC TGG

500 510  
GLY LEU GLY CYS GLY GLN LYS ASP VAL PRO GLY VAL TYR THR LYS  
GGC CTG GGC TGT GGA CAG AAG GAT GTC CCG GGT GTG TAC ACC AAG

520 527  
VAL THR ASN TYR LEU ASP TRP ILE ARG ASP ASN MET ARG PRO OP  
GTT ACC AAC TAC CTA GAC TGG ATT CGT GAC AAC ATG CGA CCG TGA

CCAGGAACACCCGACTCCTCAAAGCAAATGAGATCCCGCCTCTTCTTCTCAGAAGACA  
CTGCAAAGGCGCAGTGCTTCTCTACAGACTTCTCCAGACCCACCACCCGAGAAGCGGG  
ACGAGACCCCTACAGGAGAGGGAAGAGTGCATTTTCCAGATACTTCCCATTTTGGAAGT  
TTTCAGGACTTGGTCTGATTTTCAGGATACTCTGTGAGATGGGAAGACATGAATGCACACT  
AGCCTCTCCAGGAATGCCTCCTCCTGGGCAGAAAGTGGCCATGCCACCCTGTTTTTCAGCTA  
AAGCCCAACCTCCTGACCTGTCAACCGTGAGCAGCTTTGGAAACAGGACCACAAAAATGAA  
AGCATGTCTCAATAGTAAAAGATAACAAGATCTTTTCAGGAAGACGGATTGCATTAGAA  
ATAGACAGTATATTTATAGTCACAAGAGCCCAGCAGGGCCTCAAAGTTGGGGCAGGCTGGC  
TGGCCCGTCATGTTCTCCTCAAAGCACCTTGACGTCAAGTCTCCTTCCCCTTTCCCACT  
CCCTGGCTCTCAGAAGGTATTCTTTTGTGTACAGTGTGTAAGTGTAAATCCTTTTTCT  
TTATAAACTTTAGAGTAGCATGAGAGAATTGTATCATTTGAACAACTAGGCTTCAGCATA  
TTTATAGCAATCCATGTTAGTTTTTACTTTCTGTTGCCACAACCTGTTTTTATACTGTA  
CTTAATAAATTCAGATATATTTTTCACAGTTTTTCCAAAAAAAAAAAAA

Fig. 5C.

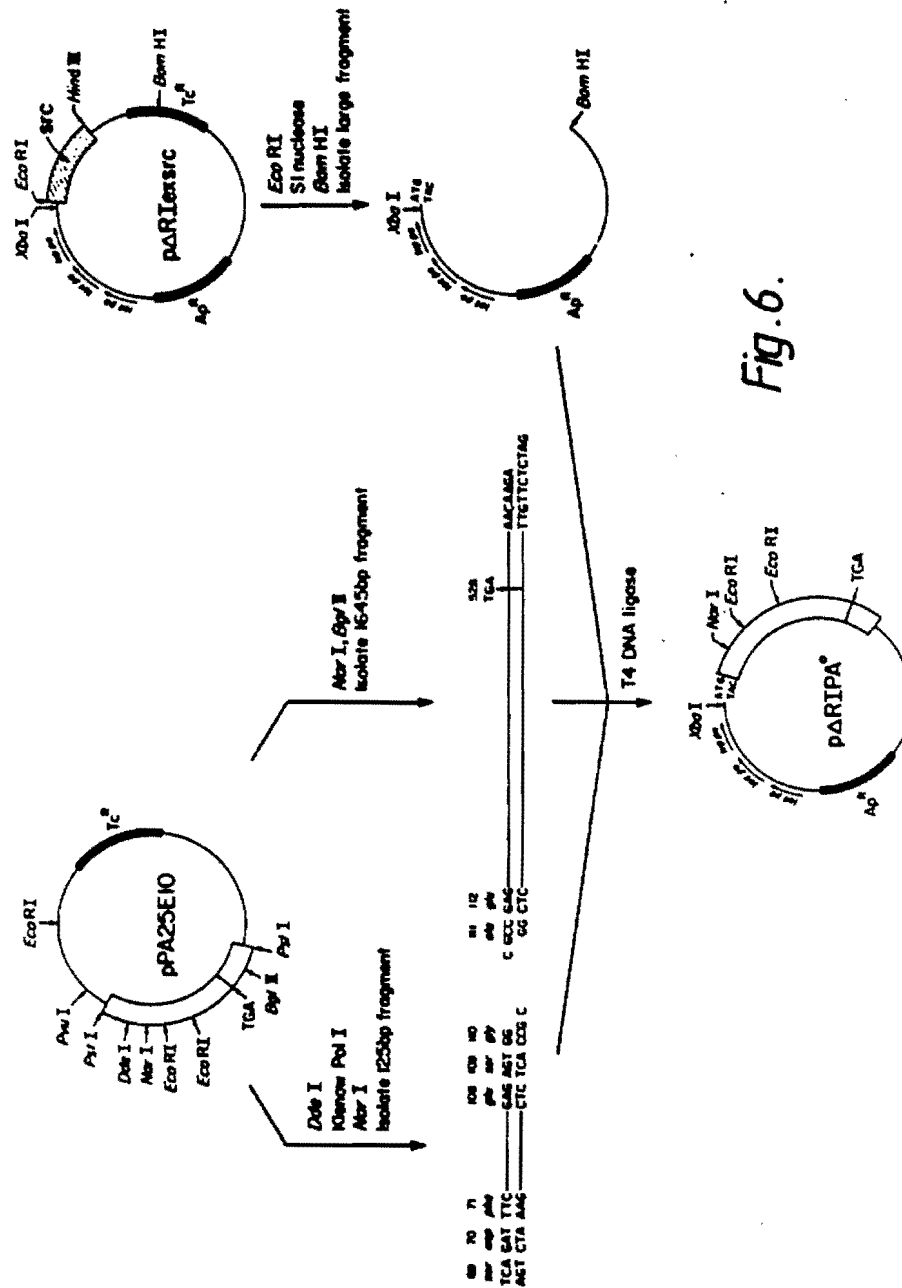
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Fig. 7.

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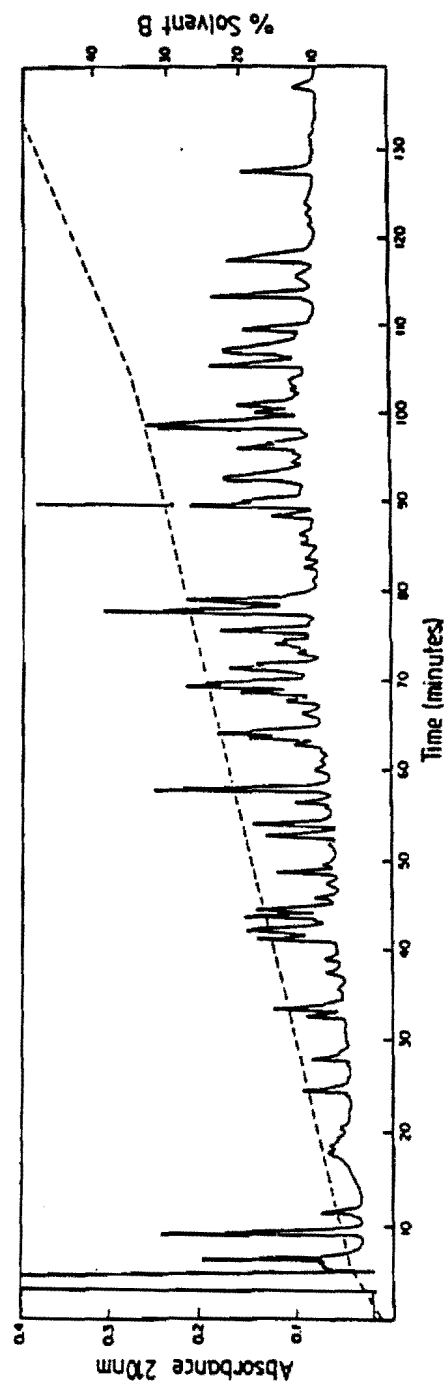


Fig. 8.



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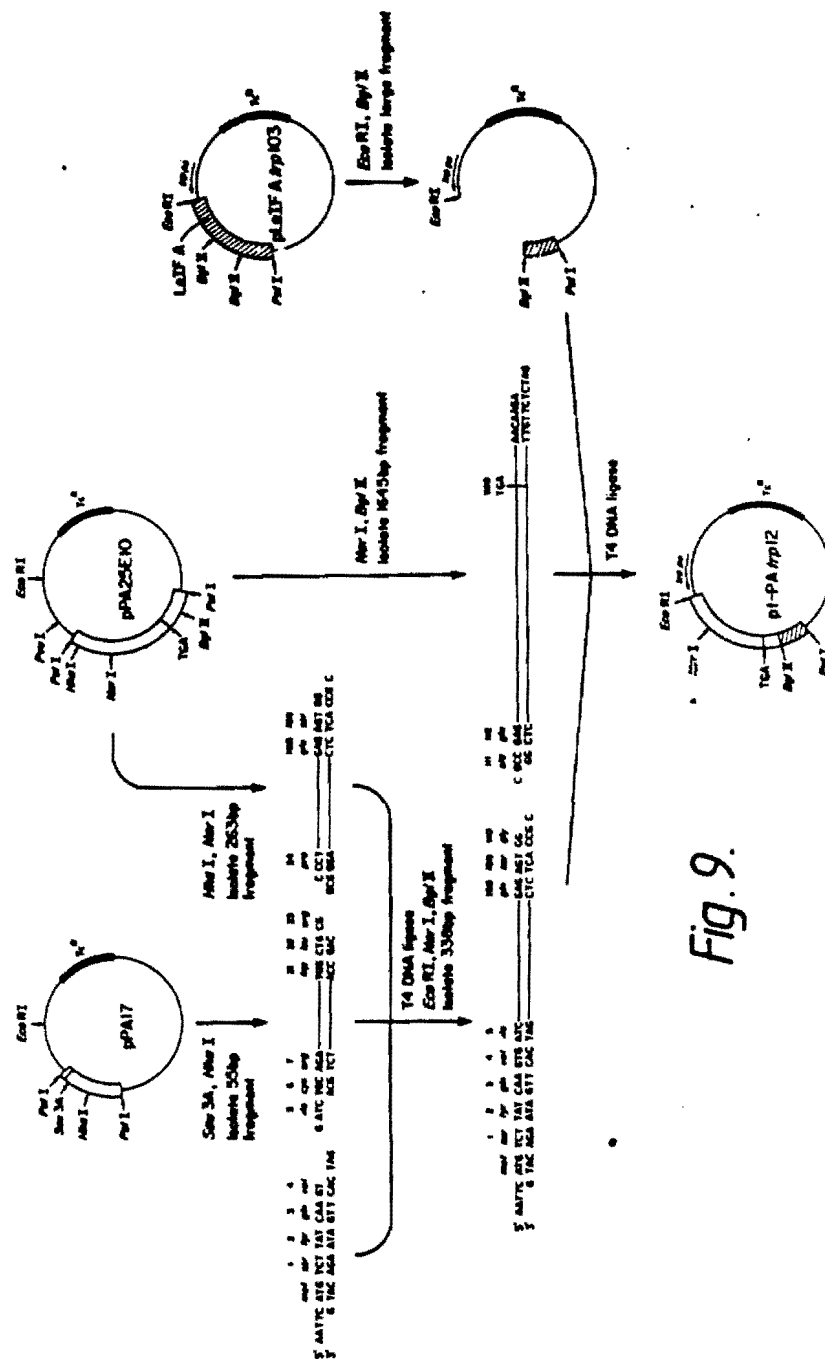


Fig. 9.

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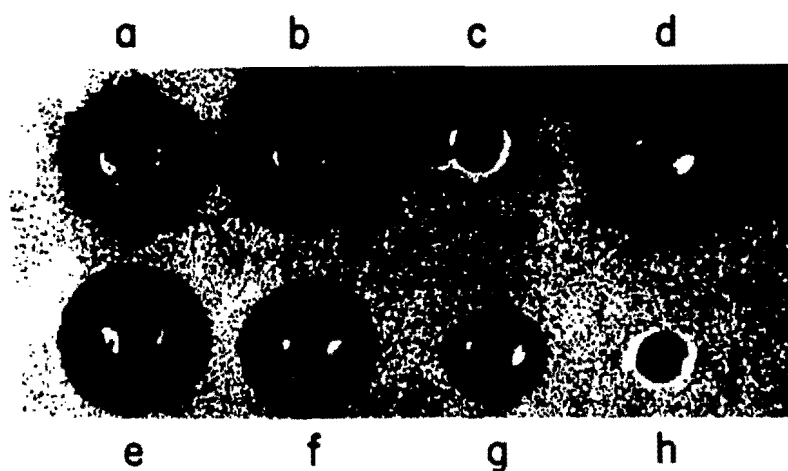


Fig. 10.

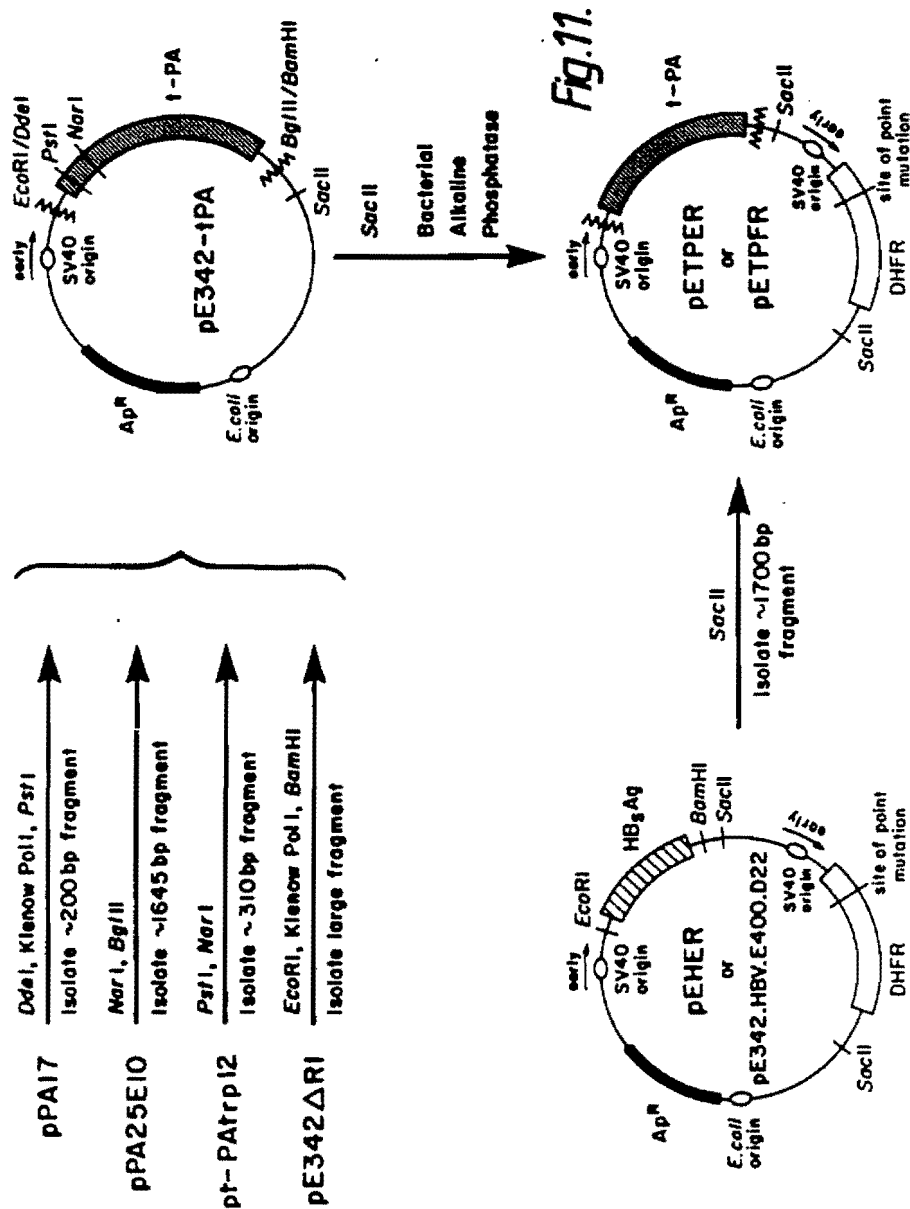
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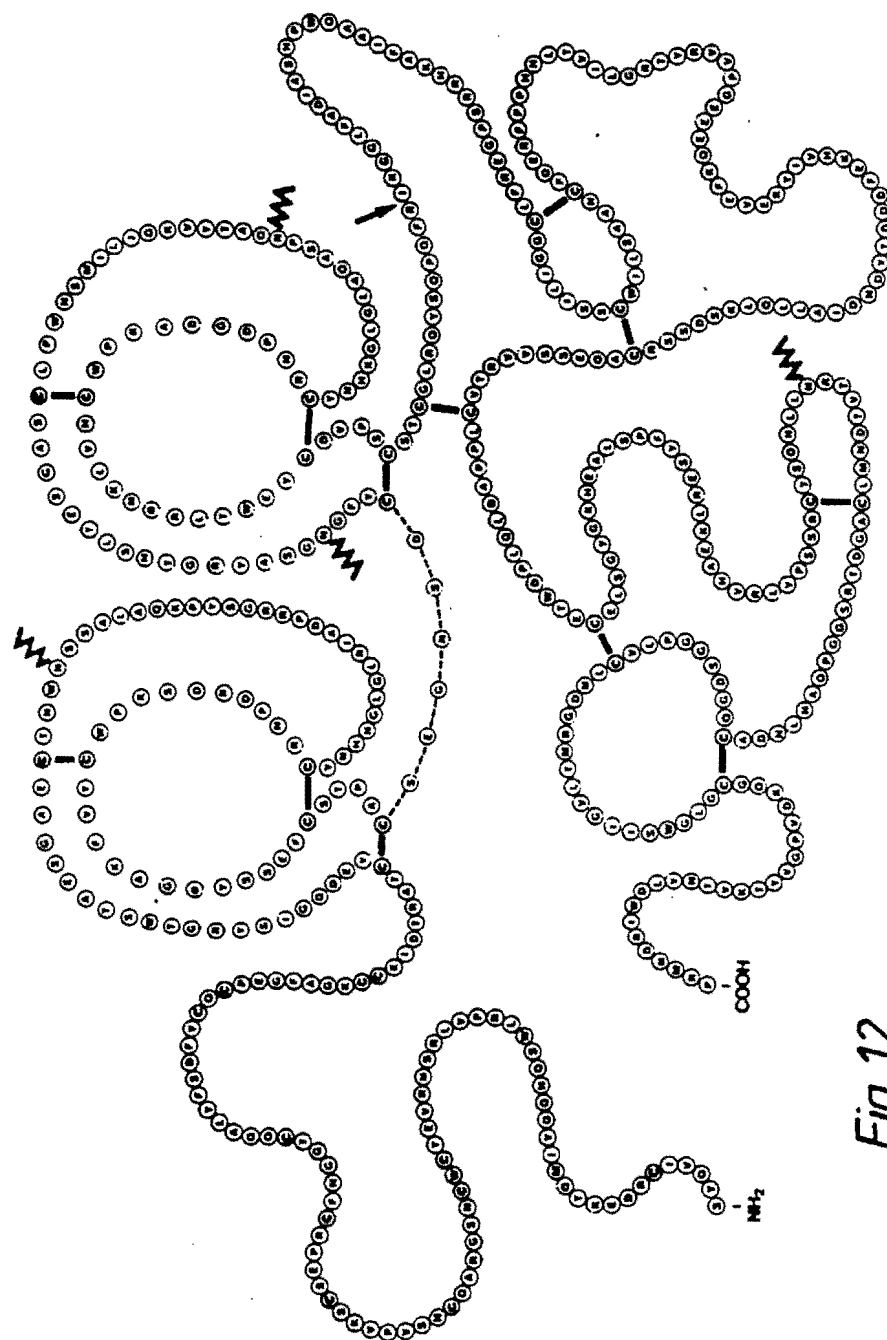


Fig. 12.

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## HUMAN TISSUE PLASMINOGEN ACTIVATOR

This is a continuation-in-part of applications Ser. No. 398,003 filed July 14, 1982 and Ser. No. 374,860, filed May 5, 1982.

## FIELD OF THE INVENTION

The present invention relates to human plasminogen activator, corresponding to that found in human serum and/or tissues, and to novel forms and compositions thereof and particularly to the means and methods for its production to homogeneity in therapeutically significant quantities.

The present invention arises in part from the discovery of the DNA sequence and deduced amino acid sequence of human plasminogen activator. This discovery enabled the production of human plasminogen activator via the application of recombinant DNA technology, in turn, enabling the production of sufficient quality and quantity of material to initiate and conduct animal and clinical testing as prerequisites to market approval, unimpeded by the restrictions necessarily inherent in the isolation methods hitherto employed involving production and extraction from existing cell culture. This invention is directed to these associated embodiments in all respects.

The publications and other materials hereof used to illuminate the background of the invention, and in particular cases, to provide additional details concerning its practice are incorporated herein by reference, and for convenience, are numerically referenced in the following text and respectively grouped in the appended bibliography.

## BACKGROUND OF THE INVENTION

## A. Human Tissue Plasminogen Activator

The fibrinolytic system is in a dynamic equilibrium with the coagulation system, maintaining an intact, patent vascular bed. The coagulation system deposits fibrin as a matrix serving to restore a hemostatic condition. The fibrinolytic system removes the fibrin network after the hemostatic condition is achieved. The fibrinolytic process is brought about by the proteolytic enzyme plasmin that is generated from a plasma protein precursor plasminogen. Plasminogen is converted to plasmin through activation by an activator.

Currently, two activators are commercially available, streptokinase and urokinase. Both are indicated for the treatment of acute vascular diseases such as myocardial infarct, stroke, pulmonary embolism, deep vein thrombosis, peripheral arterial occlusion and other venous thromboses. Collectively, these diseases account for major health hazards and risks.

The underlying etiological basis for these diseases points to either a partial, or in severe cases, total occlusion of a blood vessel by a blood clot—thrombus or thromboembolus. Traditional anticoagulant therapy, as with heparin and coumarin, does nothing to directly enhance dissolution of thrombi or thromboemboli. The thrombolytic agents referred to earlier, streptokinase and urokinase, have enjoyed practical and effective use. However, each has severe limitations. Neither has a high affinity for fibrin; consequently, both activate circulating and fibrin-bound plasminogen relatively indiscriminately. The plasmin formed in circulating blood is neutralized rather quickly and lost for useful thrombolysis. Residual plasmin will degrade several clotting

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factor proteins, for example, fibrinogen, Factor V and Factor VIII, causing a hemorrhagic potential. In addition, streptokinase is strongly antigenic and patients with high antibody titers respond inefficiently to treatment and cannot remain on continuous treatment. Urokinase therapy is expensive, owing to its involved isolation from human urine or tissue culture, and it, therefore, is not generally accepted in clinical practice. Urokinase has been the subject of numerous investigations—See, for example, references 1-6.

So-called plasminogen activators have been isolated from various human tissue, e.g., uterine tissue, blood, serum—see generally references 7-11 and from cell culture (reference 94). Compositions thereof have also been described—see references 12, 13. See also references 14-18. The plasminogen activators derived from these sources have been classified into two major groups: urokinase-type plasminogen activators (u-PA) and tissue-type plasminogen activators (t-PA) based on differences in their immunological properties. (The abbreviations t-PA and u-PA are those proposed at the XXVIII Meeting of the International Committee on Thrombosis and Hemostasis, Bergamo, Italy, July 27, 1982.)

Recently, a human melanoma line has been identified which secretes t-PA. Characterization of this melanoma plasminogen activator has shown it to be indistinguishable both immunologically and in amino acid composition from the plasminogen activator isolated from normal human tissue (Reference 19, 88).

The product was isolated in relatively pure form, characterized and found to be a highly active fibrinolytic agent (20).

Several studies (e.g. References 95 to 98) which used t-PA purified from the melanoma cell line have demonstrated its higher affinity for fibrin, compared with urokinase type plasminogen activators. More intensive investigation of human t-PA as a potential thrombolytic agent has, however, been hampered by its extremely low concentration in blood, tissue extracts, vessel perfusates and cell cultures.

It was perceived that the application of recombinant DNA and associated technologies would be a most effective way of providing the requisite large quantities of high quality human tissue-type plasminogen activator (earlier referred to as human plasminogen activator), essentially free of other human protein. Such materials would probably exhibit bioactivity admitting of their use clinically in the treatment of various cardiovascular conditions or diseases.

## B. Recombinant DNA Technology

Recombinant DNA technology has reached the age of some sophistication. Molecular biologists are able to recombine various DNA sequences with some facility, creating new DNA entities capable of producing copious amounts of exogenous protein product in transformed microbes and cell cultures. The general means and methods are in hand for the in vitro ligation of various blunt ended or "sticky" ended fragments of DNA, producing potent expression vehicles useful in transforming particular organisms, thus directing their efficient synthesis of desired exogenous product. However, on an individual product basis, the pathway remains somewhat tortuous and the science has not advanced to a stage where regular predictions of success can be made. Indeed, those who portend successful results without the underlying experimental basis, do so with considerable risk of inoperability.

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DNA recombination of the essential elements, i.e., an origin of replication, one or more phenotypic selection characteristics, an expression promoter, heterologous gene insert and remaining vector, generally is performed outside the host cell. The resulting recombinant replicable expression vehicle, or plasmid, is introduced into cells by transformation and large quantities of the recombinant vehicle obtained by growing the transformant. Where the gene is properly inserted with reference to portions which govern the transcription and translation of the encoded DNA message, the resulting expression vehicle is useful to actually produce the polypeptide sequence for which the inserted gene codes, a process referred to as expression. The resulting product may be obtained by lysing, if necessary, the host cell, in microbial systems, and recovering the product by appropriate purification from other proteins.

In practice, through the use of recombinant DNA technology, one can express entirely heterologous polypeptides—so-called direct expression—or alternatively may express a heterologous polypeptide fused to a portion of the amino acid sequence of a homologous polypeptide. In the latter cases, the intended bioactive product is sometimes rendered bioinactive within the fused, homologous/heterologous polypeptide until it is cleaved in an extracellular environment. See references (21) and (22).

Similarly, the art of cell or tissue cultures for studying genetics and cell physiology is well established. Means and methods are in hand for maintaining permanent cell lines, prepared by successive serial transfers from isolate normal cells. For use in research, such cell lines are maintained on a solid support: in liquid medium, or by growth in suspension containing support nutrimenta. Scale-up for large preparations seems to pose only mechanical problems. For further background, attention is directed to references (23) and (24).

Likewise, protein biochemistry is a useful, indeed necessary, adjunct in biotechnology. Cells producing the desired protein also produce hundreds of other proteins, endogenous products of the cell's metabolism. These contaminating proteins, as well as other compounds, if not removed from the desired protein, could prove toxic if administered to an animal or human in the course of therapeutic treatment with desired protein. Hence, the techniques of protein biochemistry come to bear, allowing the design of separation procedures suitable for the particular system under consideration and providing a homogeneous product safe for intended use. Protein biochemistry also proves the identity of the desired product, characterizing it and ensuring that the cells have produced it faithfully with no alterations or mutations. This branch of science is also involved in the design of bioassays, stability studies and other procedures necessary to apply before successful clinical studies and marketing can take place.

#### SUMMARY OF THE INVENTION

The present invention is based upon the discovery that recombinant DNA technology can be used successfully to produce human tissue plasminogen activator (t-PA), preferably in direct form, and in amounts sufficient to initiate and conduct animal and clinical testing as prerequisites to market approval. The product human t-PA is suitable for use, in all of its forms, in the prophylactic or therapeutic treatment of human beings for various cardiovascular conditions or diseases. Accordingly, the present invention, in one important aspect, is

directed to methods of treating vascular disorders in human subjects using t-PA and to suitable pharmaceutical compositions thereof.

The present invention further comprises essentially pure human tissue plasminogen activator. The product produced by genetically engineered microorganisms or cell culture systems provides an opportunity to produce human tissue plasminogen activator in a much more efficient manner than has been possible, enabling hitherto elusive commercial exploitation. In addition, depending upon the host cell, the human tissue plasminogen activator hereof may contain associated glycosylation to a greater or lesser extent compared with native material. In any event, the t-PA will be free of the contaminants normally associated with it in its non-recombinant cellular environment.

The present invention is also directed to replicable DNA expression vehicles harboring gene sequences encoding human tissue plasminogen activator in expressible form, to microorganism strains or cell cultures transformed with them and to microbial or cell cultures of such transformed strains or cultures, capable of producing human tissue plasminogen activator. In still further aspects, the present invention is directed to various processes useful for preparing said gene sequences, DNA expression vehicles, microorganism strains and cell cultures, and specific embodiments thereof. Still further, this invention is directed to the preparation of fermentation cultures of said microorganisms and cell cultures.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a 10% sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS PAGE) of 35S-methionine labelled proteins precipitable with anti t-PA IgG secreted from melanoma cells with and without protease inhibitor.

FIG. 2 shows electrophoresis of the immunoprecipitated translation products of mRNA fractions derived from melanoma cells.

FIG. 3 shows the hybridization pattern of 96 bacterial colonies transformed with cDNA using the pool of 32P labeled 14-mer as probe prepared based on a 5 amino acid sequence of human t-PA.

FIG. 4 is a restriction endonuclease map of the full length human t-PA cDNA.

FIGS. 5a, 5b and 5c show the nucleotide sequence and deduced amino acid sequence of the full length human t-PA cDNA.

FIG. 6 is a schematic of the construction of the expression plasmid pARIPA'.

FIG. 7 shows the results of a fibrin plate assay for fibrinolytic activity of *E. coli* cells transformed with pARIPA'.

FIG. 8 is an HPLC trace of peptides from human t-PA trypsin digest.

FIG. 9 shows the construction of a plasmid coding for the direct expression of mature human t-PA in *E. coli*.

FIG. 10 shows the results of a fibrin plate assay for fibrinolytic activity of the human t-PA produced by *E. coli*.

FIG. 11 shows the construction of DHFR (mutant or wild type)/t-PA encoding plasmids suitable for transforming into mammalian tissue culture cells.

FIG. 12 is a schematic diagram of human tissue plasminogen activator as prepared by the method exemplified in E.1 herein.

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## DETAILED DESCRIPTION

## A. Definitions

As used herein, "human tissue plasminogen activator" or "human t-PA" or "t-PA" denotes human extrinsic (tissue-type) plasminogen activator, produced by microbial or cell culture systems, in bioactive forms comprising a protease portion and corresponding to those tissue plasminogen activators otherwise native to human tissue. The human tissue plasminogen activator protein produced herein has been defined by means of determined DNA gene and deductive amino acid sequencing. It will be understood that natural allelic variations exist and occur from individual to individual. These variations may be demonstrated by (an) amino acid difference(s) in the overall sequence or by deletions, substitutions, insertions, inversions or additions of (an) amino acid(s) in said sequence. In addition, the location of and degree of glycosylation will depend on the nature of the host cellular environment.

The potential exists, in the use of recombinant DNA technology, for the preparation of various human tissue plasminogen activator derivatives, variously modified by resultant single or multiple amino acid substitutions, deletions, additions or replacements, for example, by means of site directed mutagenesis of the underlying DNA. Included would be the preparation of derivatives retaining the essential kringle region and serine protease region characteristic generally of the human tissue plasminogen activator described specifically herein, but otherwise modified as described above. All such allelic variations and modifications resulting in derivatives of human tissue plasminogen activator are included within the scope of this invention, as well as other related human extrinsic (tissue-type) plasminogen activators, similar physically and biologically, so long as the essential, characteristic human tissue plasminogen activator activity remains unaffected in kind. Human tissue plasminogen activator is prepared (1) having methionine as its first amino acid (present by virtue of the ATG start signal codon insertion in front of the structural gene) or (2) where the methionine is intra- or extracellularly cleaved, having its normally first amino acid, or (3) together with either its signal polypeptide or a conjugated protein other than the conventional signal polypeptide, the signal polypeptide or conjugate being specifically cleavable in an intra- or extracellular environment (See reference 21), or (4) by direct expression in mature form without the necessity of cleaving away any extraneous, superfluous polypeptide. The latter is particularly important where a given host may not, or not efficiently, remove a signal peptide where the expression vehicle is designed to express the tissue plasminogen activator together with its signal peptide. In any event, the thus produced human t-PA, in its various forms, is recovered and purified to a level fitting it for use in the treatment of various vascular conditions or diseases.

Furthermore, t-PA has forms which include both the single chain (1-chain) protein and the 2-chain protein. The latter is proteolytically derived from the 1-chain compound. It is theorized that the 2-chain protein is associated with produced fibrin and that proteolytic conversion from 1- to 2- chain material occurs at the locus of the conversion of plasminogen to plasmin. The present invention provides for the administration of the 1-chain protein for in vivo conversion as just described or for the administration of 2-chain protein, which has

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also been shown to be active. The 2-chain protein can be prepared by in vitro proteolytic conversion after the 1-chain material is produced. A so-called "kringle" area is positioned upstream from the serine protease portion and is believed to play an important function in binding the tissue plasminogen activator hereof to a fibrin matrix; hence, the observed specific activity of the present tissue plasminogen activator toward tangible, extant thrombi. The tissue plasminogen activator hereof is produced containing the enzymatically active portion corresponding to native material and the term human tissue plasminogen activator defines products comprising such portion alone or together with additional amino acid sequences up to the full length molecule.

To summarize in the present invention, human t-PA thus has a functional definition; it is capable of catalyzing the conversion of plasminogen to plasmin, binds to fibrin, and is classified as a t-PA based on immunological properties as set forth hereinabove.

"Essentially pure form" when used to describe the state of human t-PA produced by the invention means free of protein or other materials normally associated with human t-PA when produced by non-recombinant cells, i.e. in its "native" environment.

"DHFR protein" refers to a protein which is capable of the activity associated with dihydrofolate reductase (DHFR) and which, therefore, is required to be produced by cells which are capable of survival on medium deficient in hypoxanthine, glycine, and thymidine (-HGT medium). In general, cells lacking DHFR protein are incapable of growing on this medium, cells which contain DHFR protein are successful in doing so.

"Cells sensitive to MTX" refers to cells which are incapable of growing on media which contain the DHFR inhibitor methotrexate (MTX). Thus, "cells sensitive to MTX" are cells which, unless genetically altered or otherwise supplemented, will fail to grow under ambient and medium conditions suitable for the cell type when the MTX concentration is 0.2 µg/ml or more. Some cells, such as bacteria, fail to exhibit MTX sensitivity due to their failure to permit MTX inside their cell boundaries, even though they contain DHFR which would otherwise be sensitive to this drug. In general, cells which contain, as their DHFR protein, wild type DHFR will be sensitive to methotrexate if they are permeable or capable of uptake with respect to MTX.

"Wild type DHFR" refers to dihydrofolate reductase as is ordinarily found in the particular organism in question. Wild type DHFR is generally sensitive in vitro to low concentrations of methotrexate.

"DHFR protein with low binding affinity for MTX" has a functional definition. This is a DHFR protein which, when generated within cells, will permit the growth of MTX sensitive cells in a medium containing 0.2 µg/ml or more of MTX. It is recognized that such a functional definition depends on the facility with which the organism produces the "DHFR protein with low binding affinity for MTX" as well as upon the protein itself. However, as used in the context of this invention, such a balance between these two mechanisms should not be troublesome. The invention operates with respect to conferring the capability of surviving these levels of MTX, and it is not consequential whether the ability to do so is impacted by increased expression in addition to the innate nature of the DHFR produced. A convenient DHFR protein which fits this definition is

disclosed in U.S. Appl. Ser. No. 459,151, filed Jan. 19, 1983, now abandoned, incorporated herein by reference.

"Expression vector" includes vectors which are capable of expressing DNA sequences contained therein, where such sequences are operably linked to other sequences capable of effecting their expression. It is implied, although not always explicitly stated, that these expression vectors must be replicable in the host organisms either as episomes or as an integral part of the chromosomal DNA. Clearly a lack of replicability would render them effectively inoperable. In sum, "expression vector" is given a functional definition, and any DNA sequence which is capable of effecting expression of a specified DNA code disposed therein is included in this term as it is applied to the specified sequence. In general, expression vectors of utility in recombinant DNA techniques are often in the form of "plasmids" which refer to circular double stranded DNA loops which, in their vector form are not bound to the chromosome. In the present specification, "plasmid" and "vector" are used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors which serve equivalent functions and which become known in the art subsequently hereto.

"Recombinant host cells" refers to cells which have been transformed with vectors constructed using recombinant DNA techniques. As defined herein, t-PA is produced in the amounts achieved by virtue of this transformation, rather than in such lesser amounts, or, more commonly, in such less than detectable amounts, as might be produced by the untransformed host. t-PA produced by such cells can be referred to as "recombinant t-PA".

#### B. Host Cell Cultures and Vectors

The vectors and methods disclosed herein are suitable for use in host cells over a wide range of prokaryotic and eukaryotic organisms.

In general, of course, prokaryotes are preferred for cloning of DNA sequences in constructing the vectors useful in the invention. For example, *E. coli* K12 strain 294 (ATCC No. 31446) is particularly useful. Other microbial strains which may be used include *E. coli* strains such as *E. coli* B, and *E. coli* X1776 (ATCC No. 31537). These examples are, of course, intended to be illustrative rather than limiting.

Prokaryotes may also be used for expression. The aforementioned strains, as well as *E. coli* W3110 (F<sup>-</sup>,  $\lambda^{-}$ , prototrophic, ATCC No. 27325), bacilli such as *Bacillus subtilis*, and other enterobacteriaceae such as *Salmonella typhimurium* or *Serratia marcescens*, and various pseudomonas species may be used.

In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with these hosts. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* is typically transformed using pBR322, a plasmid derived from an *E. coli* species (Bollivar, et al., *Gene* 2: 95 (1977)). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells. The pBR322 plasmid, or other microbial plasmid must also contain, or be modified to contain, promoters which can be used by the microbial organism for expression of

its own proteins. Those promoters most commonly used in recombinant DNA construction include the  $\beta$ -lactamase (penicillinase) and lactose promoter systems (Chang et al., *Nature*, 275: 617 (1978); Itakura, et al., *Science*, 198: 1056 (1977); Goeddel, et al. *Nature* 281: 544 (1979)) and a tryptophan (trp) promoter system (Goeddel, et al., *Nucleic Acids Res.*, 8: 4057 (1980); EPO Appl Publ No. 0036776). While these are the most commonly used, other microbial promoters have been discovered and utilized, and details concerning their nucleotide sequences have been published, enabling a skilled worker to ligate them functionally with plasmid vectors (Siebenlist, et al., *Cell* 20: 269 (1980)).

In addition to prokaryotes, eukaryotic microbes, such as yeast cultures may also be used. *Saccharomyces cerevisiae*, or common baker's yeast is the most commonly used among eukaryotic microorganisms, although a number of other strains are commonly available. For expression in *Saccharomyces*, the plasmid YRp7, for example, (Stinchcomb, et al., *Nature*, 282: 39 (1979); Kingsman et al., *Gene*, 7: 141 (1979); Tschemper, et al., *Gene*, 10: 157 (1980)) is commonly used. This plasmid already contains the trp1 gene which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example ATCC No. 44076 or PEP4-1 (Jones, *Genetics*, 85: 12 (1977)). The presence of the trp1 lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

Suitable promoting sequences in yeast vectors include the promoters for 3-phosphoglycerate kinase (Hitzeman, et al., *J. Biol. Chem.*, 255: 12073 (1980)) or other glycolytic enzymes (Hess, et al., *J. Adv. Enzyme Reg.*, 7: 149 (1968); Holland, et al., *Biochemistry*, 17: 4900 (1978)), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triose-phosphate isomerase, phosphoglucose isomerase, and glucokinase. In constructing suitable expression plasmids, the termination sequences associated with these genes are also ligated into the expression vector 3' of the sequence desired to be expressed to provide polyadenylation of the mRNA and termination. Other promoters, which have the additional advantage of transcription controlled by growth conditions are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, and the aforementioned glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization (Holland, *ibid.*). Any plasmid vector containing yeast-compatible promoter, origin of replication and termination sequences is suitable.

In addition to microorganisms, cultures of cells derived from multicellular organisms may also be used as hosts. In principle, any such cell culture is workable, whether from vertebrate or invertebrate culture. However interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years [*Tissue Culture*, Academic Press, Kruse and Patterson, editors (1973)]. Examples of such useful host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, and W138, BHK, COS-7 and MDCK cell lines. Expression vectors for such cells ordinarily include (if necessary) an origin of replication, a pro-



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motor located in front of the gene to be expressed, along with any necessary ribosome binding sites, RNA splice sites, polyadenylation site, and transcriptional termination sequences.

For use in mammalian cells, the control functions on the expression vectors are often provided by viral material. For example, commonly used promoters are derived from polyoma, Adenovirus 2, and most frequently Simian Virus 40 (SV40). The early and late promoters of SV40 virus are particularly useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication (Fiers, et al, *Nature*, 273: 113 (1978) incorporated herein by reference. Smaller or larger SV40 fragments may also be used, provided there is included the approximately 250 bp sequence extending from the Hind III site toward the Bgl I site located in the viral origin of replication. Further, it is also possible, and often desirable, to utilize promoter or control sequences normally associated with the desired gene sequence, provided such control sequences are compatible with the host cell systems.

An origin of replication may be provided either by construction of the vector to include an exogenous origin, such as may be derived from SV40 or other viral (e.g. Polyoma, Adeno, VSV, BPV, etc.) source, or may be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter is often sufficient.

In selecting a preferred host cell for transfection by the vectors of the invention which comprise DNA sequences encoding both t-PA and DHFR protein, it is appropriate to select the host according to the type of DHFR protein employed. If wild type DHFR protein is employed, it is preferable to select a host cell which is deficient in DHFR, thus permitting the use of the DHFR coding sequence as a marker for successful transfection in selective medium which lacks hypoxanthine, glycine, and thymidine. An appropriate host cell in this case is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub and Chasin, *Proc. Natl. Acad. Sci. (USA)* 77: 4216 (1980), incorporated herein by reference.

On the other hand, if DHFR protein with low binding affinity for MTX is used as the controlling sequence, it is not necessary to use DHFR deficient cells. Because the mutant DHFR is resistant to methotrexate, MTX containing media can be used as a means of selection provided that the host cells are themselves methotrexate sensitive. Most eukaryotic cells which are capable of absorbing MTX appear to be methotrexate sensitive. One such useful cell line is a CHO line, CHO-K1 ATCC No. CCL 61.

Examples which are set forth hereinbelow describe use of *E. coli* using the lac and trp promoter system and use of CHO cells as host cells, and expression vectors which include the SV40 origin of replication as a promoter. However, it would be well within the skill of the art to use analogous techniques to construct expression vectors for expression of desired protein sequences in alternative prokaryotic or eukaryotic host cell cultures.

Satisfactory amounts of human t-PA are produced by cell cultures, however, later refinements using a secondary coding sequence serve to enhance production levels even further. The secondary coding sequence comprises dihydrofolate reductase (DHFR) which is affected by an externally controlled parameter, such as

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methotrexate, thus permitting control of expression by control of the methotrexate (MTX) concentration.

#### C. Methods Employed

If cells without formidable cell membrane barriers are used as host cells, transfection is carried out by the calcium phosphate precipitation method as described by Graham and Van der Eb, *Virology*, 52: 456 (1973). However, other methods for introducing DNA into cells such as by nuclear injection or by protoplast fusion may also be used.

If prokaryotic cells or cells which contain substantial cell wall constructions are used, the preferred method of transfection is calcium treatment using calcium chloride as described by Cohen, F. N. et al *Proc. Natl. Acad. Sci. (USA)*, 69: 2110 (1972).

Construction of suitable vectors containing the desired coding and control sequences employ standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and religated in the form desired to form the plasmids required.

Cleavage is performed by treating with restriction enzyme (or enzymes) in suitable buffer. In general, about 1 µg plasmid or DNA fragments is used with about 1 unit of enzyme in about 20 µl of buffer solution. (Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer.) Incubation times of about 1 hour at 37° C. are workable. After incubations, protein is removed by extraction with phenol and chloroform, and the nucleic acid is recovered from the aqueous fraction by precipitation with ethanol.

If blunt ends are required, the preparation is treated for 15 minutes at 15° C. with 10 units of Polymerase I (Klenow), phenol-chloroform extracted, and ethanol precipitated.

Size separation of the cleaved fragments is performed using 6 percent polyacrylamide gel described by Goeddel, D., et al, *Nucleic Acids Res.*, 8: 4057 (1980) incorporated herein by reference.

For ligation approximately equimolar amounts of the desired components, suitably end tailored to provide correct matching are treated with about 10 units T4 DNA ligase per 0.5 µg DNA. (When cleaved vectors are used as components, it may be useful to prevent religation of the cleaved vector by pretreatment with bacterial alkaline phosphatase.)

For analysis to confirm correct sequences in plasmids constructed, the ligation mixtures are used to transform *E. coli* K12 strain 294 (ATCC 31446), and successful transformants selected by ampicillin or tetracycline resistance where appropriate. Plasmids from the transformants are prepared, analyzed by restriction and/or sequenced by the method of Messing, et al, *Nucleic Acids Res.*, 9:309 (1981) or by the method of Maxam, et al, *Methods in Enzymology*, 65:499 (1980).

Amplification of DHFR protein coding sequences is effected by growing host cell cultures in the presence of approximately 20-500,000 nM concentrations of methotrexate, a competitive inhibitor of DHFR activity. The effective range of concentration is highly dependent, of course, upon the nature of the DHFR gene, protein and the characteristics of the host. Clearly, generally defined upper and lower limits cannot be ascertained. Suitable concentrations of other folic acid analogs or other compounds which inhibit DHFR could also be used. MTX itself is, however, convenient, readily available and effective.

#### D. General Description of Preferred Embodiments

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Human tissue plasminogen activator was obtained according to the following protocol:

1. Human melanoma cells actively producing tissue plasminogen activator were cultured to confluency.
2. Cell pellets from such cell cultures were extracted in the presence of ribonuclease inhibitors to isolate all cytoplasmic RNA.
3. An oligo-dT column isolated the total messenger RNA (mRNA) in polyadenylated form. This mRNA was size fractionated using acid-urea agarose gel electrophoresis.
4. The gel fraction containing tissue plasminogen activator specific RNA was identified in the following manner: The RNA from each of the gel fractions was translated in a rabbit reticulocyte lysate in vitro system supplemented with dog pancreas microsomes. The resulting translation products were then immunoprecipitated with human tissue plasminogen activator specific IgG antibody.
5. The appropriate RNA (21 to 245) was converted to corresponding single stranded complementary DNA (cDNA) from which was produced double stranded cDNA. After poly-dC tailing, it was inserted into a vector, such as a plasmid bearing one or more phenotypic markers.
6. The thus prepared vectors were used to transform bacterial cells providing a cloned cDNA library. A pool of radiolabeled synthetic deoxy oligonucleotides complementary to codons for known amino acid sequences in t-PA, such as, for example the pool of 8 14-mers,



(complementary to sequences coding for the known—see infra—amino acid sequence: tryptophan-glutamic acid-tyrosine-cysteine -aspartic acid (W-E-Y-C-D) was prepared and used to probe the colony library.

7. From the positive cDNA clones plasmid DNA was isolated and sequenced.
8. The sequenced DNA encoding t-PA was then tailored in vitro for insertion into an appropriate expression vehicle which was used to transform an appropriate host cell, which was, in turn, permitted to grow in a culture and to produce the desired human tissue plasminogen activator.
9. Human tissue plasminogen activator thus produced has ca. 251 amino acids in its enzymatic serine protease portion and a "kringle" containing sequence upstream therefrom which is presently believed to be responsible for fibrin binding. The mature protein plus its signal presequence, totals 362 amino acids.

The foregoing procedure, in itself, is successful in producing pure t-PA. Methods of the invention employing an additional coding sequence sensitive to methotrexate permit the production in host cell cultures of antigenically active t-PA protein in amounts greater than 0.1 pg per cell per day. With suitable application of amplifying conditions, amounts greater than 20 pg per cell per day can be obtained. Stated in alternate terms, gene expression levels resulting in production of more than  $9 \times 10^{-6}$  Plough units, per cell per day or, with suitable amplification, more than  $18 \times 10^{-6}$  Plough units per cell per day of t-PA activity are achieved.

Advantage is taken in this aspect of the invention of methotrexate as a drug which, while normally fatal to cells capable of its uptake, permits cells to grow in the

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presence of controlled levels of MTX by amplification of the gene coding for the DHFR coding sequence (Schimke, Robert T. et al, *Science*, 202: 1051 (1978); Biedler, J. L. et al, *Cancer Res.* 32: 153 (1972); Chang, S. E., et al, *Cell*, 7: 391 (1976)).

Of importance to this aspect of the invention is the showing that amplification of the gene for DHFR may cause amplification of associated sequences which code for other proteins. This appears to be the case when the associated protein is hepatitis B surface antigen (HBsAg) (Christman, J. et al, *Proc. Natl. Acad. Sci.*, 79: 1815 (1982)); the *E. coli* protein XGPRT (Ringold, Gordon, et al, *J. Molec. and Appl. Gen.*, 1: 165 (1981)); and an endogenous sequence from a DHFR/SV40 plasmid combination (Kaufman, R. F. et al, *J. Molec. Biol.*, 159: 601 (1982)).

Other mechanisms for conferring methotrexate resistance include diminution of the binding affinity of the DHFR protein, so that it is less susceptible to methotrexate (Flintoff, W. F. et al, *Somat. Cell Genet.*, 2: 245 (1976)) but in this instance, amplification appears to occur as well.

It would appear that the genes both for wild type DHFR and for DHFR which is resistant to MTX by virtue of its own decreased binding capacity are amplified by the presence of MTX. Hence, in principle, this aspect of the invention herein concerns using the impact of DHFR sequence amplification on associated protein coding sequences to provide a control mechanism which permits enhanced expression levels of t-PA sequences in the presence of MTX, or by virtue of prior treatment of transformed cells with MTX.

#### E. Examples

The following examples are intended to illustrate but not to limit the invention. In the examples here an *E. coli* host culture and a CHO cell line suitable for the type of DHFR protein coding sequence to be introduced were employed as host cell cultures. However, other eukaryotic and prokaryotic cells are suitable for the method of the invention as well.

#### E.1 Expression of the Human t-PA Gene in *E. coli*

##### E.1.A Figure Legends

FIG. 1 is an autoradiogram of a 10 percent SDS page displaying the immunoprecipitated [<sup>35</sup>S]-methionine labeled protein(s) secreted from human melanoma cells during a 3 hour pulse in vivo, in the presence (lane b) or absence (lane a) of the protease inhibitor aprotinin. After immunoprecipitation with tissue plasminogen activator specific IgG, three bands were observed (lane a) having molecular weights of approximately 65,000, 63,000 and 35,000. In the presence of the protease inhibitor, however, no 35,000 molecular weight species is observed. No products are immunoprecipitated when preimmune serum is used (lane c). The migrations and molecular weights of <sup>14</sup>C- labeled protein standards are shown to the left of lane a.

FIG. 2 depicts the gel electrophoresis of the immunoprecipitated translation products of RNA fractions isolated from an acid urea agarose gel. A major band was observed in fraction numbers 7 and 8 after translation in the presence of dog pancreas microsomes followed by immune precipitation with tissue plasminogen activator specific IgG. This band has a molecular weight of approximately 63,000 daltons. The size of the mRNA migrating in fractions 7 and 8 is approximately 21 to 24S. The positions of ribosomal RNA markers which were determined after electrophoresis on the RNA urea

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gel and visualized by ethidium bromide staining are labeled above the appropriate gel lanes.

FIG. 3 displays the hybridization pattern of 96 colonies with



probe. 96 individual transformants were grown in a microtiter plate, replica plated and grown on a nitrocellulose membrane. The colonies were then lysed, bacterial DNA fixed and the filters were hybridized with the  $^{32}\text{P}$ -14-mer (W-E-Y-C-D) probes. The filters were washed to remove nonhybridized probe and exposed to X-ray film. This autoradiogram is representative of the patterns obtained with 48 individual filters (4600 independent colonies). An example of a positive tissue plasminogen activator cDNA clone on filter number 25 is labelled as E10 (arrow).

FIG. 4 is a restriction endonuclease map of the full length human tissue plasminogen activator cDNA. The number and size of fragments produced by restriction endonuclease cleavage was estimated by electrophoresis through 6 percent acrylamide gels. Positions of sites were confirmed by nucleic acid sequence (presented in FIG. 5). The coding region of the largest open reading frame is boxed and the hatched region represents the putative signal peptide sequence, while the stippled region represents the putative mature tissue plasminogen activator sequence (327 amino acids). The 5' end of the mRNA is to the left while the 3' end is to the right.

FIGS. 5A, 5B, and 5C illustrate the nucleotide sequence and deduced amino acid sequence of the full length human tissue plasminogen activator cDNA. The 35 amino acids (-35 to +1) preceding the mature sequence is depicted as an uninterrupted sequence. It is believed that this 35-amino acid sequence is comprised of a hydrophilic "pro" sequence, preceding serine (+1) of the mature protein, of about 12 to 15 amino acids, in turn preceded by a "conventional" hydrophobic signal (extending 5' to -35). This type of pre-pro structure on secreted proteins has been described previously, e.g. with preproalbumin. Assuming this theory, all of the secreted tissue plasminogen activator molecules will start with the serine (+1) as the amino-terminus. A second theory is that the hydrophilic sequence could be involved with the function of tissue plasminogen activator in a manner analogous to that observed with plasminogen where a peptide of 10,000 daltons can be cleaved from the amino terminal portion of native plasminogen (Glu-plasminogen, named for the amino terminal residue), resulting in a smaller molecule, with a new amino terminus, designated Lys-plasminogen. Lys-plasminogen is more easily activated to plasmin, and also has a greater affinity for fibrin than Glu plasminogen. Plasmin has been shown to catalyze the conversion of Glu- to Lys-plasminogen. This type of control mechanism results in a "positive feedback" mechanism. The first amounts of plasmin formed, beside degrading fibrin, also result in the generation of plasminogen molecules which are more easily activated, and also bind tighter to their substrate, than native plasminogen. The result is a faster degradation of fibrin. The hydrophilic peptide of tissue plasminogen activator could be involved in a similar mechanism, its cleavage resulting in modified binding of the enzyme to fibrin. In any event the 35 amino acid sequence is considered a presequence of the mature protein.

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FIG. 6 is a schematic diagram of the construction of a tissue plasminogen activator expression plasmid pARIPA\*. The starting plasmid pPA25E10 was first digested with PstI to isolate a 376 bp. fragment that was then digested as shown in the figure.

FIG. 7 shows the result of a fibrin plate assay for fibrinolytic activity of the expression product obtained via pARIPA\* in transformed cells.

FIG. 8 is an HPLC trace of peptides from tissue plasminogen activator (hereof) trypsin digest (Absorbance at 210 nm). The arrow identifies the peak corresponding to the peptide used to design the nucleotide probe used with the colony library. The peptide represented by this peak was found to have the entire sequence: L-T-W-E Y-C D-V-P-S-C-S-T-C-G-L. The other major peaks likewise were sequenced and found to confirm the correct amino sequence of human tissue plasminogen activator. The peptide one letter code referring to amino acid designations is as follows:

Asp	D	Aspartic acid	Ile	I	Isoleucine
Thr	T	Threonine	Leu	L	Leucine
Ser	S	Serine	Tyr	Y	Tyrosine
Glu	E	Glutamic acid	Phe	F	Phenylalanine
Pro	P	Proline	His	H	Histidine
Gly	G	Glycine	Lys	K	Lysine
Ala	A	Alanine	Arg	R	Arginine
Cys	C	Cysteine	Trp	W	Tryptophan
Val	V	Valine	Gln	Q	Glutamine
Met	M	Methionine	Asn	N	Asparagine

FIG. 9 depicts the construction of a plasmid coding for the direct expression of mature human tissue plasminogen activator in *E. coli*. 50 µg of plasmid pPA17 was digested with Sau3AI, HincII and HhaI and electrophoresed on a 6 percent polyacrylamide gel. Approximately 0.5 µg of the 55 bp Sau3AI-HhaI fragment was recovered. Similarly, approximately 3 µg of the 263 bp HhaI-NarI fragment was purified from 80 µg of clone pPA25E10 by first isolating a 300 bp PstI-NarI fragment and then digesting this fragment with HhaI. All digests were performed at 37° C. for 1 hour and the reaction products resolved and electroeluted from 6 percent polyacrylamide gels. The two indicated deoxyoligonucleotides 5' - dAATTCATGTCCTTAT-CAAGT (I) and 3' - GATCACTTGATAAGACATG (II) were synthesized by the solid phase phosphotriester method (51). 100 pmole of oligonucleotide II was phosphorylated in a 30 µl reaction mixture containing 60 mM Tris (pH 8), 10 mM MgCl<sub>2</sub>, 15 mM β-mercaptoethanol and 50 µCi [ $\gamma$ - $^{32}\text{P}$ ]ATP (Amersham 5,000 Ci mmol<sup>-1</sup>) 12 units of T4 polynucleotide kinase were added and the reaction allowed to proceed at 37° C. for 15 min. One µl of 10 mM ATP and 12 units of T4 kinase were then added and the reaction allowed to proceed for an additional 30 min. After phenol/CHCl<sub>3</sub> extraction, the phosphorylated oligomer II and the 5' hydroxyl oligomer I were combined with 0.5 µg of the eluted 55 bp Sau3AI-HhaI fragment and 2 µg of the 263 bp HhaI-NarI fragment and ethanol precipitated. These fragments were ligated at room temperature for 4 hours in 60 µl of 20 mM Tris-HCl (pH 7.5), 10 mM MgCl<sub>2</sub>, 10 mM dithiothreitol, 0.5 mM ATP and 1000 units of T4 DNA ligase. The mixture was digested for 1 hour with 48 units of NarI, 20 units of EcoRI and 40 units of BglII (to eliminate polymerization through ligation of cohesive Sau3AI termini) and electrophoresed on a 6 percent gel. The 338 bp product (approximately 0.1 µg)

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was recovered by electroelution. The remainder of the t-PA coding sequences (amino acids 111-528) were isolated on a 1645 bp fragment by digesting plasmid pPA25E10 with *NarI* and *BglII*. The plasmid pLeIFAtprp103 is a derivative of the plasmid pLeIFA25 (52) in which the *EcoRI* site distal to the *LeIF A* gene has been removed (53). Three  $\mu\text{g}$  of pLeIFAtprp103 were digested with 20 units of *EcoRI* and 20 units of *BglII* for 90 min. at 37° C., electrophoresed on a 6 percent polyacrylamide gel and the large (~4,200 bp) vector fragment was recovered by electroelution. For the final construction, 80 ng of *EcoRI*-*BglII* pLeIFAtprp103 was ligated with 100 ng of the 1645 bp *NarI* *BglII* fragment and 20 ng of the 338 bp *EcoRI*-*NarI* fragment for 10 hours at room temperature. This ligation mixture was used to transform *E. coli* K-12 Strain 294. Plasmid DNA was prepared from 38 of these transformants and digested with *EcoRI*. Ten of these plasmids contained the desired 600 bp and 472 bp *EcoRI* fragments. DNA sequence analysis verified that one of these plasmids (pt-PAtrp12) had the desired nucleotide sequence at the junctions between the *trp* promoter, synthetic DNA and cDNA.

FIG. 10 shows the result of a fibrin plate assay for fibrinolytic activity of a tissue plasminogen activator expression product hereof. An overnight culture of *E. coli* W3110 (ATCC 27325) containing a t-PA expression vector in Luria broth containing 5  $\mu\text{g ml}^{-1}$  tetracycline was diluted 1:100 in M9 medium containing 0.2 percent glucose, 0.5 percent casamino acids and 5  $\mu\text{g ml}^{-1}$  tetracycline. The cells were grown at 37° C. to an  $A_{550}$  of 0.2 and indole acrylic acid was added to a final concentration of 20  $\mu\text{g/ml}$ . Samples were collected by centrifugation at  $A_{550}=0.514-0.6$  ( $\sim 2 \times 10^8$  cells  $\text{ml}^{-1}$ ) and immediately frozen. The cell pellets were suspended in 6M guanidine hydrochloride at  $5 \times 10^8$  cells/ml, sonicated for 10 sec, incubated at 24° for 30 min and then dialyzed for 4 hrs against 25 mM Tris-HCl pH 8.0, 250 mM NaCl, 0.25 mM EDTA and 0.01 percent Tween 80. After dialysis the samples were centrifuged at  $13,000 \times g$  for 2 min and 10  $\mu\text{l}$  of the supernatants analyzed for tissue plasminogen activator activity. Following the procedure of Granelli-Piperno and Reich (87), the plate was incubated for 3.5 hours at 37° C. and lysis zones measured. Plasmin produces a clear lysis zone in the fibrin plate and the area of this zone can be correlated to the amount tissue plasminogen activator in the sample.

#### E.1.B Source of Tissue Plasminogen Activator mRNA

Human melanoma cells (Bowes Melanoma Cell line, ATCC accession number CRL9607) were used. The melanoma cells were cultured to confluent monolayers in 100 ml Earles Minimal Essential Media supplemented with sodium bicarbonate (0.12 percent final concentration), 2 mM glutamine and 10 percent heat-inactivated fetal calf serum. To confirm that the melanoma cells were actively producing human tissue plasminogen activator, human melanoma cells were cultured to confluency in a 24 well microtiter dish. Either in the presence or absence of 0.33  $\mu\text{M}$  the protease inhibitor aprotinin, the cells were washed once with phosphate buffered saline and 0.3 ml of serum free methionine free medium was added. 75  $\mu\text{Ci}$  of [<sup>35</sup>S]-methionine was added and the cells were labeled at 37° for 3 hours. At the end of the 3 hour labelling period the media was removed from the cells and treated with either tissue plasminogen activator specific IgG or pre-immune serum for immunoprecipitation (54). The immuno-

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precipitated products were displayed by electrophoresis on a 10 percent SDS-acrylamide gel. The slab gel was fixed, dried and subjected to fluorography.

#### E.1.C Messenger RNA Isolation and Size Fractionation

Total RNA from melanoma cell cultures was extracted essentially as reported by Ward et al. (55). Cells were pelleted by centrifugation and then resuspended in 10 mM NaCl, 10 mM Tris-HCl pH 7.5, 1.5 mM MgCl<sub>2</sub>. Cells were lysed by the addition of NP-40 (1 percent final concentration), and nuclei were pelleted by centrifugation. The supernatant contained the total RNA which was further purified by multiple phenol and chloroform extractions. The aqueous phase was made 0.2 M in NaCl and then total RNA was precipitated by the addition of two volumes of ethanol. Oligo-dT cellulose chromatography was utilized to purify mRNA from the total RNA preparations (54). Typical yields from 10 grams of cultured melanoma cells were 5 to 10 milligrams of total RNA and 50-200 micrograms of Poly(A) plus mRNA.

Fractionation of PolyA+ mRNA (200  $\mu\text{g}$ ) (56) was performed by electrophoresis through urea-agarose gels. The slab agarose gel (57, 58) was composed of 1.75 percent agarose, 0.025 M sodium citrate, pH 3.8 and 6 M urea. Electrophoresis was performed for 7 hours at 25 milliamps and 4° C. The gel was then fractionated with a razor blade. The individual slices were melted at 70° and extracted twice with phenol and once with chloroform. Fractions were then ethanol precipitated and subsequently assayed by in vitro translation in a rabbit reticulocyte lysate system, Bethesda Research Lab. (59,60), supplemented with dog pancreas microsomes as follows: Translations were performed using 25  $\mu\text{Ci}$  of [<sup>35</sup>S] methionine and 500 nanograms of each gel slice RNA in a final volume of 30  $\mu\text{l}$  containing 25 mM HEPES, 48.3 mM potassium chloride, 10 mM creatine phosphate, 19 amino acids at 50 mM each, 1.1 mM magnesium chloride 16.6 mM EDTA, 0.16 mM dithiothreitol 8.3 mM hemin, 16.6  $\mu\text{g/ml}$  creatine kinase, 0.33 mM calcium chloride, 0.66 mM EGTA, 23.3 mM sodium chloride.

Incubations were carried out at 30° C. for 90 minutes. Dog pancreas microsomal membranes prepared from rough microsomes using EDTA for removal of the ribosomes (61) were treated with nuclease as described (62) and were present in the translation mixture at a final concentration of 7  $A_{260}$  units/ml. Translation products or immunoprecipitated translation products were analyzed by electrophoresis on 10 percent polyacrylamide gels in sodium dodecyl sulfate as previously described (63). The unstained slab gels were fixed, dried and subjected to fluorography (64).

The resulting translation products from each gel fraction were immunoprecipitated with rabbit anti-human tissue plasminogen activator specific IgG. One major immunoprecipitated polypeptide band was observed in the translation of RNA fraction numbers 7 and 8 (migration of 21 to 24 S) having a molecular weight of approximately 63,000 daltons. This band was not observed when preimmune IgG was used for immunoprecipitation which suggested these polypeptides were tissue plasminogen activator specific.

#### E.1.D Preparation of a Colony Library Containing Tissue Plasminogen Activator Sequences

Five  $\mu\text{g}$  of gel fractionated mRNA (gel slice 7 mRNA) was used for the preparation of double stranded cDNA by standard procedures (52,65,66). The cDNA was size fractionated on a 6 percent polyacry-

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amide gel. The cDNA greater than 350 base pairs in length (125 ng) was electroeluted. 30 ng of cDNA was extended with deoxy(C) residues using terminal deoxynucleotidyl transferase (67) and annealed with 300 ng of the plasmid pBR322 (68) which had been similarly tailed with deoxy(G) residues at the Pst I site (67). The annealed mixture was then transformed into *E. coli* K12 strain 294 (ATCC No. 31446). Approximately 4,600 transformants were obtained.

## E.1.E Preparation of DNA Probe

Purified human tissue plasminogen activator was obtained according to the procedure of disclosed references (19, 20).

The molecule was scanned in order to locate regions best suited for making synthetic probes, as follows:

To make the proteins susceptible to digestion by trypsin it was reduced and carboxymethylated. A 2 mg sample of tissue plasminogen activator was first dialyzed against 0.01 percent Tween 80 over night at room temperature. The lyophilized protein was then dissolved in 12 ml of 0.56 M Tris-HCl buffer (pH 8.6), 8 molar in urea and 5 mM EDTA. The disulfide bonds were reduced by the addition of 0.1 ml of  $\beta$ -mercaptoethanol. This reaction was carried out under nitrogen for 2 hours at 45° C. The reduced disulfides were alkylated to the carboxymethyl derivative by the addition of 1.0 ml of 1.4 M iodoacetic acid in 1N NaOH. After 20 min at room temperature the reaction was stopped by dialysis against 0.01 percent Tween 80 for 18 hours at room temperature and lyophilized.

The resulting lyophilized carboxymethylated protein was redissolved in 3 ml of 0.1 M sodium phosphate buffer (pH 7.5). Trypsin (TPCK) was added (1 to 50 ratio) and digested at 37° C. Aliquots (0.1 ml) were taken at 3 hours, 6 hours, and 12 hr. A second addition of trypsin was made at 12 hr. The reaction was stopped after 24 hr by freezing the sample until it could be injected on the HPLC. The progress of the digestion was determined by SDS gels on the aliquots. All gels were blank except for a faint band on the 3 hour aliquot. This indicated that the 24 hour digestion was complete and no large peptides remained.

A sample (ca. 0.5 ml) was injected into a high resolution Altex C-8 ultrasphere 5 $\mu$  column with two runs. A gradient of acetonitrile was made gradual (1 percent to 5 percent in 5 min, 5 percent to 35 percent in 100 min, 35-50 percent in 30 min). In one of the two preparative runs, the eluant was monitored at two wavelengths (210 nm and 280 nm). The ratio of the two wavelength absorptions was used to indicate the tryptophan containing peptides.

The peptide peaks most likely to contain tryptophan, or that were believed useful for other reasons, were sequenced first. This enabled the determination of the sequence around most of the tryptophans. After sequencing about 25 of the best possible peptide peaks, all the sequence data that could be aligned was pooled to obtain a preliminary model of the primary structure of tissue plasminogen activator. From this data and model, several possible probes were located.

## E.1.F Identification of Bacterial Clones Containing Tissue Plasminogen Activator cDNA Sequences

The colonies were individually inoculated into wells of microtiter plates containing LB (93)+5  $\mu$ g/ml tetracycline and stored at -20° C. after addition of DMSO to 7 percent. Two copies of the colony library were grown up on nitrocellulose filters and the DNA from

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each colony fixed to the filter by the Grunstein Hogness procedure (69).

The

<sup>32</sup>P-labelled-TCG(CA)(G)TA(C)TCCCCA

probe was prepared (from the synthetic oligomer) (W-E-Y-C-D) 14-mer pool as described above. Filters containing 4,600 transformants were prehybridized for 2 hours at room temperature in 50 mM sodium phosphate pH 6.8, 5 $\times$  SSC (80), 150  $\mu$ g/ml sonicated salmon sperm DNA, 5 $\times$  Denhardt's solution (85) 10 percent formamide and then hybridized with 50 $\times$  10<sup>6</sup> counts per minute of the labelled probe in the same solution. After an overnight incubation at room temperature, the filters were washed 3 times at room temperature in 6 $\times$  SSC, 0.1 percent SDS for 30 minutes, once in 2 $\times$  SSC and then exposed to Kodak XR-5 x-ray film with Dupont Lightning Plus intensifying screens for 16 hours.

Plasmid DNA was isolated by a rapid method (71) from all colonies showing a positive hybridization reaction. The cDNA inserts from these clones were then sequenced after subcloning fragments into the M13 vector mp 7 (73) and by the Maxam Gilbert chemical procedure (74). FIG. 3 displays filter number 25 showing the hybridization pattern of a positive tissue plasminogen activator clone. The cDNA insert in clone 25E10 was demonstrated to be the DNA coding for tissue plasminogen activator by comparing its amino acid sequence with peptide sequence (See Supra) obtained from purified tissue plasminogen activator and by its expression product produced in *E. coli* as described in more detail infra. The cDNA insert of clone 25E10 (Plasmid pPA25E10) was 2304 base pairs in length with the longest open reading frame encoding a protein of 508 amino acids (MW of 56,756) and containing a 772 bp 3' untranslated region. This cDNA clone lacked the N-terminal coding sequences.

Bacterial clone *E. coli* (pPA25E10), as well as a sample of plasmid pPA25E10, have been deposited with the American Type Culture Collection and accorded accession numbers 67587 and 40401, respectively.

E.1.G Direct Expression of a Human Tissue Plasminogen Activator Clone in *E. coli*

With reference to FIG. 6, 50  $\mu$ g of pPA25E10 (supra) were digested with Pst I and the 376 bp fragment isolated by electrophoresis on a 6 percent polyacrylamide gel. Approximately 3  $\mu$ g of this fragment was isolated from the gel by electroeluting, digested with 30 units of Dde I for 1 hr at 37° phenol and chloroform extracted, and ethanol precipitated. The resulting Dde I sticky ends were extended to blunt ends by adding 5 units of DNA polymerase I (Klenow fragment) and 0.1 mM each of dATP, dCTP, dGTP, dTTP to the reaction mixture and incubating at 4° C. for 8 hours. After extraction with phenol and chloroform, the DNA was digested with 15 units of Nar I for 2 hours and the reaction mixture electrophoresed on a 6 percent polyacrylamide gel. Approximately 0.5  $\mu$ g of the desired 125 bp blunt end Nar I fragment was recovered. This fragment codes for amino acids number 69 through 110 of the mature full length tissue plasminogen activator protein.

For isolation of the 1645 bp Nar I - Bgl II fragment, 30  $\mu$ g of pPA25E10 were digested with 30 units of Nar I and 35 units of Bgl II for 2 hours at 37° and the reac-

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tion mixture electrophoresed on a 6 percent polyacrylamide gel. Approximately 6 µg of the desired 1645 bp Nar I - Bgl II fragment were recovered.

The plasmid pSRCE16 (79) in which the Eco RI sites proximal to the trp promoter and distal to the SRC gene have been removed by repair with DNA polymerase I (28), and the self-complementary oligodeoxynucleotide AATTATGAATTCAT (synthesized by the phosphotriester method (75)) was inserted into the remaining Eco RI site immediately adjacent to the Xba I site. 20 µg of pdeltaRISRC were digested to completion with Eco RI, phenol and chloroform extracted, and ethanol precipitated. The plasmid was then digested with 100 units of nuclease S1 at 16° C. for 30 minutes in 25 mM sodium acetate (pH 4.6), 1 mM ZnCl<sub>2</sub> and 0.3M NaCl to create a blunt end with the sequence ATG. After phenol and chloroform extraction and ethanol precipitation, the DNA was digested with Bam HI, electrophoresed on a 6 percent polyacrylamide gel, and the large (4,300 bp) vector fragment recovered by electroelution.

The expression plasmid was assembled by ligating together 0.2 µg of vector, 0.06 µg of the 125 bp blunt end - Nar I fragment and 0.6 µg of the 1645 bp Nar I - Bgl II fragment with 10 units of T<sub>4</sub> DNA ligase for 7 hours at room temperature and used to transform *E. coli* strain 294 (ATCC No. 31446) to ampicillin resistance. Plasmid DNA was prepared from 26 of the colonies and digested with Xba I and Eco RI. Twelve of these plasmids contained the desired 415 bp Xba I-Eco RI and 472 bp Eco RI-fragments. DNA sequence analysis verified that several of these plasmids had an ATG initiation codon correctly placed at the start of amino acid number 69 (serine). One of these plasmids, pARIPA<sup>+</sup> was tested and produced the desired tissue plasminogen activator (FIG. 7).

Bacterial clone *E. coli* (pARIPA<sup>+</sup>), as well as a sample of plasmid pARIPA<sup>+</sup>, have been deposited with the American Type Culture Collection and accorded accession numbers 67585 and 40400, respectively. E.1.H Full Length Tissue Plasminogen Activator cDNA

#### a. Preparation of a Colony Library Containing N-terminal Tissue Plasminogen Activator Sequences

0.4 µg of the synthetic oligonucleotide 5' TTCTGAGCACAGGGCG 3' was used for priming 7.5 µg of gel fraction number 8 mRNA (supra) to prepare double stranded cDNA by standard procedures (65, 66). The cDNA was size fractionated on a 6 percent polyacrylamide gel. A size fraction greater than 300 base pairs (36 ng) was electroeluted. 5 ng cDNA was extended with deoxy(C) residues using terminal deoxycytidyl transferase (67) and annealed with 50 ng of the plasmid pBR322 (68) which had been similarly tailed with deoxy(G) residues at the Pst I site (67). The annealed mixture was then transformed into *E. coli* K12 strain 294. Approximately 1,500 transformants were obtained.

#### b. Southern Hybridization of Human Genomic DNA

Since the cDNA priming reaction had been done using a synthetic fragment that hybridized 13 base pairs from the N-terminal of clone pPA25E10, no convenient restriction fragment was available in this 29 base pair region (which includes the 16-mer sequence) for screening the cDNA clones. Therefore, it was necessary to isolate a human tissue plasminogen activator genomic clone in order to identify any primer extended cDNA

clones containing N-terminal tissue plasminogen activator coding sequences.

The first step in this process involved establishing the fact that only a single homologous tissue plasminogen activator gene is present in human genomic DNA. To determine this, a Southern hybridization was performed. In this procedure (77), 5 µg of high molecular weight human lymphocyte DNA (prepared as in 80) was digested to completion with various restriction endonucleases, electrophoresed on 1.0 percent agarose gels (81) and blotted to a nitrocellulose filter (77). A <sup>32</sup>P-labelled DNA probe was prepared (76) from the 5' end of the cDNA insert of pPA25E10 (a 230 bp Hpa II - Rsa I fragment) and hybridized (82) with the nitrocellulose filter. 35 × 10<sup>6</sup> counts per minute of the probe were hybridized for 40 hours and then washed as described (82). Two endonuclease digestion patterns provide only a single hybridizing DNA fragment: Bgl II (5.7 Kbp) and Pvu II (4.2 Kbp). Two hybridizing DNA fragments were observed with Hinc II (5.1 Kbp and 4.3 Kbp). Taken together, these data suggest the presence of only a single tissue plasminogen activator gene in the human genome, and that this gene contains at least one intervening sequence.

#### c. Screening of the Human λ Phage Library for Tissue Plasminogen Activator Genes.

The strategy used to identify λ phage recombinants carrying tissue plasminogen activator genes consisted in detecting nucleotide homology with a radioactive probe prepared from the tissue plasminogen activator cDNA 8 pPA25E10. One million recombinant λ phage were plated out on DP 50 Sup F at a density of 10,000 pfu/15 cm plate, and nitrocellulose filter replicas were prepared for each plate by the method of Benton and Davis (78). A <sup>32</sup>P-labelled DNA probe was prepared by standard procedures (83) from a 230 base pair Hpa II - Rsa I fragment located 34 base pairs from the 5' end of the plasmid pPA25E10. Each nitrocellulose filter was prehybridized at 42° for 2 hours in 50 mM sodium phosphate (pH 6.5), 5X SSC (77), 0.05 mg/ml sonicated salmon sperm DNA, 5X Denhardt's solution (84), 50 percent formamide and then hybridized with 30 × 10<sup>6</sup> counts per minute of the labelled probe in the same solution containing 10 percent sodium dextran sulfate (85). After an overnight incubation at 42° C., the filters were washed 4 times at 50° in 0.2X SSC, 0.1 percent SDS for 30 minutes, once in 2 x SSC at room temperature and then exposed to Kodak XR-5 X-ray film with Dupont Cronex intensifying screens overnight. A total of 19 clones were obtained which hybridized with the probe. Phage DNA was prepared as previously described (86) from 6 recombinants. λ Clone C was selected for preparation of a Pvu II fragment for colony screening. 30 µg of DNA was digested with Pvu II for 1 hour at 37°, and electrophoresed on 1.0 percent agarose gels. A 4.2 Kilobase pair fragment previously shown to contain tissue plasminogen activator sequences was electroeluted and purified. A <sup>32</sup>P labelled probe was prepared by standard procedures (83) for colony hybridizations as described infra.

#### d. Screening of Colony Library for 5' Tissue Plasminogen Activator Sequences.

The colonies were transferred from plates and grown on nitrocellulose filters and the DNA from each colony fixed to the filter by the Grunstein - Hogness procedure (69). A <sup>32</sup>P-labelled probe was made by calf-thymus priming (83) a 4.2 kilobase pair Pvu II fragment from an isolated tissue plasminogen activator λ genomic clone.

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Filters containing the 1,500 transformants were hybridized with  $112 \times 10^6$  cpm of  $^{32}\text{P}$ -genomic Pvu II fragment. Hybridization was for 16 hours using conditions described by Fritsch et. al (82). Filters were extensively washed and then exposed to Kodak XR-5 X-ray film with Dupont Lightning-Plus intensifying screens for 16-48 hours. Eighteen colonies clearly hybridized with the genomic probe. Plasmid DNA was isolated from each of these colonies and was bound to nitrocellulose filters and hybridized with the  $^{32}\text{P}$ -labelled synthetic oligonucleotide (16-mer) used for the original priming reaction. Of the 18 clones, seven hybridized with the kinased 16-mer. Upon sequence analysis after subcloning fragments into the m13 vector mp7 (73), one clone (pPA17) was shown to contain the correct 5' N terminal region of tissue plasminogen activator, a signal leader sequence and an 84 bp 5' untranslated region. From the two clones pPA25E10 and pPA17 the complete nucleotide sequence FIG. 3 and restriction pattern (FIG. 4) of a full length tissue plasminogen activator clone were determined.

Bacterial clone *E. coli* (pPA17), as well as a sample of plasmid pPA17, have been deposited with the American Type Culture Collection and accorded accession numbers 67586 and 40402, respectively.

The native tissue plasminogen activator molecule has the potential to be stabilized by 17 disulfide bridges based on homology with other serine proteases. There are four potential N-glycosylation sites, three located in the "kringle" regions at asn117, asn184, asn218 and one potential site in the light chain region, at asn444. Variations in the structure of the oligosaccharide ligands may be responsible for the different molecular forms (65,000 and 63,000 mol. wt. species).

#### E.1.1 Direct Expression of Full Length Tissue Plasminogen Activator cDNA Clone in *E. coli*

A reconstruction of the entire coding sequence was possible employing the common HhaI restriction endonuclease site shared by both partial clones pPA17 and pPA25E10. A 55 bp Sau3AI-HhaI restriction fragment corresponding to amino acids 5-23 was isolated from the plasmid pPA17. The Sau3AI restriction site was located at codon four of the presumed mature coding sequence and was used to remove the signal peptide coding region. A 263 bp HhaI-NarI fragment (coding for amino acids 24-110) was also isolated from plasmid pPA25E10. Two synthetic deoxyoligonucleotides were designed which restore the codons for amino acids 1-4, incorporate an ATG translational initiation codon and create an EcoRI cohesive terminus. These three fragments were then ligated together to form a 338 bp fragment coding for amino acids 1-110. This fragment and a 1645 bp NarI-BglII fragment from pPA25E10 were then ligated between the EcoRI and BglII sites of the plasmid pLelFAtpr103 (53) to give the expression plasmid pt PAtrp12. The cloned t-PA gene is transcribed under the control of a 300 bp fragment of the *E. coli* trp operon which contains the trp promoter, operator, and the Shine-Dalgarno sequence of the trp leader peptide but lacks the leader peptide ATG initiation codon (52).

A sample of plasmid pt-PAtrp12 has been deposited with the American Type Culture Collection and accorded accession number 40404.

#### E.1.J Sequence Analysis

Sequence analysis was based on the Edman degradation. (83b) The sample was introduced into the cup of the Beckman 890B or 890C spinning cup sequencer. Polybrene TM (poly N,N,N',N'-tetramethyl-N-trime-

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thylenehexamethylene diammonium diacetate) was used as a carrier in the cup. (63C) The sequencer was modified with a cold trap and some program changes to reduce background peaks. The reagents were Beckman's sequence grade 0.1 molar Quadrol buffer, phenylisothiocyanate, and heptafluorobutyric acid.

The collected Edman cycles were manually converted to 2-anilino-5-thiazolinone derivatives. The 1-chlorobutane was dried under nitrogen. Then 1.0N HCl in water was added to the 2-anilino-5-thiazolinone and heated to 70° C. for 10 min to convert it into the 3-phenyl-2-thiohydantoin (PTH derivative). The PTH-amino acid residue was then dissolved in 50 percent acetonitrile and water and injected into a reverse-phase high-pressure liquid chromatograph. Each PTH-amino acid was then identified by comparison to the retention times of a standard mixture of PTH-amino acids that was introduced into the conversion vial and treated the same way as a cycle from the sequencer.

#### E.1.K. Assays for Detection of Expression of Tissue Plasminogen Activator

##### 1. Direct Assay of Plasmin Formation

###### a. Theory

A sensitive assay for tissue plasminogen activator can be obtained by monitoring the tissue plasminogen activator catalyzed conversion of plasminogen to plasmin. Plasmin is an enzyme for which there are chromogenic substrate assays. These assays are based on the proteolytic cleavage of a tripeptide from a chromophoric group. The rate of cleavage is directly related to both the specificity and the concentration of the protease being tested. The basis of the assay is the determination of the amount of plasmin formed following incubation of the tissue plasminogen activator containing solution with a solution of plasminogen. The greater the amount of activator, the greater the amount of plasmin formed. Plasmin is measured by monitoring its cleavage of the chromogenic substrate S2251 (purchased from F&abi Group, Inc., Greenwich, Conn.).

###### b. Procedure

An aliquot of the sample is mixed with 0.10 ml of 0.7 mg/ml plasminogen (in 0.05M Tris.HCl, pH 7.4, containing, 0.012M NaCl) and the volume adjusted to 0.15 ml. The mixture is incubated at 37° C. for 10 minutes. 0.35 ml of S2251 (1.0 mM solution in above buffer) is added, and the reaction continued for 30 minutes at 37° C. Glacial acetic acid (25  $\mu\text{L}$ ) is added to terminate the reaction. The samples are centrifuged and the absorbance at 405 nm is measured. Quantitation of the amount of activity is obtained by comparison with a standard urokinase solution. Activity was recorded in Plough units, wherein 90,000 Plough units is equal to the activity exhibited by 1 mg of purified tissue plasminogen activator.

##### 2. Indirect Assay of Plasmin Formation

###### a. Theory

A sensitive assay for tissue plasminogen activator activity has been developed (87). The assay is based on determination of plasmin formation by measuring the extent of plasmin digestion of fibrin in an agar plate containing fibrin and plasminogen. Plasmin produces a clear lysis zone in the fibrin plate. The area of this lysis zone can be correlated to the amount of tissue plasminogen activator in the sample.

###### b. Procedure

Following the procedure of Granelli-Piperno and Reich (87), the plates were incubated 3.5 hours at 37° C.



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and lysis zones measured. Quantitation was obtained by comparison to a standard urokinase solution.

### E.1.L. Detection of Tissue Plasminogen Activator Activity

#### 1. Bacterial Growth and Sample Preparation.

A colony of *E. coli* containing the plasmid (pARI-PA<sup>+</sup>) was inoculated into a test tube containing 5 mL of LB growth media containing 20 µg/ml ampicillin. The cells were grown overnight at 37° C. An aliquot of this culture was diluted 1:100 into 300 ml of M9 media containing 20 µg/ml ampicillin. The cells were grown in a shaker flask at 37° C. for four hours, with a resulting absorbance at 550 nm of 0.419. The tryptophan analog indole acrylic acid was added to a concentration of 30 µg/ml. The cells were incubated 90 minutes, with a resulting absorbance at 550 nm of 0.628. The cells were harvested by centrifugation and resuspended in 0.8 ml of 0.01M Tris, pH 8.0, containing 0.01M EDTA. The resulting suspension was stirred rapidly at room temperature for 18 hours. The sample was centrifuged and the supernatant assayed for tissue plasminogen activator activity.

#### 2. Activity Detection.

Table 1 shows the results of the activation of plasminogen by *E. coli* extracts when assayed. An activity is generated which is dependent on the presence of plasminogen. This activity is not affected by pre-immune serum of a rabbit but is markedly inhibited by antiserum which was raised against purified melanoma cell derived tissue plasminogen activator (88). This demonstrates that the *E. coli* extracts are producing a plasminogen activating activity which is inhibited by antibodies against the tissue plasminogen activator.

FIG. 7 shows the result of a fibrin plate assay for fibrinolytic activity. A standard amount of urokinase was added to the center row in concentrations, from left to right, of 0.24, 0.14, 0.10, 0.05 and 0.02 Plough Units. The bottom row is samples of natural tissue plasminogen activator, with the same amount of enzyme in each well. The wells contain, from left to right, tissue plasminogen activator, anti-plasminogen activator plus pre-immune serum, and tissue plasminogen activator plus tissue plasminogen activator antibodies. The wells in the top row each contain 8 µl of the recombinant tissue plasminogen activator *E. coli* extracts. The first well is the extract alone, the second well has preimmune serum added, and the third well has the tissue plasminogen activator antibodies added. It is obvious that the pre-immune serum does not affect natural or recombinant tissue plasminogen activator, and that tissue plasminogen activator antibodies inhibit the activity of natural as well as the *E. coli* extracts. Based on the urokinase standards, the extracts contain slightly less than 2.5 Plough units per ml. This compares favorably with the value obtained in Table 1 of 1.3 Plough units per ml.

Table 1 sets forth the results of assays performed as described above in E.1.K.1.b.:

TABLE 1

Plasminogen Activation by <i>E. coli</i> Extracts of Cultures Containing pARI-PA			
Sample	A <sub>405</sub>	Percent Activity <sup>1</sup>	Calculated Plough Units/mL
Extract (with no plasminogen)	0.043	(0)	—
Extract	0.451	(100)	1.3
Extract plus preimmune serum	0.477	106	—
Extract plus	0.079	9	—

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TABLE 1-continued

Plasminogen Activation by <i>E. coli</i> Extracts of Cultures Containing pARI-PA			
Sample	A <sub>405</sub>	Percent Activity <sup>1</sup>	Calculated Plough Units/mL
anti t-PA antibodies			

<sup>1</sup>Percent activity calculated by subtracting the blank (0.043) from the values obtained and dividing by the value obtained from the extract.

FIG. 10 represents the results of a fibrin plate assay performed with extracts from 10 L fermentation cultures of *E. coli* containing a tissue plasminogen activator expressing plasmid. The fibrinolytic activity of the tissue plasminogen activator containing extract is represented in FIG. 10 by Well A. This fibrinolytic activity is inhibited by anti t-PA IgG (Well C) but not by pre-immune IgG (Well B) or anti urokinase IgG (Well D) and no activity is seen from an extract prepared from cells containing as a control the leukocyte interferon plasmid pLeIFAtpr103 (Well H).

#### E.2 Production of tPA Using DHFR Protein with a Low Binding Affinity for MTX

##### E.2.A Vector Construction

The sequence encoding human tissue plasminogen activator (t-PA) is inserted into an expression plasmid containing a mutant DHFR with low binding affinity for MTX, described in the now abandoned copending application U.S. Ser. No. 459,151, filed Jan. 19, 1983, corresponding to European Patent Application Publ. No. 117,060 incorporated herein by reference, by the following procedure (see FIG. 11):

Three fragments from overlapping t-PA plasmids, pPA25E10, and pPA17, and pt-PATrp12 (supra) were prepared as follows: Plasmid pPA17 was digested with Dde I, filled in using Klenow DNA polymerase 1, and subcut with Pst I; the approximately 200 bp fragment containing 5' terminal t-PA sequence thus generated was isolated. The second t-PA fragment was obtained by digesting pt-PATrp12 with Pst I and Nar I and isolating the approximately 310 bp fragment. The third t-PA fragment was obtained by digesting pPA25E10 with Nar I and Bgl II and isolating the approximately 1645 bp fragment which contains, in addition to much of the t-PA coding region, some 3' non-translated sequences.

Plasmid pE342 which expresses HBV surface antigen (also referred to as pHbS348-E) has been described by Levinson et al, patent application Ser. No. 326,980, filed Dec. 3, 1981, now abandoned in favor of continuing application Ser. No. 603,529, filed Apr. 24, 1984, now U.S. Pat. No. 4,741,901 issued May 31, 1988, and corresponding to European Patent Application Publ. No. 73,656, which is incorporated herein by reference. (Briefly, the origin of the Simian virus SV40 was isolated by digesting SV40 DNA with HindIII, and converting the HindIII ends to EcoRI ends by the addition of a converter (AGCTGAATTC). This DNA was cut with PvuII, and RI linkers added. Following digestion with EcoRI, the 348 base-pair fragment spanning the origin was isolated by polyacrylamide gel electrophoresis and electroelution, and cloned in pBR322. Expression plasmid pHbS348-E was constructed by cloning the 1986 base-pair fragment resulting from EcoRI and BglII digestion of HBV (*Animal Virus Genetics*, (Ch. 5) Acad. Press, N.Y. (1980)) (which spans the gene encoding HBsAg) into the plasmid pML (Lusky et al., *Nature*, 293: 79 (1981) at the EcoRI and BamHI sites. (pML is a



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derivative of pBR322 which has a deletion eliminating sequences which are inhibitory to plasmid replication in monkey cells). The resulting plasmid (pRI-Bgl) was then linearized with EcoRI, and the 348 base pair fragment representing the SV40 origin region was introduced into the EcoRI site of pRI-Bgl. The origin fragment can insert in either orientation. Since this fragment encodes both the early and late SV40 promoters in addition to the origin of replication, HBV genes could be expressed under the control of either promoter depending on this orientation (pHBS348-E representing HBs expressed under control of the early promoter). pE342 is modified by partially digesting with EcoRI, filling in the cleaved site using Klenow DNA polymerase I, and ligating the plasmid back together, thus removing the EcoRI site preceding the SV40 origin in pE342. The resulting plasmid, designated pE342ΔRI, is digested with EcoRI, filled in using Klenow DNA polymerase I, and subcut with BamHI. After electrophoresing on acrylamide gel, the approximately 3500 bp fragment is electroeluted, phenol-chloroform extracted, and ethanol precipitated as above.

The thus prepared p342E 3500 bp vector, and above described t-PA fragments comprising approximately 2160 bp were ligated together using standard techniques. A plasmid containing the three t-PA encoding fragments in the proper orientation was isolated, characterized, and designated pE342-t-PA. This plasmid was digested with SacII and treated with bacterial alkaline phosphatase (BRL). To provide the DHFR sequence (along with control sequences for its expression) an approximately 1700 bp fragment was generated by SacII digestion of pEHER. (pEHER is a plasmid expressing mutant DHFR described in U.S. Ser. No. 459,151 (supra). This fragment was ligated into the pE342-t-PA plasmid to create pETPAER400, a plasmid which is analogous to pEHER except that the HBsAg coding region has been replaced by the cDNA sequences from t-PA.

#### E.2.B Expression and Amplification of the t-PA Sequence

pETPAER400 (pETPER) was transfected into both dhfr<sup>-</sup> CHO-DUX B11 cells and DHFR<sup>+</sup> CHO-K1 (ATCC CCL61) cells by the method of Graham and Van der Eb (supra). Transformed dhfr<sup>-</sup> cells were selected by growth in glycine, hypoxanthine and thymidine deficient medium. Transformed DHFR<sup>+</sup> cells were selected by growth in  $\approx 100$  nM MTX. Colonies which arose on the appropriate selection medium were isolated using cloning rings and propagated in the same medium to several generations.

For amplification cells from the colonies are split into media containing  $5 \times 10^4$ ,  $10^5$ ,  $2.5 \times 10^5$ ,  $5 \times 10^5$ , and  $10^6$  nM MTX and passaged several times. Cells are plated at very low ( $10^2$ - $10^3$  cells/plate) cell densities in 10 cm dishes and the resulting colonies are isolated.

#### E.2' C. Assay Methods

Expression of t-PA in the transfected amplified colonies may conveniently be assayed by the methods similar to those set forth in E.1.K.1.b (supra).

Coamplification of DHFR and t-PA sequences is assayed by isolating DNA from confluent monolayers of amplified colonies as follows: Confluent monolayers in 150 mm plates are washed with 50 ml sterile PBS and lysed by the addition of 5 ml of 0.1 percent SDS, 0.4M CaCl<sub>2</sub>, 0.1M EDTA, pH 8. After 5-10 minutes, the mixture is removed, phenol extracted, chloroform extracted, and ethanol precipitated. The DNA is resus-

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pended in 1 ml (per 150 mm plate) 10 mM Tris-HCl pH 8, 1 mM EDTA (TE), RNase added to 0.1 mg/ml, and the solution incubated 30 minutes at 37°. SDS is then added to 0.1 percent and pronase (Sigma) is added to 0.5 mg/ml. After 3-16 hours incubation at 37°, the solution is again phenol extracted, chloroform extracted, and ethanol precipitated. The DNA pellet is resuspended in 0.5 ml water and digested with restriction enzymes. Approximately 5-10 µg of digested DNA is electrophoresed in an agarose gel [1 percent agarose in Tris-acetate buffer (40 mM Tris, 1 mM EDTA, made to pH 8.2 with acetic acid)]; Crouse, et al, *J. Biol. Chem.*, 257: 7887 (1982)). After bromophenol blue dye had migrated  $\frac{1}{2}$  of the way down the gel, the gel is removed and stained with ethidium bromide. After visualizing the DNA with ultraviolet light, the DNA is transferred from the gel to nitrocellulose filters according to the procedure of Southern (*J. Mol. Biol.* 98: 503. (1975)). The filters are then hybridized with a nick translated probe made from the 1700 bp SacII fragment of pEHER (prepared and hybridized as described above), or from the approximately 1970 bp BglII fragment of pETPER.

#### E.3 Production of t-PA in Conjunction with Wild Type DHFR Protein

##### E.3.A. Vector Construction

In a manner analogous to that used in the construction of pETPER, plasmid PETPFR was constructed containing the DNA sequence encoding wild type DHFR. The construction was as described in Example E.2.A. except that in place of plasmid pEHER as a source for the DHFR protein gene sequence, the plasmid pE342.HBV.E400.D22 described in copending U.S. Ser. No. 459,152, filed Jan. 19, 1983, now U.S. Pat. No. 4,713,339 issued Dec. 15, 1987, corresponding to European Patent Application Publ. No. 117,058, incorporated herein by reference, was substituted. The plasmid pE342.HBV.E400.D22 is the same as pEHER except for a single base pair difference between wild type and mutant DHFR. Thus the resulting plasmid PETPFR is analogous in every way to pETPER except that the DNA sequence encoding for wild type DHFR is substituted for that of the mutant.

A sample of plasmid PETPFR has been deposited with the American Type Culture Collection and accorded accession number 40403.

##### E.3.B Expression of t-PA sequence

PETPFR was used to transfect DHFR deficient CHO cells (Urlaub and Chasin (supra)) using the calcium phosphate precipitation method of Graham and Van der Eb. Twenty-one colonies which arose on the selective medium (-HGT) were assayed by detection of plasmin formation as assessed by the digestion of fibrin in an agar plate containing fibrin and plasminogen, described by Granelli-Piperno, et al, *J. Exp. Med.*, 148: 223 (1978).

Four of the most strongly positive clones were then assayed quantitatively for plasmin formation on a per cell basis according to the method set forth in E.1.K.1.b.

Upon such quantitative determination it was found that the four clones tested exhibited the same or comparable t-PA secretion into the medium, determined as units/cell/day. Subclones were prepared by transferring inocula from two of the clones into separate plates containing -HGT medium. Two of the resulting subclones, 18B and 1 were used for further analysis.

##### E.3.C. Amplification and t-PA Production Levels

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The above subclones were plated at  $2 \times 10^5$  cells per 100 mm plates in 50 nM MTX to promote amplification. Those cells which survived, when assayed as described above, gave, in all cases, about 10 times the unamplified amount of tissue plasminogen activator activity. Two of these clones were chosen for further study and were named 1-15 and 18B-9.

Subclone 1-15 was further amplified by seeding  $2 \times 10^5$  cells in 100 mm plates containing 500 nM MTX. Assay of the cells thus amplified yielded a further increase (of about 3 fold) in t-PA production; when assayed quantitatively by the method of C.I.C., levels were in the range of  $7 \times 10^{-6}$  units/cell/day. A portion of these amplified cells was then transferred and maintained in the presence of 10,000 nM MTX. Subclones of 1-15, and 18B-9 were further tested after being maintained for approximately 1-2 months at the conditions specified in Table 3.

TABLE 3

Cell Line	Growth Conditions	ng t-PA/cell/day*
1-15 <sub>500</sub>	500 nM MTX	$28.5 \times 10^{-3}$
1-15 <sub>500</sub>	500 nM MTX	$26.0 \times 10^{-3}$
1-15 <sub>500</sub>	(-HGT medium, no MTX)	$8.3 \times 10^{-3}$
1-15 <sub>500</sub>	(-HGT medium, no MTX)	$18.0 \times 10^{-3}$
1-15 <sub>10,000</sub>	10 $\mu$ M MTX	$29.3 \times 10^{-3}$
1-15 <sub>10,000</sub>	10 $\mu$ M MTX	$49.0 \times 10^{-3}$
18B-9	50 nM MTX	$14.3 \times 10^{-3}$
18B-9	50 nM MTX	$14.4 \times 10^{-3}$
18B-9	(-HGT medium, no MTX)	$14.3 \times 10^{-3}$
18B-9	(-HGT medium, no MTX)	$14.4 \times 10^{-3}$
1	(-HGT medium, no MTX)	$1.0 \times 10^{-3}$
1	(-HGT medium, no MTX)	$0.7 \times 10^{-3}$

\*t-PA in the culture medium was assayed quantitatively in a radioimmunoassay as follows: Purified t-PA and purified iodinated tracer t-PA derived from melanoma cells were diluted serially to include concentration of 12.5 to 400 ng/ml in a buffer containing phosphate buffered saline, pH 7.3, 0.5 percent bovine serum albumin, 0.01 percent Tween 20, and 0.02 percent NaH<sub>2</sub>PO<sub>4</sub>. Appropriate dilutions of medium samples to be assayed were added to the radioactively labelled tracer proteins. The antigens were allowed to incubate overnight at room temperature in the presence of a 1:10,000 dilution of the IgG fraction of a rabbit anti-t-PA antiserum. Antibody-antigen complex was precipitated by absorption to goat anti-rabbit IgG Immuno-beads (BioRad) for two hours at room temperature. The beads were cleared by the addition of saline diluent followed by centrifugation for ten minutes at 2000 x g at 4° Celsius. Supernatants were discarded and the radioactivity in the precipitates was monitored. Concentrations were assigned by comparison with the reference standard.

The cell lines are as follows: Cell line "1" is an unamplified clone from the original set of four. "1-15<sub>500</sub>" is an amplified subclone of cell line "1" which was amplified initially in 50 nM MTX to give 1-15 and then transferred for further amplification into 500nM MTX. 1-15<sub>10,000</sub> is subclone of 1-15<sub>500</sub> which has been further amplified the presence of 10,000 nM MTX. Cell line 18B-9 is a subclone of one of the original four detected which had been amplified on 50 nM MTX.

A sample of CHO cell line 1-15<sub>500</sub> has been deposited with the American Type Culture Collection and accorded accession number CRL 9606.

All of the amplified cells show increased levels of t-PA production over that exhibited by the unamplified cell culture. Even the unamplified culture produces amounts of t-PA greater than 0.5 pg/cell/day; amplification results in levels approaching 50 pg/cell/day.

#### F. Pharmaceutical Compositions

The compounds of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the human tissue plasminogen activator product hereof is combined in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation, inclusive of other human proteins, e.g. human serum albumin, are described for example in Remington's *Pharmaceutical Sciences* by E. W. Martin, which is

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hereby incorporated by reference. Such compositions will contain an effective amount of the protein hereof together with a suitable amount of vehicle in order to prepare pharmaceutically acceptable compositions suitable for effective administration to the host.

For example, the human tissue plasminogen activator hereof may be parenterally administered to subjects suffering from cardiovascular diseases or conditions. Dosage and dose rate may parallel that currently in use in clinical investigations of other cardiovascular, thrombolytic agents, e.g., about 440 IU/kg. body weight as an intravenous priming dose followed by a continuous intravenous infusion at about 440 IU/kg./hr. for 12 hours, in patients suffering from pulmonary embolism.

As one example of an appropriate dosage form for essentially homogeneous human tissue plasminogen activator in parenteral form applicable herein, a vial containing 25000 IU tissue plasminogen activator activity, 25 mg. mannitol and 45 mg. NaCl, may be reconstituted with 5 ml. sterile water for injection and mixed with a suitable volume of 0.9 percent Sodium Chloride Injection or 5 percent Dextrose Injection for intravenous administration.

#### G. Detailed Description of Recombinant Human t-PA

The structure of the particular embodiment of human t-PA prepared in the examples herein has been studied in some detail, both by elucidation of the gene coding sequence and by protein biochemistry techniques. The current understanding of the protein structure is illustrated in FIG. 12.

It has also previously been demonstrated by Collen and coworkers (88) that two chain human t-PA is formed by proteolytic cleavage of the single chain molecule into two polypeptides connected by disulfide bonding. The present work permits the conclusion that the heavy chain (30882 mol. wt.) is derived from the NH<sub>2</sub> terminal part and the light chain (28126 mol. wt.) comprises the COOH-terminal region. N-terminal sequencing of the two chain molecule suggests that the two chain form is generated by cleavage of a single arginyl-isoleucine bond (FIG. 12; arrow depicted).

The primary structure of a portion of the heavy chain region of human t-PA (FIG. 12) reveals a high degree of sequence homology with the "kringle" regions of plasminogen (89) and prothrombin (40, 41). "Kringle" region refers to a characteristic triple disulfide structure originally discovered in the "pro"-fragment of prothrombin, first described in detail by Magnusson et al. (91, 92). From the primary sequence of t-PA, two so-called "kringle" regions, of 82 amino acids each, that share a high degree of homology with the 5 "kringle" regions of plasminogen are apparent. The remaining N-terminal 91 amino acids share little homology to the conventional "kringle" region. One can speculate however that this region may also assume a structure containing multiple disulfide bonds as 11 additional cysteine residues are found here.

The catalytic site of the light chain of human t-PA, the so-called serine protease region, as in other serine enzymes, is most likely formed by the histidine<sub>322</sub>, aspartic<sub>371</sub> and serine<sub>471</sub> residues. Furthermore, the amino acid sequences surrounding these residues are very homologous to corresponding parts of other serine proteases such as trypsin, prothrombin and plasminogen.

Notwithstanding that reference has been made to particular preferred embodiments, it will be understood

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that the present invention is not to be construed as limited to such, rather to the lawful scope of the appended claims.

## Bibliography

1. U.S. Pat. No. 3,355,361.
2. U.S. Pat. No. 3,926,727.
3. U.S. Pat. No. 4,029,767.
4. U.S. Pat. No. 4,258,030.
5. U.S. Pat. No. 4,271,150.
6. European Patent Application Publn. No. 0037687.
7. Rijken, D. C., "Plasminogen Activator from Human Tissue," Krips Repro Meppel, 1980.
8. U.S. Pat. No. 3,555,000.
9. U.S. Pat. No. 3,998,947.
10. U.S. Pat. No. 4,245,051.
11. European Patent Application Publn. No. 0023860.
12. U.S. Pat. No. 4,083,961.
13. U.S. Pat. No. 4,177,262.
14. U.S. Pat. No. 4,082,612.
15. Wallen, P., *Proc. Sero Symp.* 9, 91 (1977).
16. Thorsen, S., et al., *Thrombos. Diathes. haemorrh.* 28, 65 (1972).
17. Collen, *Thrombos. Haemostas.* 43, 77 (1980).
18. Wiman et al., *Nature* 272, 549 (1978).
19. European Patent Application Publn. No. 0041766.
20. Weimar, W., et al., *The Lancet* Vol. II, No. 8254, p. 1018 (1981).
21. British Patent Application Publn. No. 2007676A.
22. Wetzel, *American Scientist* 68, 664 (1980).
23. *Microbiology*, 2d Ed., Harper and Row Publications, Inc., Hagerstown, Md. (1973), esp. pp. 1122 et seq.
24. *Scientific American* 245, 106 (1981).
25. British Patent Application Publn. No. 2055382A.
26. German Offenlegungsschrift No. 2644432.
27. Chang et al., *Nature* 275, 617 (1978).
28. Itakura et al., *Science* 198, 1056 (1977).
29. Goeddel et al., *Nucleic Acids Research* 8, 4057 (1980).
30. European Patent Application Publn. No. 0036776.
31. Siebenlist et al., *Cell* 20, 269 (1980).
32. Stinchcomb et al., *Nature* 282, 39 (1979).
33. Kingsman et al., *Gene* 7, 141 (1979).
34. Tachumper et al., *Gene* 10, 157 (1980).
35. Mortimer et al., *Microbiological Reviews* 44, 519 (1980).
36. Miozzari et al., *Journal of Bacteriology* 134, 48 (1978).
37. Jones, *Genetics* 85, 23 (1977).
38. Hitzeman, et al., *J. Biol. Chem.* 255, 12073 (1980).
39. Hess et al., *J. Adv. Enzyme Regul.* 7, 149 (1968).
40. Holland et al., *Biochemistry* 17, 4900 (1978).
41. Bostian et al., *Proc. Natl. Acad. Sci. (USA)* 77, 4504 (1980).
42. *The Molecular Biology of Yeast* (Aug 11-18, 1981), Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
43. Chambon, *Ann. Rev. Biochemistry*, 44, 613 (1975).
44. *Tissue Culture*, Academic Press, Kruse and Patterson eds, (1973).
45. Gluzman, *Cell* 23, 175 (1981).
46. Bolivar et al., *Gene* 2, 95 (1977).
47. Lusky et al., *Nature* 293, 79 (1981).
48. Gluzman et al., Cold Spring Harbor Symp. Quant. Biol. 44, 293 (1980).
49. Fiers et al., *Nature* 273, 113 (1978).
50. Reddy et al., *Science* 200, 494 (1978).
51. Crea et al., *Nucleic Acids Research* 8, 2331 (1980).
52. Goeddel et al., *Nature* 287, 411 (1980).
53. Gray et al., *Nature* 295, 503 (1982).
54. Oppermann et al., *Virology* 108, 47 (1981).
55. Ward et al., *J. Virol.* 9, 61 (1972).
56. Aviv et al., *Proc. Natl. Acad. Sci. (USA)* 69, 1408 (1972).
57. Lehrach et al., *Biochemistry* 16, 4743 (1977).
58. Lynch et al., *Virology* 98, 251 (1979).
59. Lodish, *Ann. Rev. of Biochem.* 45, 40 (1976).
60. Pelham et al., *Eur. J. Biochem.* 43, 247 (1974).
61. Blobel, et al., *J. Cell Biology* 67, 852 (1975).
62. Shields et al., *J. Biol. Chemistry* 253, 3753 (1978).
63. Laemmli, *Nature* 227, 680 (1970).
64. Bonner et al., *Eur. J. Biochem.* 46, 83 (1974).
65. Goeddel et al., *Nature* 281, 544 (1979).
66. Wickens et al., *J. Biol. Chem.* 253, 2483 (1978).
67. Chang et al., *Nature* 275, 617 (1978).
68. Bolivar et al., *Gene* 2, 95 (1977).
69. Grunstein et al., *Proc. Natl. Acad. Sci. U.S.A.* 72, 3961 (1975).
70. Hanahan et al., *Gene* 10, 63 (1980).
71. Birnboim et al., *Nucleic Acids Res.* 7, 1513 (1979).
72. Smith, *Methods Enzymol.* 65, 499 (1980).
73. Messing et al., *Nucleic Acids Res.* 9, 309 (1981).
74. Maxam et al., *Methods in Enzymol.* 65, 499 (1980).
75. Crea et al., *Proc. Natl. Acad. Sci.* 75, 5765 (1978).
76. Lawn et al., *Cell* 15, 1157 (1978).
77. Southern, *J. Mol. Biol.* 98, 503 (1975).
78. Benton et al., *Science* 196, 180 (1977).
79. McGrath and Levinson, *Nature* 295, 423 (1982).
80. Blin et al., *Nucleic Acid Res.* 3, 2303 (1976).
81. Lawn et al., *Science* 212, 1159 (1981).
82. Fritsch et al., *Cell* 19, 959 (1980).
83. Taylor et al., *Biochim. Biophys. Acta* 442, 324 (1976).
84. Edman et al., *European J. Biochem.* 1, 80 (1967).
85. Denhardt, *Biochem. Biophys. Res. Comm.* 23, 641 (1966).
86. Wahl et al., *Proc. Natl. Acad. Sci. (USA)* 76, 3683 (1979).
87. Davis et al., *Advanced Bacterial Genetics*, Cold Spring Harbor Laboratory, New York (1980).
88. Graneli-Piperno et al., *J. Exp. Med.* 148, 223 (1978).
89. Rijken et al., *J. Biol. Chem.* 256, 7035 (1981).
90. Sottrup-Jensen et al., *Progress in Chemical Fibrinolysis and Thrombolysis*, Vol. 3, Raven Press, N.Y. p. 191 (1978).
91. Sottrup-Jensen et al., *Proc. Natl. Acad. Sci. (USA)* 72, 2577 (1975).
92. Magnussen et al., *Proteolysis and Physiological Regulation*, Ribbons et al., Eds., Academic Press, New York, p. 203 (1976).
93. Magnussen et al., *Proteases and Biological Control*, Cold Spring Harbor Laboratory, N.Y., p. 123 (1975).
94. Miller, *Experiments in Molecular Genetics*, p. 431-3, Cold Spring Harbor Lab., Cold Spring Harbor, N.Y. (1972).
95. Reich, E., *Proteases and Biological Control*, (Ibid) p. 333-341.
96. Matsuo, O., et al., *Throm. Haemostasis* 45 225 (1981).
97. Koringe, C., et al., *Throm. Haemostasis* 46 561, 662 (1981).
98. Hoylaerts, M., et al., *J. Biol. Chem.* 257 2912 (1982).
99. Koringe, C., et al., *Thromb. Haemostasis* 46 685 (1981).

We claim:

1. A DNA isolate consisting essentially of a DNA sequence encoding human tissue plasminogen activator.

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2. The DNA isolate of claim 1 wherein said human tissue plasminogen activator has the amino acid sequence 1-527 set forth in FIGS. 5a, 5b and 5c.

3. A recombinant expression vector containing a DNA sequence encoding human tissue plasminogen activator, wherein the vector is capable of expressing human tissue plasminogen activator in a transformed microorganism or cell culture.

4. The recombinant expression vector of claim 3 wherein said human tissue plasminogen activator has the amino acid sequence 1-527 set forth in FIGS. 5a, 5b and 5c.

5. A microorganism transformed with the vector of claim 3, said microorganism being capable of expressing human tissue plasminogen activator.

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6. An *E. coli* microorganism according to claim 5.

7. The microorganism of claim 5 wherein said human tissue plasminogen activator has the amino acid sequence 1-527 set forth in FIGS. 5a, 5b and 5c.

8. A cell culture capable of expressing human tissue plasminogen activator, obtained by transforming a mammalian cell line with a vector according to claim 3.

9. A cell culture according to claim 8 wherein the cell line is a Chinese Hamster Ovary cell line.

10. The cell culture of claim 8 wherein said human tissue plasminogen activator has the amino acid sequence 1-527 set forth in FIGS. 5a, 5b and 5c.

11. The cell culture of claim 9 wherein said human tissue plasminogen activator has the amino acid sequence 1-527 of FIGS. 5a, 5b and 5c.

\* \* \* \* \*

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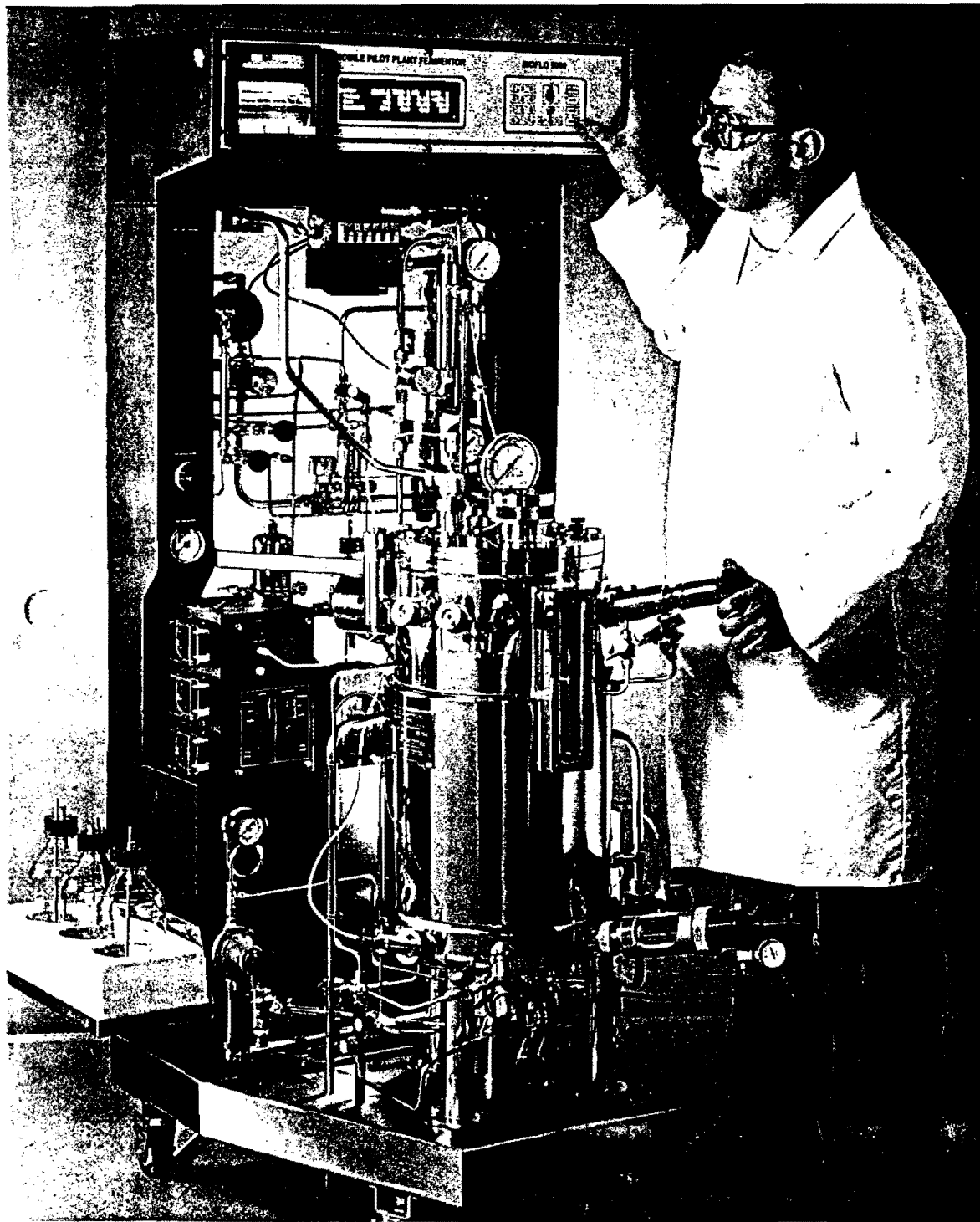
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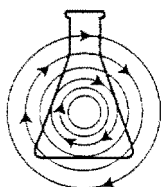


# BIOFLO<sup>®</sup> 5000

*Mobile Pilot Plant Fermentor*



- For Process Development and Small-Scale Production
- 40, 80 or 120 L Capacities
- Automatic In-Place Sterilization
- Space Efficient
- Equipped with Resterilizable Valves for Addition, Sample and Harvest
- Thermal Mass Flow Meter or Controller



**NEW BRUNSWICK SCIENTIFIC**

*Where Quality and Innovation Have Become Tradition*

# BIOFLO® 5000 PILOT PLANT FERMENTOR

New Brunswick Scientific offers the most compact and complete pilot plant fermentor in the industry. Designed for culturing both aerobic and anaerobic organisms, the versatile BioFlo 5000 fermentor is capable of process development and small-scale production of bacterial, insect, yeast, plant, fungal and algal cells. This sterilizable-in-place system is available in 40, 80 and 120 liter (total volume) capacities, yet occupies just 7.3 square feet (0.70 m<sup>2</sup>) of floor space. It comes equipped with all accessories needed for out-of-the-box start up, as well as for convenient and contamination-free operation. Automated data logging, process control and optimization are made possible with an optional software package. With its outstanding array of standard features, this proven system provides an exceptional value.

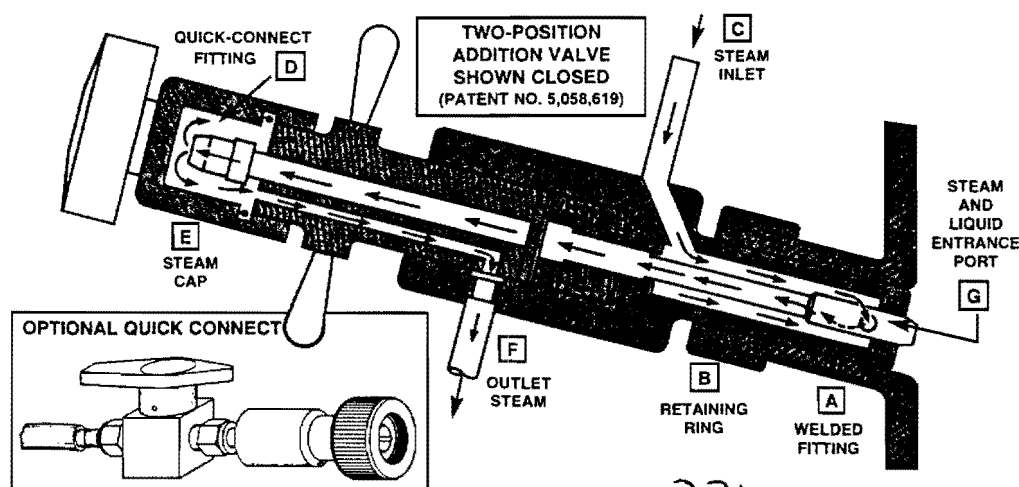
## UNIQUE ADVANTAGES

- Guaranteed high oxygen transfer rates (OTR) of 350 mM O<sub>2</sub>/L/Hr or higher enable high-density microbial culture\*. Up to 1,500 mM O<sub>2</sub>/L/Hr attainable via O<sub>2</sub> supplementation utilizing an optional NBS 2-gas mixer
- Capable of rapid temperature shifts in growth phase, to allow for heat induction (1°C/minute in range of 23°C to 45°C\*)
- Capable of remote control, data logging and process programming using optional AFS-BioCommand® software
- Smallest footprint of any pilot plant fermentor
- Most complete, fully accessorized, pilot plant fermentor
- Set-up and start-up assistance provided, with optional validation and training packages available. Process development and scale-up assistance can also be provided
- Most comprehensive warranty in the industry. One year on parts and labor
- Only NBS has an in-house ASME-accredited vessel shop, microbiology and cell culture labs, software and instrumentation development group, and integrated cGMP bioprocess area to provide added customer support

\* Optional on 120 L systems

## STANDARD FEATURES INCLUDE

1. **Easy-To-Read, 8" (20.3 cm) Vacuum Fluorescent Display** is clearly visible under all light conditions. Simultaneously displays current value, setpoint and control mode for (4) parameters
2. **Membrane Keypad** facilitates activation of setpoint entry, calibration and sterilization cycles. Watertight and easily cleanable
3. **Swing-Away Headplate** provides easy access to vessel interior
4. **Resterilizable Addition/Inoculation, Harvest and Sampling Valves** for aseptic operation are readily removable and cleanable. Straight-through flow path attains complete sterilization of the valve and transfer lines. (See diagram.) All valves utilize quick connect fittings. Sample valve provides variable flow
5. **Large Window** allows viewing the culture above and below maximum working level
6. **Sterilizable-In-Place Vessel**, ASME coded, available in 40, 80 and 120 L capacities. Type 316L jacketed stainless steel provides rapid sterilization and precise temperature; Internal finish of 15 - 20 Ra facilitates cleaning and prevents residue build-up
7. **DO, pH, Foam, Level, Temperature RTD Sensors**  
Choice of Ingold or Broadley-James pH and DO Probes. In-Line Amplifiers for pH and DO sensors, compatible with polarographic and galvanic DO probes. (pH probe and pressurizable housing shown)
8. **Mobile System with Lockable Casters** facilitates preparation, clean-up, maintenance and servicing
9. **Addition Bottles and Support Stand** for acid, base and antifoam provided for added convenience
10. **Three Peristaltic Pumps** provided for acid, base and antifoam addition
11. **Two Switched Outlets** for optional remote pumps to control nutrient and level
12. **In-line Sterilizable Air and Exhaust Filters** designed for 100% removal of viable organisms
13. **Lamp** illuminates vessel contents

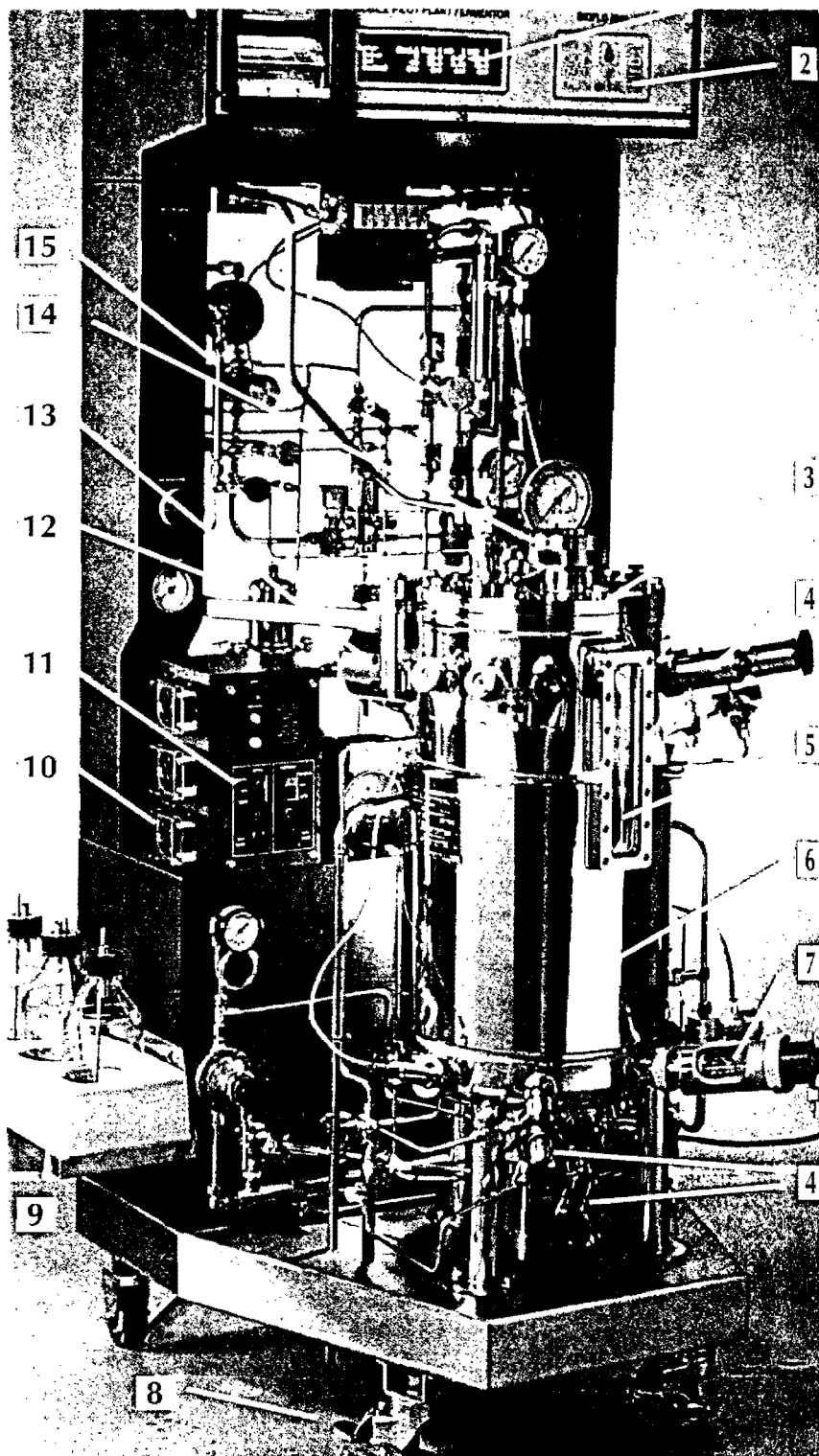


**Resterilizable Valve** is coupled to a welded fitting (A) by a retaining ring (B). A steam inlet line (C) delivers pressurized steam to the interior and exterior of the valve before and after each use. Liquid is transferred via quick-connect fitting (D) maintained sterile by steam cap (E). Outlet steam (F) is fed to a condensate trap (not shown). This design eliminates "blind" pockets and crevices to ensure sterile operation. The two-position Addition Valve (shown closed) is spring-loaded so that Entrance Port (G) opens a steam path through the hollow tube (as shown). When in the forward position, the tube is extended into the vessel for addition of sterile liquid. An optional Quick Connect (inset) can be readily coupled to the valve for sterile and adjustable flow of additives.

# MENTOR

WITH EVERY BIOFLO 5000

- 14** Extra-Large Powder/Fill Port with septum
- 15** Exhaust Line with heat exchanger and view glass. Heaters keep moisture-laden gases above the dew-point and prevent condensation and clogging of the exhaust filter  
*Features 16 - 28 are not visible in the photo*
- 16** Choice of Thermal Mass Flow Controller or Thermal Mass Flow Meter
- 17** Automatic In-Place Steam Sterilization of vessel, process piping, valves and filters
- 18** Safety override permits manual operation of the pneumatic control valves in the sterilization/cooling system to protect personnel and culture
- 19** Automatic Cool Down from sterilization temperature to growth temperature
- 20** Steam Traps, multiple traps guarantee the sterilization temperature is maintained in all process lines
- 21** Powerful Bottom-Drive Motor, 1.5 hp; allows easy access to headplate ports and vessel interior
- 22** Double Mechanical Agitator Seal, carbon-tungsten carbide, mounted in a pressurized steam/steam-condensate environment, maintains constant pressure and protects contents of both the vessel and the environment from contamination
- 23** Rupture Disk Safety Device, side mounted, of 316L stainless steel, prevents over-pressurization of vessel; includes discharge tube to convey liquid to bottom of reactor
- 24** Three Additional Ports, 19 & 25 mm Ingold-type, allow for insertion of extra sensors
- 25** High-Level Foam Control for the exhaust line shuts off air and reduces agitation while signaling alarm
- 26** Rear Connection for All Utilities. Open frame piping facilitates cleaning, maintenance and servicing
- 27** Pre-Filter Regulator Kits for air, water and steam for removal of particulates or impurities
- 28** Serial Port for data logging and computer control



## HEADPLATE PENETRATIONS

- Foam Probe Port \*
- Level Probe Port \*
- Diaphragm-Type Pressure Gauge
- High Foam-Sensing Probe \* in Exhaust Gas Line or in Optional Exhaust Gas Condenser
- Combination Filling and Inoculation Port

## BOTTOM PENETRATIONS

- Bottom Agitator Double Mechanical Seal Housing
- Resterilizable Bottom Drain Valve with Quick Connect

## SIDE WALL PENETRATIONS

- Ring Sparger and Gas Overlay
- Three Septum Addition Ports
- Resterilizable Addition/Inoculation Port with Quick Connect
- Rupture Disc
- Five Ingold-Type Probe Ports for pH\*, DO\* and other sensors
- Resterilizable Sample Port with Quick Connect
- Jacket Pressure-Relief Valve

\* Sensors supplied



VESSEL	Total Capacity	40 Liters	80 Liters	120 Liters
	Working Volume	10 - 30 Liters	20 - 60 Liters	25 - 100 Liters
	Aspect Ratio (H:D)	2:1	2:1	3:1
	Geometry	Cylindrical with dished bottom		
	Pressure Rating	Vessel is ASME-coded, rated for 40 psig (2.8 kg/cm <sup>2</sup> ) Jacket rated for 50 psig (3.5 kg/cm <sup>2</sup> ) / 149° C		
	Viewing Window	Rectangular, sidewall mounted above and below the maximum working level. 40 L: 8.5" x 1.25" (21.6 x 3.2 cm); 80 & 120 L: 11.25" x 2.5" (29.2 x 6.4 cm)		
	Baffles	(4) 316 L stainless steel removable baffles		
MATERIALS & FINISH	Dimensions Inches cm	11.625" ID x 23.25" H 29.53 x 59.06 cm	15.625" ID x 28.625" H 39.69 x 72.72 cm	
	Vessel	316 L stainless steel, orbitally welded and electropolished		
	Process Piping	All piping fabricated of 316 L stainless steel including valves and fittings		
IMPELLERS	Gaskets/O-Rings	Gaskets of Silicone; O-Rings of EPDM		
	Diameter	4.87" (12.38 cm)	6.44" (16.35 cm)	
PENETRATIONS	Type	(2) Six-bladed 316 L stainless steel, Rushton blade, standard Marine blade and pitched blade optional		
	Headplate	(1) Powder/Liquid Fill Port with Septum; (1) Pressure Gauge; (1) Exhaust/High Foam; (1) Level; (1) Foam		
	Upper Side Wall	(3) 19 mm septum ports; (1) 25 mm port and resterilizable valve; (1) 19 mm spare port, plugged		
	Lower Side Wall	(1) 19 mm port and resterilizable septum valve; 25 mm ports, plugged: (4) in 40 L vessels and (5) in 80 & 120 L vessels		
AGITATION	Bottom	Bottom drive; Steam-sterilizable ball drain valve		
	Drive	1.5 HP, AC motor, bottom-entry drive		
	Sensor	Magnetic speed pick-up sensor		
	Range	80 - 800 rpm ± 2 rpm	55 - 550 rpm ± 2 rpm	
	Control	PID regulation of speed via microprocessor feedback circuit		
TEMPERATURE	Bearing Housing	Double mechanical seal		
	Sensor	Platinum RTD in thermowell		
	Control	Microprocessor-based via PID control of tempered water, employing a pulse-width modulation of steam and cooling control valve		
	Range	5°C above coolant supply temperature to 85°C, ± 0.2°C. (See also sterilization, below)		
AIR FLOW	Shift	1°C/minute in growth phase in a range from 23° C to 45°C <sup>Ⓓ</sup>		
	Inlet Filter	Sterilizable-in-place absolute 0.2 µ filter with 316 L stainless steel housing		
	Outlet Filter	Depth filter		
	Control	Choice of Thermal Mass Controller or Thermal Mass Flow Meter		
PRESSURE	Range	0 - 60 SLPM	0-90 SLPM	
	Manual back pressure control standard. Automatic back pressure control optional			
STERILIZATION	Automatic sterilization and cool down			
	Selectable temperature from 105°C - 130°C with duration of 1 - 120 minutes			
COMMUNICATIONS PORT				
RS 422 BioCommand port for automatic data logging and control				
WEIGHT	Net	630 lbs. (286 Kg)	670 lbs. (304 Kg)	880 lbs. (400 Kg)
	Gross	680 lbs. (309 Kg)	700 lbs. (318 Kg)	920 lbs. (418 Kg)
DIMENSIONS, OVERALL	Inches	34" Wide x 32" Deep x 70" High		
	cm	86.4 Wide x 81.3 Deep x 178 High		
ELECTRICAL	Recorder Output	0 - 1 V AC for logging pH, DO, temperature and agitation on optional recorder		
	Connections	Receptacle for optional nutrient pump, 120 V AC (2 Amps) or 220/230 V AC (1 Amp) Receptacle for optional harvest pump, 120 V AC (2 Amps) or 220/230 V AC (1 Amp)		
	Power	200 V / 208 V 50/60 Hz or 230 V 60 Hz AC, 1 Phase, 20 Amps Meets UL, CSA, VDE and British Standards		
UTILITIES				
Requires appropriate utilities for steam, air, water, exhaust, water return and drain				

\* Specifications subject to change without notice.

① In the 120 L system, this is an optional feature.

## OPTIONAL ACCESSORIES

- Gas Overlay
- Exhaust Condenser, water-cooled installed in headplate to minimize evaporation of culture medium
- Resterilizable Quick Connect for inoculation/ addition, sampling and harvest valves
- Adaptable to Recirculation of Tempered Water with optional chiller to conserve water
- Aerosol Containment System to prevent aerosol formation when sampling
- Steam Generator for utility and clean steam
- 2-Gas Controller for oxygen supplementation and gas mixing
- Low-Shear Impellers, marine blade or pitched blade for insect cell culture
- Auxiliary Pump, assignable peristaltic pump
- Validation Documentation, IQ & OQ packages
- cGMP Validation, hardware upgrades
- Chart Recorder, 4 channel for data logging



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E-Mail: [nbsch@online.sh.cn](mailto:nbsch@online.sh.cn)

# ML-6100 — THE POWER OF PROCESS

The entire process is regulated by the ML-6100 controller. This controller is capable of operating up to sixteen process loops. Setpoints are easily specified along with high and low-limit alarms that warn of any deviation.

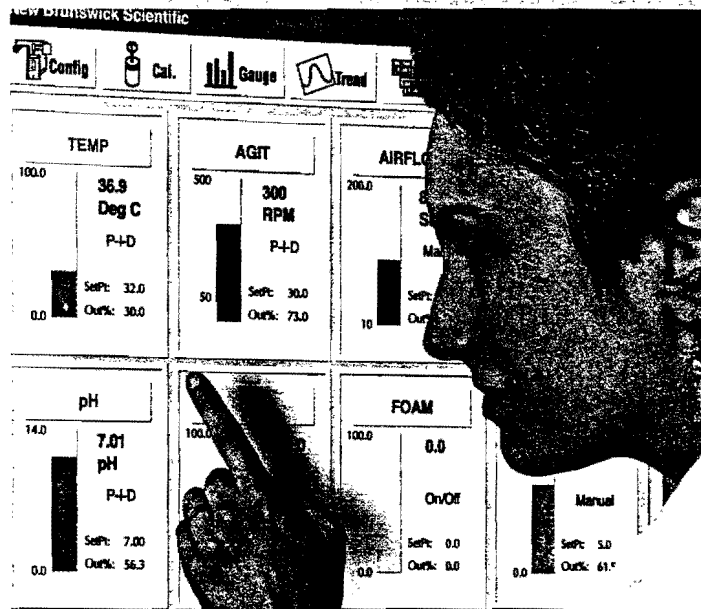
## Intuitive Control

The menu-driven system is designed for simplified control using on-screen programming of *Loop Configuration, Pump and Sensor Calibration, Display Configuration, X-Y Trend Graphs, Cascade Control, Sterilization, and Alarms*. Change setpoints by touching the appropriate gauge to bring up a numerical display for that parameter. Then, simply key in the new setpoint.

The touch-screen controller has the built-in intelligence to guide even an inexperienced operator through every step of the process.

When adding such instrumentation as glucose, optical density or CO<sub>2</sub> analyzers, the Loop Configuration Screen will help link each facet of the control loop. The controller can be adapted to the signal requirements of all instrumentation right at the touch-screen. Just choose the appropriate loop from a menu of options and set the input, the control algorithm and the output. The display screen shows you exactly where each connection is made, obviating the need for a service representative to install an optional circuit board.

There is no longer a need to order special factory-installed control loops at extra cost. Now, you can immediately create and name your own specialized loops electronically from a menu of options. The controller accommodates a wide range of input/output signals from different sensors and actuators, and may be connected without requiring



From the main screen the operator can easily access programs for sterilization, cascade control, loop configuration, calibration, trend graphs, and alarms.

## NBS TOUCH-SCREEN CONTROLLER WORKS SMARTER AND SIMPLER THAN ANY OTHER CONTROLLER

additional interface electronics. These include RTDs (temperature sensors); foam and level (conductance) probes; tachometers, pH and D.O. transmitters; as well as pumps and other devices that use voltage or current outputs. (See Loop Configuration Screen #5).

### Cascade Control

Create cascades for up to five parameters from a single screen. Control any parameter using serial, parallel, or over-

lap schemes. For example, control D.O. through air flow, agitation, pressure or nutrient feed.

### Trend Graph Display

X-Y graphs are displayed through the Trend Graph menus which are programmed to plot data against time. Up to four graphs may be displayed simultaneously in real time, which can help to determine the influence of one variable upon another. With this information available right at the fermentor, the data may be used to make rapid process adjustments during the fermentation.

## SPECIFICATIONS, TOUCH-SCREEN CONTROLLER

Console Types	Controller interface available in various console configurations: 1) In a floor-mounted water-tight mobile console; 2) As a benchtop console; or 3) As a flat-screen monitor mounted directly on the piping console.
Signal Inputs	Signal conditioning included for DO, pH, RTD, Tachometer, and conductance probes. Controller accepts 4 - 20 mA, 0 - 5 and 0 - 10 VDC, conductance, resistance, 0 - 30 kHz, fiber optic signals.
Signal Outputs	Supports 0 - 5 V, 0 - 10 V (analog and digital), 4 - 20 mA. Available modes include PID, ON-OFF, PWM, Dead Band, Gas Mix, multi-channel outputs, and cascades. Alarm output.

## ADVANCED FEATURES OF THE TOUCH-SCREEN CONTROLLER

- Add ancillary equipment, from simple pumps to complex analyzers, right on screen without factory installation or added cost
- Control and display up to 16 parameters on a large color VGA display
- Graphic user-interface replaces alpha-numeric-based controllers
- Select high and low process limits and alarms
- Simplified, menu-driven calibration of D.O., pH and other parameters
- Automatic sterilization screen enhances scale-up by duplicating thermal conditions of industrial reactors
- Valve sequences and sterilization time easily programmed directly from the touch screen
- All control operations easily performed from the main screen for simplified control
- Multiple controller configurations can be easily recalled to accommodate different processes
- Password feature ensures process security
- Battery back-up prevents loss of setpoints in event of power failure
- Serial port for data logging and control using optional AFS BioCommand® software package.

### VALIDATION

The ML-6100 is a validatable process controller for cGMP facilities

# CONTROL RIGHT AT YOUR FINGERTIPS



- 236

# Custom Fermentation and Cell Culture Equipment

New Brunswick Scientific is structured to provide specialized equipment design and construction services for a full range of custom-built cGMP compliant fermentors and bioreactors (10 to 10,000 L range) which satisfy regulatory validation requirements by FDA, EMEA and national authorities.

## Design Philosophy

A design philosophy similar to the computer industry's "open systems" approach is practiced by New Brunswick Scientific. Customers can purchase fermentors, bioreactors or other skid-mounted systems designed and fabricated utilizing proven off-the-shelf components. This approach ensures that technical and service support, including replacement components, are readily available from multiple sources. The selection of proven components increases reliability and accelerates validation.

## Engineering Capability

A dedicated engineering group, specializing in custom system design, is staffed with mechanical, electrical, automation and process engineering experts. A complete support staff consisting of AutoCAD designers and clerical personnel back up the group's engineers.

AutoCAD R14 is used as the primary design tool, with all of the engineering drawings supplied in hard copy and on magnetic media. Software tools are used in the design of pressure vessels and process piping. Detailed protocols have been developed for executing validatable cGMP compliant projects to ensure that the systems meet regulatory validation criteria.

An experienced project manager is assigned to each project. Serving as the client's primary contact during the project's execution cycle and field start-up of the equipment, the project manager provides continuity through completion of the entire scope of work.

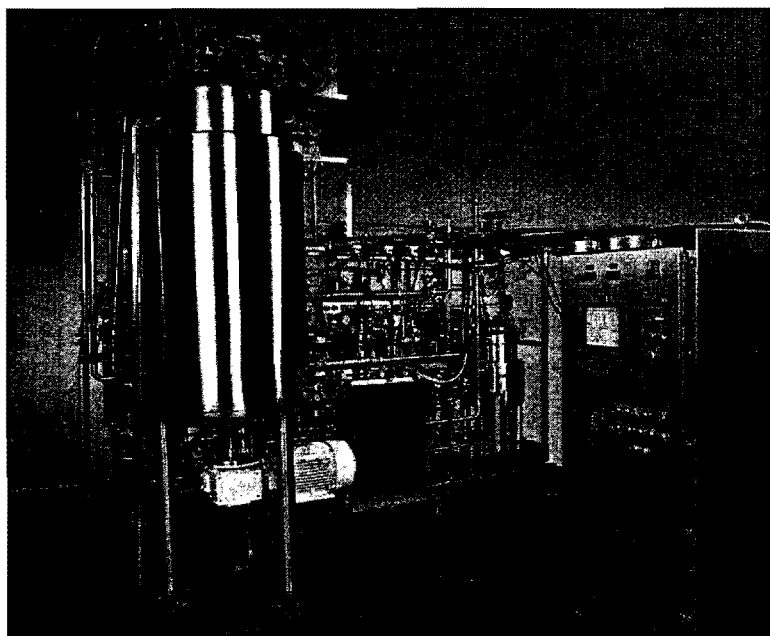
## Process Instrumentation

Our fully-staffed automation group utilizes industry-proven PLC (programmable logic controller) based systems to solve a broad range of control applications. NBS has standardized on such industry leaders as Allen Bradley and Siemens to offer our customers added flexibility in system configuration, processing power and I/O capacity.

NBS integrates process control, data logging, recipe management and Internet applications with Microsoft Windows-based, 32-bit object-oriented graphical human-machine interfaces (HMI) such as Wonderware's In Touch and Intellution's Fix software programs. These industry-standard applications offer our customers the latest in technology including VBA, Active X, OPC and DDE.

## Manufacturing Capability

NBS utilizes the manufacturing capabilities of its 250,000 square foot manufacturing plant, located in Edison, New Jersey, for the



execution of its projects. The plant includes the most advanced manufacturing machinery in the fermentation business, including automated CNC milling machines, orbital welding devices and an ASME certified vessel shop which enables NBS to directly control quality and delivery of its projects.

The manufacturing facility routinely executes custom-engineered projects including:

- cGMP Compliant Fermentors and Bioreactors
- ASME Code Compliant Process Vessels
- Clean-in-Place Systems
- Validatable Process Control Systems
- Addition, Mixing, Hold and Kill Tanks
- Sterile Filtration Systems

## Validation Services

New Brunswick has considerable experience in providing validatable bioprocess systems, which are in full compliance with cGMP regulations. To more fully support the client's IQ and OQ efforts, NBS can supply the following documentation:

- Boroscope Weld Mapping with Videotape
- As-Built Engineering Drawing
- Material Certificates
- Instrumentation Calibration Log
- ASME Pressure Vessel Calculations
- Temperature Maps
- Bill of Materials/OEM Part Numbers
- CIP Coverage Test Reports
- Biological Challenge Test Report

The material used in the manufacturing of any cGMP compliant system is carefully controlled. Incoming raw materials are carefully inspected, tagged, segregated and secured in a locked area within our facility. Material certificates are carefully controlled to assure full traceability.

In addition, NBS performs control loop tuning

using actual cultures or biological solutions. Process corrections can therefore be made before shipment, speeding installation, start-up, and validation.

## Field Service

For decades, NBS has sold and installed fermentation and bioreactor suites all over the world. These installations are serviced by New Brunswick Scientific's ten U.S.-based field service offices and world-wide service offices located in the UK, the Netherlands, Belgium, France, Germany and China.

Due to the customized nature of most of our projects, service engineers from our field service organizations are typically trained on each individual piece of equipment at the factory prior to shipment. After delivery, service engineers participate in the commissioning of the equipment at the customer's site.

Spare parts are stocked in our Edison, NJ headquarters, as well as at our domestic service centers and worldwide offices ready for shipment to the customer at short notice. Complete spare parts lists, with recommended on-hand quantities, are prepared during the detailed engineering phase of each project.

## Support Services

New Brunswick Scientific can offer a diverse range of consulting engineering services, from preliminary conceptual design studies to complete bioprocess facility design and project management.

To help support our customer's PQs and for clients with biological processes that are not fully characterized, we offer process development and optimization services that are unique in our industry. We are the only fermentor manufacturer with biochemical engineers, molecular biologists, cell biologists and microbiologists on staff who operate a modern fully-equipped pilot plant, a microbiological fermentation lab and a cell culture lab up to 130 L.

\*\*\*Correspondence with New Brunswick Scientific Co. All other correspondence occurred over the phone with Ted Shields.\*\*\*

X-Sender: tshields@207.162.131.33 (Unverified)

Date: Wed, 29 Mar 2000 08:19:15 -0500

To: maudette@seas.upenn.edu

From: Ted Shields <tshields@blast.net>

Subject: Fermentor Pricing you requested

X-Status:

X-Keywords:

X-UID: 84

Dear Melissa,

Here are the "ballpark" prices you requested, if you need any additional information please don't hesitate to ask.

BF-5000 (30 liter max working volume)    \$ 85,000

400 Liter Industrial Fermentor            \$ 125,000

5000 Liter Industrial Fermentor          \$ 250,000

These are strictly estimates, actual prices can vary substantially depending on options and configuration.

Sincerely,

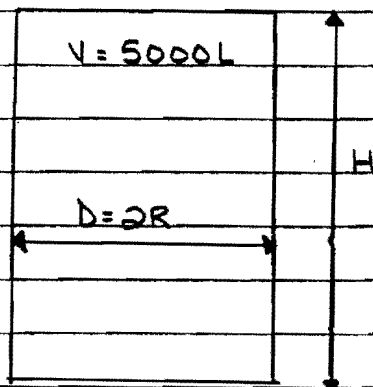
New Brunswick Scientific. Co.

Ted Shields

Calculations for Heating water needs in the jacket of the 5000L fermentor.

1) Determine dimensions of vessel

- assume cylinder
- height-diameter ratio 2:1



$$V = \pi r^2 h$$

$$= \pi \left(\frac{D}{2}\right)^2 H$$

$$5000L \times \frac{1m^3}{1000L} = \pi \left(\frac{D}{2}\right)^2 2D$$

$$\frac{5m^3}{\pi} \left(\frac{4}{2}\right) = D^3$$

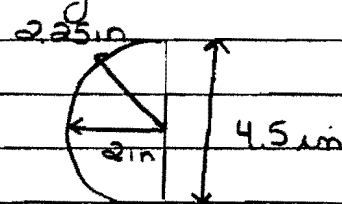
$$D = 1.47m \text{ (approx)}$$

$$\therefore H = 2.94m$$

2) Half-Pipe jacket - 4" piping (SS)

$$ID = 4 \text{ in}$$

$$O.D. = 4.5 \text{ in}$$



find circumference of reactor (wall 0.25 in thick)

$$C = 2\pi r = 2\pi \left[ \frac{1.47m \times 39.37in}{2} + 0.25in \right]$$

$$= 183.38 \text{ in}$$

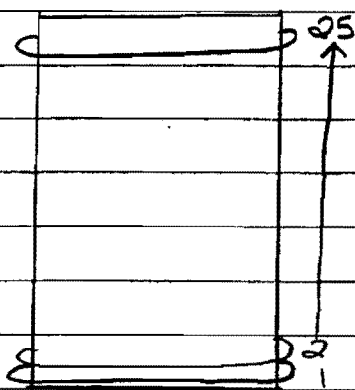
Height of reactor

$$2.94m \times \frac{39.37in}{1m} = 115.74 \text{ inch}$$

# of half pipes around reactor

$$\frac{115.74 \text{ in}}{4.5 \text{ in}} = 25 \text{ half pipes}$$

$$233$$



\* No half pipes on the top of the reactor.

3) Find surface area (to environment) of the half pipe along the sides of the reactor.

\* 25 pipes

each pipe once around

$$D = 4.5 \text{ in}$$

$$H = 183.38 \text{ in}$$

$$SA = 2\pi rh = 2\pi (2.25)(183.38 \text{ in}) \quad (\text{whole pipe - ends not included})$$

$$= 2592.5 \text{ in}^2$$

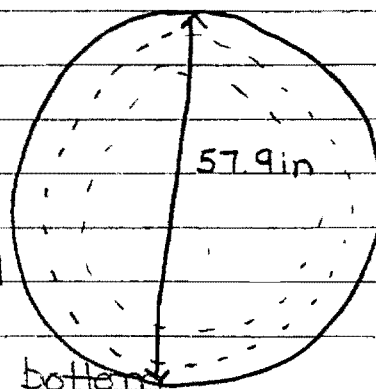
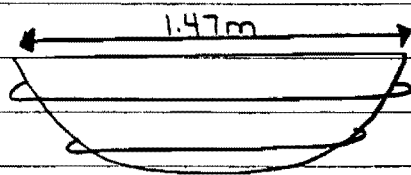
$$\text{half pipe} = 1296.24 \text{ in}^2$$

for 25 half pipes

$$= 1296.24 \text{ in}^2 \times 25$$

$$= 32405.9 \text{ in}^2 \quad (\text{heat transfer area on sides})$$

4) half pipe also on bottom of the reactor - find area



$$\# \text{ of half pipes} = \frac{57.9 \text{ in}}{9 \text{ in}} = 6.4$$

6 pipes around the bottom

Pipe 1 - use circumference at the center of the pipe

$$(57.9 - 4.5) = \frac{53.4 \text{ in} (\pi)(2)}{2}$$

$$h = 167.76 \text{ in}$$

$$2\pi r h = \frac{2\pi (2.25)(167.76 \text{ in})}{2}$$

$$\text{Area pipe 1} = 1185.8 \text{ in}^2$$

Pipe 2 -

$$(57.9 - (9 + 4.5)) = \frac{44.4 \text{ in} (2\pi)}{2}$$

$$= 139.5 \text{ in}$$

$$= \frac{2\pi (2.25)(139.5)}{2}$$

$$\text{Area pipe 2} = 986 \text{ in}^2$$

Pipe 3

$$(57.9 - (18 + 4.5)) = \frac{35.4 (\pi)(2)}{2}$$

$$= 111.2 \text{ in}$$

$$= \frac{2\pi (2.25)(111.2)}{2}$$

$$\text{Area pipe 3} = 786.1 \text{ in}^2$$

(each pipe is 200 in<sup>2</sup> less in area)

$$\text{pipe 4 area} = 586.25 \text{ in}^2$$

$$\text{pipe 5 area} = 386.4 \text{ in}^2$$

$$\text{pipe 6 area} = 186.6 \text{ in}^2$$

$$\text{total area for bottom} = 1185.8 + 986 + 786.1 + 586.25 + 386.4 + 186.6$$

$$\text{total (bottom)} = 3131.15 \text{ in}^2$$

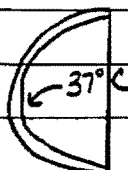


Total heat transfer area:

$$32405.92 + 3131.15 = \boxed{35537 \text{ in}^2}$$

5) Heat loss to environment

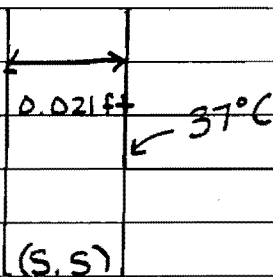
$$T_{\infty} = 20^{\circ}\text{C} \\ \pm 5^{\circ}\text{C}$$



\* Natural Convection in air

$$= 2 \frac{\text{BTU}}{\text{hr} \cdot \text{ft}^2 \cdot \text{F}^{\circ}}$$

$$T_{\infty} = 68^{\circ}\text{F} \\ (59 - 77)^{\circ}\text{F}$$



$$h = 2 \frac{\text{BTU}}{\text{hr} \cdot \text{ft}^2 \cdot \text{F}}$$

$$K = 13.4 \frac{\text{W}}{\text{m} \cdot \text{K}} \times \frac{0.57782 \text{ BTU}/\text{h} \cdot \text{ft}}{1 \frac{\text{W}}{\text{m} \cdot \text{K}}}$$

wall of jacket

$$K = 7.74 \text{ BTU}/\text{h} \cdot \text{ft}^2 \cdot \text{F}$$

$$q_x'' = \frac{(98.6 - 68)}{\frac{1}{h_i} + \frac{L}{K}} = \frac{98.6 - 68}{\frac{1}{2 \frac{\text{BTU}}{\text{hr} \cdot \text{ft}^2 \cdot \text{F}}} + \frac{0.021 \text{ ft}}{7.74 \text{ BTU}/\text{h} \cdot \text{ft}^2 \cdot \text{F}}}$$

@ 20°C

$$q_x'' = 60.9 \frac{\text{BTU}}{\text{ft}^2 \cdot \text{h}}$$

@ 15°C

$$q_x'' = \frac{98.6 - 59 \text{ F}}{\frac{1}{2 \frac{\text{BTU}}{\text{hr} \cdot \text{ft}^2 \cdot \text{F}}} + \frac{0.021 \text{ ft}}{7.74 \text{ BTU}/\text{h} \cdot \text{ft}^2 \cdot \text{F}}}$$

$$q_x'' = 78.77 \frac{\text{BTU}}{\text{ft}^2 \cdot \text{F}}$$

@ 25°C

$$q_x'' = \frac{98.6 - 77 \text{ F}}{\frac{1}{2 \frac{\text{BTU}}{\text{hr} \cdot \text{ft}^2 \cdot \text{F}}} + \frac{0.021 \text{ ft}}{7.74 \text{ BTU}/\text{h} \cdot \text{ft}^2 \cdot \text{F}}}$$

$$q_x'' = 42.96 \text{ BTU}/\text{ft}^2 \cdot \text{h} \rightarrow 310$$

(c) Water flow in jacket (without insulation)

$$Area = 35537 \text{ in}^2$$

$$= 35537 \text{ in}^2 \times \frac{1 \text{ ft}^2}{144 \text{ in}^2} = 246.8 \text{ ft}^2 \quad C_p = 4.178 \frac{\text{kJ}}{\text{kg} \cdot \text{K}}$$

$$\Delta T = 0.5^\circ \text{C}$$

Air @  $15^\circ \text{C}$

$$q_x = \dot{m} C_p (T_{\text{out}} - T_{\text{in}})$$

$$\frac{78.77 \text{ BTU}}{\text{ft}^2 \cdot \text{h}} (246.8 \text{ ft}^2) = \dot{m} \left[ \frac{4.178 \text{ kJ}}{\text{kg} \cdot \text{K}} \times \frac{1000 \text{ J}}{1 \text{ kJ}} \times \frac{2.388 \times 10^{-4} \text{ BTU}}{1 \text{ J/kg} \cdot \text{K}} \right] (100.4 - 99.5)$$

$$\dot{m} = 21643.4 \frac{\text{lbm}}{\text{hr}} \left[ \frac{1 \text{ kg/sec}}{793.66 \text{ lbm/hr}} \right]$$

max flow  $\dot{m} = 2.71 \text{ kg/sec}$

Air @  $20^\circ \text{C}$

$$\frac{60.9 \text{ BTU}}{\text{ft}^2 \cdot \text{h}} (246.8 \text{ ft}^2) = \dot{m} \left[ \frac{4.178 \text{ kJ}}{\text{kg} \cdot \text{K}} \times \frac{1000 \text{ J}}{1 \text{ kJ}} \times \frac{2.388 \times 10^{-4} \text{ BTU}}{1 \text{ J/kg} \cdot \text{K}} \right] (100.4 - 99.5)$$

$$\dot{m} = 116733.3 \text{ lbm/hr}$$

$$\dot{m} = 2.11 \text{ kg/sec}$$

Air @  $25^\circ \text{C}$

$$\frac{42.96 \text{ BTU}}{\text{ft}^2 \cdot \text{h}} (246.8 \text{ ft}^2) = \dot{m} \left[ \frac{4.178 \text{ kJ}}{\text{kg} \cdot \text{K}} \times \frac{1000 \text{ J}}{1 \text{ kJ}} \times \frac{2.388 \times 10^{-4} \text{ BTU}}{1 \text{ J/kg} \cdot \text{K}} \right] (100.4 - 99.5)$$

$$\dot{m} = 11803.9 \text{ lbm/hr}$$

min flow  $\dot{m} = 1.487 \text{ kg/sec}$

7) Flow Rates with 2 in of insulation

Glass fiber blanket ( $\rho = 116$ )

heat transfer area = side + bottom

$$\frac{2\pi(157.9+4.5+4)(115.4)}{2} + \pi \left[ \frac{157.9+4.5+4}{2} \right]^2$$

$$\text{Area} = 27535 \text{ in}^2 = 191.2 \text{ ft}^2$$

$$h_{air} = 2 \text{ BTU/hr} \cdot \text{ft}^2 \cdot \text{F}$$

$$k_{ss} = 13.4 \text{ W/m} \cdot \text{K} = 7.74 \text{ BTU/h} \cdot \text{ft} \cdot \text{F}$$

$$k_{in} = 0.046 \text{ W/m} \cdot \text{K} = 0.0266 \text{ BTU/h} \cdot \text{ft} \cdot \text{F}$$

temp @ 15°C

$$q_x'' = \frac{(98.6 - 59)}{\frac{1}{2} + \frac{(2/12)}{0.0266} + \frac{(0.021)}{7.74}} = 5.85 \text{ BTU/hr} \cdot \text{ft}^2$$

$$\frac{5.85 \text{ BTU}}{\text{hr} \cdot \text{ft}^2} (191.2 \text{ ft}^2) = \dot{m} \left[ 4.178 \frac{\text{kJ}}{\text{kg} \cdot \text{K}} \times \frac{1000 \text{ J}}{\text{kJ}} \times \frac{2.388 \times 10^{-4}}{1 \text{ J/kg} \cdot \text{K}} \right] (100.4 - 99.5)$$

$$\dot{m} = 1245.66 \text{ lbm/hr}$$

Max  
Flow

$$\dot{m} = 0.16 \text{ kg/sec}$$

temp @ 20°C

$$q_x'' = \frac{(98.6 - 68)}{\frac{1}{2} + \frac{(2/12)}{0.0266} + \frac{(0.021)}{7.74}} = 4.52 \text{ BTU/h} \cdot \text{ft}^2$$

$$\frac{4.52 \text{ BTU}}{\text{h} \cdot \text{ft}^2} (191.2 \text{ ft}^2) = \left[ 4.178 \times \frac{2.388 \times 10^{-4}}{1} \right] \dot{m} (100.4 - 99.5)$$

$$\dot{m} = 962.5 \text{ lbm/hr}$$

$$\dot{m} = 0.121 \text{ kg/sec}$$

Temp @

$$q_x'' = \frac{[98.6 - 77]}{\frac{1}{2} + \frac{(2/12)}{0.0266} + \frac{0.021}{1.14}} = 3.19 \text{ BTU/ft}^2 \cdot \text{hr}$$

$$\frac{3.19 \text{ BTU}}{\text{ft}^2 \cdot \text{hr}} (191 \text{ ft}^2) = \dot{m} \left[ \frac{4178 \times 2.3886 \times 10^{-4}}{1} \right] (100.4 - 99.5)$$

$$\dot{m} = 679 \text{ lbm/hr}$$

min  
flow

$$\dot{m} = 0.0856 \text{ kg/sec}$$

# Recycle Stream Material Balance Calculations for heating jacket on 5000L fermentor

Water flow rate (max)

$$0.16 \text{ kg/sec}$$

$$\text{average} = 0.121 \text{ kg/sec}$$

steam needed to heat stream to  $38^\circ\text{C}$  from  $30^\circ\text{C}$

(using  $30^\circ\text{C}$  to estimate the temperature loss over the tubing)

$$C_p: 4.178 \frac{\text{kJ}}{\text{kg} \cdot \text{K}}$$

energy needed to heat stream of water

$$(0.121 \frac{\text{kg}}{\text{sec}}) (4.178 \frac{\text{kJ}}{\text{kg} \cdot \text{K}}) (38 - 30)$$

$$= 4.044 \text{ kJ/sec}$$

Heat of vap for steam:  $2173 \text{ kJ/kg}$

$$\frac{4.044 \text{ kJ/sec}}{2173 \text{ kJ/kg}} = 0.00186 \frac{\text{kg}}{\text{sec}}$$

Batch runs for 240 hrs.

$$0.00186 \frac{\text{kg}}{\text{sec}} \times \frac{60 \text{ sec}}{1 \text{ min}} \times \frac{60 \text{ min}}{1 \text{ hr}} \times 240 \text{ hr}$$

1608 kg/batch of steam needed per batch

Total Air and CO<sub>2</sub> Requirements

5000 L Fermentor:

$$\text{Need: } (10.06 - 0.2) \times 10^{-12} \frac{\text{mol O}_2}{\text{hr} \cdot \text{cell}}$$

max. O<sub>2</sub> consumption

$$3.5 \times 10^6 \frac{\text{cell}}{\text{mL}} \times \frac{1000 \text{ mL}}{\text{L}} \times 4000 \text{ L} = 1.4 \times 10^{13} \text{ cells in fermentor}$$

$$0.15 \times 10^{-12} \frac{\text{mol O}_2}{\text{hr} \cdot \text{cell}} \times 1.4 \times 10^{13} \text{ cells}$$

$$= 2.1 \frac{\text{mol O}_2}{\text{hr}} \quad (\text{total})$$

Need an inlet flow of 5% CO<sub>2</sub> I brought up from 0.03% and  
O<sub>2</sub> = 21% air

$$\frac{5}{100} = \frac{0.03 + x}{100 + x} \longrightarrow 500 + 5x = 3 + 100x$$

$$x = 5.23$$

New % compositions:

$$\text{O}_2 : 0.20 \longrightarrow 2.1 \text{ mol O}_2 / \text{hr}$$

$$\text{N}_2 : 0.74 \longrightarrow 7.77 \text{ mol N}_2 / \text{hr}$$

$$\text{CO}_2 : 0.05 \longrightarrow 0.525 \text{ mol CO}_2 / \text{hr}$$

total inlet flow 10.5 mol/hr

Compressed Air:

$$\frac{2.1 \text{ mol O}_2}{0.2} : \frac{9.975 \text{ mol air}}{\text{hr.}}$$

$$9.975 \frac{\text{mol (air)}}{\text{hr}} \times \frac{29 \text{ g}}{\text{mol}} = 289.3 \text{ g air/hr.}$$

$$\# \text{ of hours reactor is operating} = 24 \text{ hr} \times 10 \text{ days} \\ = 240 \text{ hrs.}$$

$$240 \text{ hrs} \times \frac{289.3 \text{ g air}}{\text{hr.}} = 69.426 \frac{\text{kg air}}{\text{batch}}$$

# of tanks needed

$$1 \text{ tank} = 8.70 \text{ m}^3 \times \frac{1}{0.833 \text{ m}^3/\text{kg}} = 10.44 \text{ kg}$$

$$\frac{69.4 \text{ kg}}{\text{batch}} \times \frac{1 \text{ tank}}{10.44 \text{ kg}} = \boxed{6.65 \text{ tanks/batch}}$$

$$6.65 \text{ tanks} \times 50 \text{ batches}$$

$$= \boxed{350 \frac{\text{tanks}}{\text{Year}} \text{ (compressed air)}}$$

CO<sub>2</sub>

$$0.525 \frac{\text{mol}}{\text{h CO}_2} - (0.00028)(10.5) = 0.522 \frac{\text{mol CO}_2}{\text{batch}} \text{ added}$$

$$0.522 \frac{\text{mol CO}_2}{\text{h}} \times \frac{44 \text{ g}}{\text{mol}} = 22.968 \text{ g/h CO}_2$$

$$240 \text{ hours} \times \frac{22.9 \text{ g}}{\text{h CO}_2} = 5.512 \text{ kg/batch}$$

amount in each tank:

$$\frac{601 \text{ lb}}{2.204 \text{ lb}} \times \frac{1 \text{ kg}}{2.204 \text{ lb}} = 27.21 \text{ kg}$$

$$\frac{5.512 \text{ kg}}{\text{batch}} \times \frac{1 \text{ tank}}{27.21 \text{ kg}} = \boxed{0.202 \frac{\text{tank}}{\text{batch}}}$$

$$= 25 \frac{\text{tanks}}{\text{Year}} \quad 247$$

400L fermentor

$$3.5 \times 10^6 \frac{\text{cells}}{\text{mL}} \times \frac{1000 \text{ mL}}{\text{L}} \times 300 \text{ L} = 1.05 \times 10^{12} \text{ cells total}$$

$$1.05 \times 10^{12} \text{ cells} \times 0.15 \times 10^{-12} \frac{\text{mol O}_2}{\text{hr} \cdot \text{cell}} = 0.1575 \text{ mol O}_2/\text{hr}$$

$$\frac{0.1575 \text{ mol O}_2}{0.20} = 0.7875 \frac{\text{mol total air}}{\text{hr}} \times \frac{29 \text{ g}}{\text{mol}}$$

$$= 22.83 \frac{\text{g air}}{\text{hr}}$$

$$22.83 \frac{\text{g air}}{\text{hr}} \times 192 \text{ hrs} \times \frac{\text{tank}}{10.44 \text{ kg}} \times \frac{1 \text{ kg}}{1000 \text{ g}} = \boxed{0.384 \frac{\text{tank}}{\text{batch}}}$$

$$0.384 \frac{\text{tank}}{\text{batch}} \times 50 \text{ batches} = \boxed{20 \frac{\text{tanks}}{\text{Year}}}$$

CO<sub>2</sub>

$$0.7875 \text{ mol} \times 0.05 = 0.0393 \frac{\text{mol CO}_2}{\text{hr}}$$

$$0.0393 \text{ mol CO}_2 - (0.7875)(0.00028) = 0.0391 \frac{\text{mol CO}_2}{\text{hr}} \times \frac{44 \text{ g}}{\text{mol}}$$

$$= 1.719 \frac{\text{g CO}_2}{\text{hr}} \times 192 \text{ hr} \times \frac{\text{tank}}{27.21 \text{ kg}} \times \frac{1 \text{ kg}}{1000 \text{ g}}$$

$$= \boxed{0.012 \frac{\text{tank}}{\text{batch}}} \times 50 \text{ batches}$$

$$= \boxed{2 \text{ tanks/Year}}$$



40L fermentor:

$$3.5 \times 10^6 \frac{\text{cells}}{\text{mL}} \times \frac{1000 \text{ mL}}{1 \text{ L}} \times 30 \text{ L} = 1.05 \times 10^{11} \text{ cells total}$$

O<sub>2</sub> Needs:

$$0.15 \times 10^{-12} \frac{\text{mol O}_2}{\text{cell} \cdot \text{hr}} \times 1.05 \times 10^{11} \text{ cells} = 0.01575 \text{ mol O}_2$$

$$\frac{0.01575 \text{ mol O}_2}{0.20} = 0.07875 \frac{\text{total mol (air)}}{\text{hr}}$$

CO<sub>2</sub>

$$0.07875 \times 0.05 = 0.00393 \frac{\text{mol CO}_2}{\text{hr}} \times \frac{44 \text{ g}}{\text{mol}} = 0.17 \frac{\text{g}}{\text{hr}} \times \frac{144 \text{ hr}}{\text{batch}} = 24.48 \text{ g/batch}$$

$$24.48 \text{ g CO}_2 \times \frac{\text{tank}}{27.21 \text{ g}} \times \frac{1 \text{ kg}}{1000 \text{ g}} = \boxed{0.001 \frac{\text{tank}}{\text{batch}}} \times 50 \text{ batches}$$

$$= \boxed{1 \text{ tank CO}_2 / \text{year}}$$

Total air:

$$0.07875 - 0.00393 = 0.0748125 \frac{\text{mol air}}{\text{hr}} \times \frac{29 \text{ g}}{\text{mol}}$$

$$2.16 \frac{\text{g air}}{\text{hr}} \times \frac{144 \text{ hr}}{\text{batch}} \times \frac{1}{10.44 \text{ kg}} \times \frac{1 \text{ kg}}{1000 \text{ g}} \times 50 \frac{\text{batches}}{\text{year}}$$

$$= \boxed{3 \text{ tanks / year}}$$

TOTALS:

$$\text{Compressed Air: } 350 + 20 + 3 = 373 \text{ tanks/year}$$

$$\text{CO}_2 = 25 + 2 + 1 = 28 \text{ tanks CO}_2 / \text{year}$$

Design Calculations for the Heat Exchanger used to Heat up the Media for the 3 Fermentors

## 1) Overall Energy Balance:

Q = total Energy needed to heat up media (BTU/hr)

$$m := 2 \cdot \frac{1}{264.17} \cdot \frac{1}{1.00 \cdot 10^{-3}} \cdot \left( \frac{60}{1} \right) \quad \text{Volumetric Flow of 2gal/min converted into kg/hr}$$

$$m = 454.253 \quad \text{kg/hr}$$

$$H_{\text{out}} := 159.1 \quad \text{Enthalpy of water at 38 C in KJ/kg}$$

$$H_{\text{in}} := 16.8 \quad \text{Enthalpy of water at 4 C in KJ/kg}$$

$$Q := m \cdot (H_{\text{out}} - H_{\text{in}})$$

$$Q = 6.464 \cdot 10^4 \quad \text{KJ/hr}$$

$$\text{total} := Q \cdot \frac{1000}{1} \cdot \left[ \frac{9.486 \cdot 10^{-4}}{1} \right] \quad \text{Conversion of KJ/hr into BTU/hr}$$

$$\text{total} = 6.132 \cdot 10^4 \quad \text{BTU/hr}$$

Calculate the flow rate of the warm water

$$H_{\text{outw}} := 200.9 \quad \text{Enthalpy of water at 48 C in KJ/kg}$$

$$H_{\text{inw}} := 230.2 \quad \text{Enthalpy of water at 55 C in KJ/kg}$$

$$m_w := \frac{-Q}{H_{\text{outw}} - H_{\text{inw}}}$$

$$m_w = 2.206 \cdot 10^3 \quad \frac{\text{kg}}{\text{hr}} \quad \text{Flow rate of the water on the shell side}$$

## 2) Estimate overall heat transfer coefficient for a water-water heat exchanger

 $U_i = \text{BTU/F} \cdot \text{ft}^2 \cdot \text{hr}$ 

$$U_i := 70$$

## 3) Assume 1-1 heat exchanger

## 4) Calculate Ai from equation 8.7

Calculation of the correction factor

$$R := \frac{(55 - 48)}{(38 - 4)} \quad R = 0.206 \quad S := \frac{(38 - 4)}{(55 - 4)} \quad S = 0.667$$

$$F_T := 0.95 \quad \text{From graph on page 323 of Seider and Seader}$$

Calculation of the log mean temperature

$$\Delta T_{LM} := \frac{((48 - 38) - (55 - 4))}{\ln \left[ \frac{(48 - 38)}{(55 - 4)} \right]}$$

$$\Delta T_{LM} = 25.165 \text{ } ^\circ\text{C}$$

$$\Delta T_{LMF} := 25.165 \cdot 1.8 + 32 \quad \text{Convert temp to F}$$

$$\Delta T_{LMF} = 77.297 \text{ } ^\circ\text{F}$$

$$A_i := \frac{\text{total}}{U_i \cdot \Delta T_{LMF} \cdot F_T}$$

$$A_i = 11.929 \text{ } \text{ft}^2$$

$$A_{im} := A_i \cdot \left( \frac{1}{3.2808^2} \right) \quad \text{Conversion to m}^2 \text{ from ft}^2$$

5) Choose  $u_i$  to be between 1 and 10 ft/sec

$$u_i := 0.6 \text{ } \text{ft/sec}$$

$$u_{im} := u_i \cdot \frac{1}{3.2808} \quad \text{Conversion of u into m/sec from ft/sec}$$

$$u_{im} = 0.183 \text{ } \text{m/sec}$$

6) Calculate  $A_{ci}$  for the shell side

$m_w$  = the flow rate of the water that is being cooled (kg/hr)

$\rho$  = the average density of the water stream

$$\rho_{avg} := \frac{\left[ \frac{1}{1.011 \cdot 10^{-3}} + \frac{1}{1.021 \cdot 10^{-3}} \right]}{2} \text{ } \text{kg/m}^3$$

$$A_{ci} := \frac{\frac{m_w}{3600}}{\rho_{avg} \cdot u_{im}}$$

$$A_{ci} = 3.404 \cdot 10^{-3} \text{ } \text{m}^2$$

Assuming 3/4in Steel Pipe tubing

O.D. = 0.75 in

I.D. = 0.62 in

$$ID := 0.62 \cdot \frac{1}{39.37}$$

$$ID = 0.016 \text{ } \text{meters}$$

$$OD := 0.75 \cdot \frac{1}{39.37}$$

$$OD = 0.019 \text{ } \text{meters}$$

$$N_t := \frac{(4 \cdot A_{ci})}{\pi \cdot (ID)^2}$$

$$N_t = 17.478$$

7) Assume Tube Length  $L = 3$  ft

$$A_t := \pi \cdot ID \cdot \frac{4}{\left(\frac{3.2808}{1}\right)} \quad A_t = 0.06 \quad \text{m}^2$$

$$N_p := \frac{A_{im}}{A_t \cdot N_t}$$

$$N_p = 1.051$$

8) Determine the diameter of the shell

Using table in the Process Design Book found on page 333, the diameter of the shell was found from extrapolating the given data to apply to smaller number of tubes. The following equation was found by graphing the points in excel and drawing a trend line.

Guess Value

$$x := 5$$

Given

$$18 = 0.7578 \cdot x^2 - 2.7436 \cdot x + 3.701 \quad \text{Equation of the line from Excel using the number of tubes found above (NT)}$$

$$\text{Find}(x) = 6.516 \quad \text{Shell ID in inches}$$

9) Calculate  $h_i$ ,  $h_o$ ,  $U_i$

$$D_{\text{shell}} := 6.6 \quad \text{Diameter of shell in inches} \quad \text{clearance} := 0.25 \quad \text{inches}$$

$$b := 4 \quad \text{length of tubes in ft.} \quad P_t := 1 \quad \text{inch}$$

$$A_{\text{cfo}} := \frac{D_{\text{shell}} \cdot \text{clearance} \cdot b \cdot \frac{12}{1}}{P_t}$$

$$A_{\text{cfo}} = 79.2 \quad \text{in}^2$$

$$m_w = 2.206 \cdot 10^3 \quad \frac{\text{kg}}{\text{hr}} \quad \text{Flow rate of the water on the shell side}$$

$$G_o := \frac{m_w}{A_{\text{cfo}}}$$

$$G_o = 27.855 \quad \frac{\text{kg}}{\text{hr} \cdot \text{in}^2}$$

$$G_{\text{om}} := G_o \cdot \frac{39.37^2}{13600}$$

$$G_i := \rho_{\text{avg}} \cdot u_{\text{im}}$$

$$G_i = 180.007 \quad \frac{\text{kg}}{\text{m}^2 \cdot \text{s}}$$

$$G_{\text{om}} = 11.993 \quad \frac{\text{kg}}{\text{s} \cdot \text{m}^2}$$

Properties of the fluids:

$$\mu_h := \frac{(567.2 \cdot 10^{-6} + 504.6 \cdot 10^{-6})}{2}$$

$$\mu_h = 5.359 \cdot 10^{-4} \frac{\text{kg}}{\text{m} \cdot \text{s}}$$

$$k_h := \frac{(648 \cdot 10^{-3} + 641 \cdot 10^{-3})}{2}$$

$$k_h = 0.645 \frac{\text{W}}{\text{m} \cdot \text{K}}$$

$$C_{ph} := \frac{(4183 + 4180)}{2}$$

$$C_{ph} = 4.181 \cdot 10^3 \frac{\text{J}}{\text{kg} \cdot \text{K}}$$

$$\mu_c := \frac{(1560 \cdot 10^{-6} + 695 \cdot 10^{-6})}{2}$$

$$\mu_c = 1.127 \cdot 10^{-3} \frac{\text{kg}}{\text{m} \cdot \text{s}}$$

$$k_c := \frac{(577.2 \cdot 10^{-3} + 629 \cdot 10^{-3})}{2}$$

$$k_c = 0.603 \frac{\text{W}}{\text{m} \cdot \text{K}}$$

$$C_{pc} := \frac{(4200 + 4178)}{2}$$

$$C_{pc} = 4.189 \cdot 10^3 \frac{\text{J}}{\text{kg} \cdot \text{K}}$$

Viscosity of Fluids

Thermal Conductivity

Heat Capacity

The Nusault numbers are calculated for the shell and tube side of the exchanger

$$Nu_o := 0.36 \cdot \left[ \frac{OD \cdot G_{om}}{\mu_h} \right]^{0.55} \cdot \left[ \frac{C_{ph} \cdot \mu_h}{k_h} \right]^{\frac{1}{3}} \quad h_o := \frac{Nu_o \cdot k_h}{OD}$$

$$Nu_o = 15.243$$

$$Nu_i := 0.023 \cdot \left[ \frac{ID \cdot G_i}{\mu_c} \right]^{0.8} \cdot \left[ \frac{C_{pc} \cdot \mu_c}{k_c} \right]^{0.4} \quad h_i := \frac{Nu_i \cdot k_c}{ID}$$

$$Nu_i = 27.516$$

The calculation of the overall heat transfer coefficient

$$U_{ie} := \frac{1}{\frac{1}{h_i} + \frac{ID}{h_o \cdot OD}} \quad U_{ie} = 391.849 \frac{\text{W}}{\text{m}^2 \cdot \text{K}}$$

$$U_i := U_{ie} \cdot 0.17612 \quad \text{Conversion of the units back into BTU/h} \cdot \text{ft}^2 \cdot \text{F}$$

$$U_i = 69.012 \frac{\text{BTU}}{\text{h} \cdot \text{ft}^2 \cdot \text{F}}$$

## Purchase Cost for Heat Exchanger

$$Q = U A \Delta T_m$$

from design calculations

$$U = 70 \text{ BTU/F} \cdot \text{ft}^2 \cdot \text{hr}$$

$$\Delta T_m = 77.3^\circ \text{F}$$

$$A = \frac{6.132 \times 10^4 \text{ BTU/hr}}{(77.3^\circ \text{F})(70 \text{ BTU/F} \cdot \text{ft}^2 \cdot \text{hr})}$$

$$Q = m(H_{\text{out}} - H_{\text{in}})$$

$$454.253 \frac{\text{kg}}{\text{hr}} (159.1 - 16.8) \frac{\text{kJ}}{\text{kg}}$$

$$A = 11.34 \text{ ft}^2 \left( \frac{1 \text{ m}^2}{3.2808 \text{ ft}^2} \right) = 6.132 \times 10^4 \text{ BTU/hr}$$

$$A = 1.053 \text{ m}^2$$

$$\text{Pressure Factor } F_p = 1.0$$

$$\text{Material Factor } F_m$$

$$\text{Stainless steel / Stainless steel} = 3.0$$

$$\text{Pressure Factor - Material Factor Product}$$

$$F_p \times F_m = 3.0$$

$$F_{pm} = 6.0$$

from chart on page 340

## Purchase Cost

$$\$850.00$$

$$C_{BM} = C_p (1.65 + 1.5 F_m)$$

$$\$850.00 (1.65 + 1.5(3))$$

$$C_{BM} = \$5227.50 \text{ (for 1982)}$$

$$\$5227.50 \quad \begin{array}{r} (389) \\ \hline 315 \end{array}$$

$$= \$6455.55$$

QUALITY  
PURE & SIMPLE



WHO WE  
ARE

THE WALKER  
COMMITMENT

STATIONARY  
PRODUCTS

STAINLESS STEEL  
COMPONENTS

TRANSPORTATION  
PRODUCTS

CARLISLE BARRIER  
SYSTEMS

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HONESTY, INTEGRITY  
& FULFILLMENT

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SIMPLE  
TO DO BUSINESS WITH US

WALKER

A CARLISLE COMPANY

# WALKER

Process Tanks

Storage Tanks

Agitation Systems

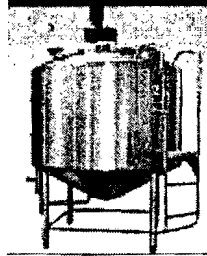
Field Service  
& Repair

...And More

## STATIONARY PRODUCTS

Walker Stationary Products Group specializes in engineering, design, fabrication and service of storage and processing tanks and equipment for the food, dairy, beverage, pharmaceutical and chemical industries.

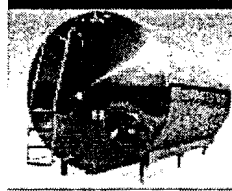
BLENDING



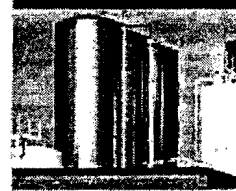
MIXING



RECEIVING



STORAGE



### STATIONARY PRODUCTS

888-667-5689 Fax: 888-667-5691

- ① 5000L - Stainless Steel  
Blending (w/ agitator)  
- Refrigerated to 4°C if possible

- ② Storage Tank ~5000L  
Stainless Steel

## Cooling Requirements for Media Mixing Tank

dimensions of Tank

$$D = 1.47 \text{ m}$$

$$H = 2.94 \text{ m}$$

Surface Area:

$$2\pi rh + 2\pi r^2$$

$$2\pi \left( \frac{1.47}{2} \right) (2.94) + 2\pi \left( \frac{1.47}{2} \right)^2$$

$$A = 16.97 \text{ m}^2$$

heat loss to the environment

$$\frac{5 \text{ BTU}}{\text{hr} \cdot \text{ft}^2} \cdot 16.97 \text{ m}^2 \left( \frac{5 \text{ BTU}}{\text{hr} \cdot \text{ft}^2} \right) \times \frac{3.28^2 \text{ ft}^2}{1 \text{ m}^2} = 912.94 \frac{\text{BTU}}{\text{hr}}$$

Refrig needed (per hour)

$$\frac{912.94}{12,000} = 0.08 \text{ ton} \left( \frac{\text{BTU}}{\text{hr}} \right)$$

total hrs.

$$24 \text{ hours} \times 7 \text{ Days} = 168 \text{ hours/batch}$$

$$= 13 \text{ tons}$$

Refrig. needed to cool initially

$$C_p = \frac{4182 \text{ J}}{\text{kg} \cdot \text{K}} \times \frac{4210 \text{ J}}{2 \text{ kg} \cdot \text{K}} : \frac{4196 \text{ J}}{\text{kg} \cdot \text{K}} \times \frac{2.886 \times 10^{-4} \text{ BTU}}{1 \text{ J/kg} \cdot \text{K}} \frac{\text{BTU}}{\text{lbm}}$$

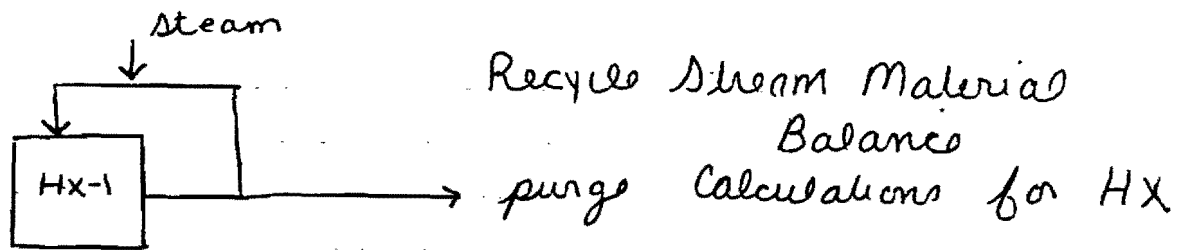
$$C_p = 1.00 \text{ BTU/lbm} \cdot \text{F}$$

$$X = \frac{1.00 \text{ BTU}}{\text{lbm} \cdot \text{F}} [68.8 - 40] \cdot \text{F} \cdot 4500 \text{ L} \times \frac{1 \text{ kg}}{2.2046 \text{ lb}} \times \frac{2.2046 \text{ lb}}{1 \text{ kg}}$$

$$X = \frac{286156.8 \text{ BTU/hr}}{3} ; \quad \text{405885.00 BTU}$$

x = 23.84 ref (load) to cool media  
purchase 8 tons  
251





Water flow rate:  $2.206 \times 10^3 \frac{\text{kg}}{\text{hr}}$

steam heat stream from  $40^\circ\text{C}$  up to  $55^\circ\text{C}$   
(the stream exits the exchanger at  $48$ , but an  $8^\circ\text{C}$  temp loss is estimated over the piping.)

$C_p$  of water:  $4.178 \frac{\text{kJ}}{\text{kg} \cdot \text{K}}$

energy needed to heat stream

$$2.206 \times 10^3 \frac{\text{kg}}{\text{hr}} \times (4.178) \frac{\text{kJ}}{\text{kg} \cdot \text{K}} (55 - 40)$$

$$= 138250.02 \text{ kJ/hr.}$$

Heat of vap of steam:  $2173 \text{ kJ/kg}$

$$\text{steam flow} = \frac{138250.02 \text{ kJ/hr}}{2173 \text{ kJ/kg}}$$

$$= 63.62 \text{ kg/hr.}$$

Batch runs for 1020.9 min or 17 hours

amount of steam required per batch

$$63.62 \frac{\text{kg}}{\text{hr}} \times \frac{17 \text{ hr}}{\text{batch}} = 1081 \frac{\text{kg}}{\text{batch}}$$

## Design Calculations and Utility Requirements for the Fermentor Section of the tPA Process

All Pumps used in the Fermentor Section of the tPA process are identical. The flow rates, pressure drops, and size of each of the pumps is within a close enough range that the same type of pump can be used for each application. The pumps are Randolph Austin Series 610-362 peristaltic pumps.

Pump Characteristics for P-1, P-2, P-3, P-4, P-5, and P-6:

$$\begin{aligned} F &:= 1001.4 \cdot \frac{\text{lb}}{\text{hr}} && \text{Flow through Pump} && \text{Assume an average pressure drop} \\ &&& && \Delta P := 15 \cdot \text{psi} \\ \rho &:= 63.72 \cdot \frac{\text{lb}}{\text{ft}^3} && \text{Denisity of Water} \\ \eta &:= 0.80 && \text{Pump efficiency} \end{aligned}$$

Using the specifications provided by Randolph Austin Co. for the Series 610-362 Vari-flow speed control peristolic pump:

$$\begin{aligned} \text{Motor power} &:= 0.25 \cdot \text{hp} && \text{Total power1} := \text{Motor power} \cdot 7920 \cdot \text{hr} \\ \text{Flow}_{\text{max}} &:= 255 \cdot \frac{\text{gal}}{\text{hr}} && \text{Total power1} = 1.476 \cdot 10^3 \cdot \text{kW} \cdot \text{hr} \\ \text{Flow}_{\text{min}} &:= 12 \cdot \frac{\text{gal}}{\text{hr}} && \text{UtilityCost} := \text{Total power1} \cdot 0.04 \cdot \frac{1}{\text{kW} \cdot \text{hr}} \end{aligned}$$

Costing the pump

$$\text{UtilityCost} = 59.059$$

The Cost of the pump as quoted by Randolph Austin Company is \$1595.00

### Pump P-7, Heat Exchanger water Pump

$$\begin{aligned} F &:= 4862.02 \cdot \frac{\text{lb}}{\text{hr}} && \text{Flow through Pump} && \text{Assume an average pressure drop} \\ &&& && \Delta P := 15 \cdot \text{psi} \\ \rho &:= 63.72 \cdot \frac{\text{lb}}{\text{ft}^3} && \text{Denisity of Water} \\ \eta &:= 0.80 && \text{Pump efficiency} \end{aligned}$$

Using the specifications provided by the Corcoran Company for the Model 3000D-HD1 (AA05):

$$\begin{aligned} \text{Motor power} &:= 2 \cdot \text{hp} \\ \text{Total power7} &:= \text{Motor power} \cdot 7920 \cdot \text{hr} \end{aligned}$$

$$\text{Total power}_7 = 1.181 \cdot 10^4 \text{ kW} \cdot \text{hr}$$

$$\text{UtilityCost} := \text{Total power}_7 \cdot 0.04 \cdot \frac{1}{\text{kW} \cdot \text{hr}}$$

$$\text{UtilityCost} = 472.475$$

### Costing the pump

The Cost of the pump as quoted by Corcoran Company to be \$3250.00.

Total Energy needs for all the pumps per year: (50 Batches)

$$\text{totalUtility} := (\text{Total power}_1 \cdot 6 + \text{Total power}_7) \cdot 2 \quad \text{Cost per pump} := 1595.00$$

$$\text{totalUtility} = 4.134 \cdot 10^4 \text{ kW} \cdot \text{hr}$$

(there are 14 pumps total in the plant, 7 in each fermentor train)

$$\text{UtilityCost} := \text{totalUtility} \cdot 0.04 \cdot \frac{1}{\text{kW} \cdot \text{hr}}$$

$$\text{UtilityCost} = 1.654 \cdot 10^3$$

$$\text{TotalPC} := \text{Cost per pump} \cdot 12 + 2 \cdot 3250$$

$$\text{TotalPC} = 2.564 \cdot 10^4$$

Utility Requirement for the Fermentors, Blending Tank, and Harvest Pumps.

40L Fermentor

$$\text{Agitationmotor}_{40} := 1.5 \cdot \text{hp}$$

$$\text{Power}_1 := \text{Agitationmotor}_{40} \cdot 7920 \cdot \text{hr}$$

$$\text{Power}_1 = 8.859 \cdot 10^3 \text{ kW} \cdot \text{hr}$$

$$\text{UtCost}_1 := \text{Power}_1 \cdot 0.04 \cdot \frac{1}{\text{kW} \cdot \text{hr}}$$

$$\text{UtCost}_1 = 354.357$$

400L Fermentor

$$\text{Agitationmotor}_{400} := 5 \cdot \text{hp}$$

$$\text{Power}_2 := \text{Agitationmotor}_{400} \cdot 7920 \cdot \text{hr}$$

$$\text{Power}_2 = 2.953 \cdot 10^4 \text{ kW} \cdot \text{hr}$$

$$\text{UtCost}_2 := \text{Power}_2 \cdot 0.04 \cdot \frac{1}{\text{kW} \cdot \text{hr}}$$

$$\text{UtCost}_2 = 1.181 \cdot 10^3$$

5000L Fermentor

$$\text{Agitationmotor}_{5000} := 10 \cdot \text{hp}$$

$$\text{Power}_3 := \text{Agitationmotor}_{5000} \cdot 7920 \cdot \text{hr}$$

$$\text{Power}_3 = 5.906 \cdot 10^4 \text{ kW} \cdot \text{hr}$$

$$\text{UtCost}_3 := \text{Power}_3 \cdot 0.04 \cdot \frac{1}{\text{kW} \cdot \text{hr}}$$

3

5000 L Harvest Pump

$$\text{Harvestmotor}_{5000} := 5 \cdot \text{hp}$$

$$\text{Power}_4 := \text{Harvestmotor}_{5000} \cdot 7920 \cdot \text{hr}$$

$$\text{Power}_4 = 2.953 \cdot 10^4 \text{ kW} \cdot \text{hr}$$

$$\text{UtCost}_4 := \text{Power}_4 \cdot 0.04 \cdot \frac{1}{\text{kW} \cdot \text{hr}}$$

3

$$UtCost_3 = 2.362 \cdot 10^3$$

$$UtCost_4 = 1.181 \cdot 10^3$$

The power for the media blending tank is equivalent to the largest fermentor.

Blending Tank

$$Agitationmotor_{tank} := 10 \cdot hp$$

$$Power_5 := Agitationmotor_{tank} \cdot 7920 \cdot hr$$

$$Power_5 = 5.906 \cdot 10^4 \cdot kW \cdot hr$$

$$UtCost_5 := Power_5 \cdot 0.04 \cdot \left( \frac{1}{kW \cdot hr} \right)$$

$$UtCost_5 = 2.362 \cdot 10^3$$

Blending Tank Refrigerator

$$refrig := 8 \cdot ton$$

$$energytocool := refrig \cdot \left( 1 \cdot \frac{kW}{ton} \right) \cdot 7920 \cdot hr$$

$$energytocool = 6.336 \cdot 10^4 \cdot kW \cdot hr$$

$$energy_{cost} := energytocool \cdot 0.04 \cdot \left( \frac{1}{kW \cdot hr} \right)$$

$$energy_{cost} = 2.534 \cdot 10^3$$

Total Utility Requirements for the Fermentors, Pumps, and Blending Tanks:

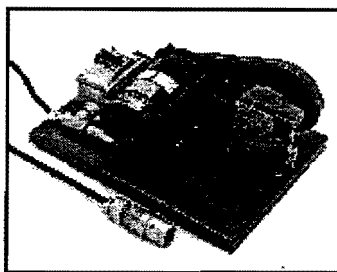
$$Total := totalUtility + Power_1 + Power_2 + Power_3 + Power_4 + Power_5 + energytocool \cdot 2$$

$$Total = 5.401 \cdot 10^5 \cdot kW \cdot hr$$

$$TotalCost := Total \cdot 0.04 \cdot \left( \frac{1}{kW \cdot hr} \right)$$

$$TotalCost = 2.161 \cdot 10^4$$

The total Energy Requirement (with a large over estimation) is  $5.4 \cdot 10^5$  kW\*hr per year and the cost of the electricity is \$21610/year.



Cat. No.  
500-200,  
610-200,  
750-200

### **Explosion Proof Model- Fixed Speed**

Cat. No. 500-200 shown

Cat. Nos. -200 are complete with pulley - belt - 1/4 hp - 115/volt - 60 hz - single phase - sleeve bearing - explosion proof motor and switch with 10 feet of flexible three wire electrical conduit and explosion proof plug. All class I group D electrical equipment. Units are mounted on a durable metal base. Series 750 model is equipped with 1/3 hp motor. Refer to page 8 for flow rates, specifications and shipping weight.

Dimensions: 500-200 - L 18-3/4" x W 12-1/2" H 8"  
610-200 - L 18-3/4" x W 12-1/2" H 8-1/2"  
750-200 - L 24-1/2" x W 14" x H 11-1/4"

### **Technical data**

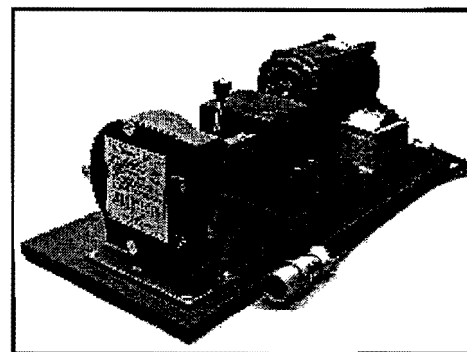
Cat. No.  
500-201,  
610-201,  
750-201

### **Explosion Proof Model and Speed Control**

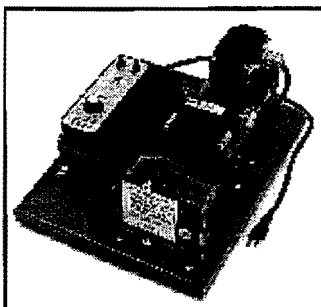
Cat. No. 750-201 shown

Cat. Nos. -201 are complete with a zero max speed control, 1/4 hp - 115/volt - 60 hz single phase - sleeve bearing - explosion proof motor and explosion proof switch with 10 feet of flexible three wire electrical conduit and explosion proof plug. All class I group D electrical equipment. Units are mounted on a durable metal base. Series 750 model is equipped with 1/2 hp motor. Refer to page 8 for flow rates, specifications and shipping weight.

Dimensions: 500-201 - L 25-1/2" x W 12" x H 8-3/4"  
610-201 - L 24-1/2" x W 12" x H 8-3/4"  
750-201 - L 32" x W 14" x H 12-1/2"



### **Technical data**



Cat. No.  
500-332, 500-342, 500-352, 500-362  
610-332, 610-342, 610-352, 610-362  
750-332, 750-342, 750-352, 750-362

### **Vari-flow DC Speed control Model**

Cat. No. 610-362 shown

The Vari-Flow models meet IPC-44 specifications and are available with a variety of DC motors, each spanning a specific rpm range. Units are complete



model					
500-400	Air drive model	10-90psi	7.13- 31.1	8.3- 56.5	17 lb.

**Back to Intermediate Volume Page**

## Series 610 - Models

Cat.#.	Model	Pump Speed rpm.	Motor hp.	Output gph		Ship. wt.
				Flow Rate 3/8" ID Tubing	1/2" ID Tubing	
610-PHO	Pumphead only					8 lb.
610-000	Pumphead and pulley					8 lb.
610-100	Std. model- single phase motor	430	1/4	125	200	29 lb.
610-101	Zero-max speed control model	0-400	1/4	0-116	0-186	40 lb.
610-200	Explosion proof model	430	1/4	125	200	69 lb.
610-201	Expl. proof model/speed control	0-400	1/4	0-116	0-186	75 lb.
610-332	Vari-flow speed control model	3-125	1/4	2.4-41	3.2-65	38 lb.
610-342	Vari-flow speed control model	4-165	1/4	3-56	4.7-86	38 lb.
610-352	Vari-flow speed control model	6-250	1/4	4.4-81	7-123	38 lb.
610-362	Vari-flow speed control model	12-500	1/4	8-174	12-255	38 lb.
610-400	Air drive model - ARO		10-90psi	28.4-150	35-225	19 lb.
610-425	Air drive model - Gast (Belt Drive)		10-90psi	30-155	40-230	35 lb.
610-450	Air drive model - Gast (Direct Drive)		10-90 psi	27-144	34-224	20 lb.

\$1595.00







**R.S.  
CORCORAN  
COMPANY**

*Problem  
Solver!*

### **Problem:**

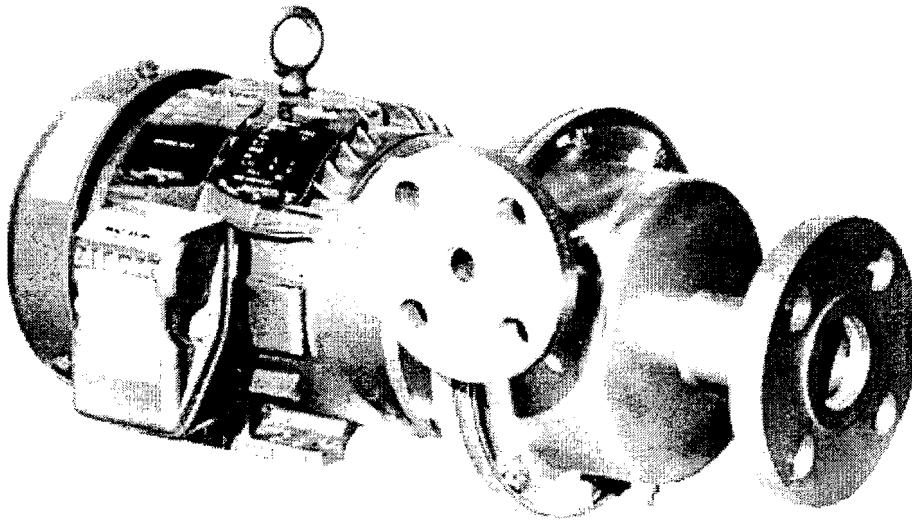
Shipboard "Plastics waste processor" required fresh water recirculation (up to 350°F) at 200 PSI inlet pressure at rates of 12 GPM @ 125 FT TH and 21 GPM @ 117 FT TH.

### **Solution:**

**Model:** 3000D-HD1 (AA05)

**Material of Construction:**  
316 Stainless Steel

**Size:** 1/4" x 1" RF FL (300#)



### **Features:**

1. Lightweight (65 lbs.) heavy-duty design: 350 PSI hydrostatic test
2. LOW FLOW/HIGH HEAD design: enclosed impeller (5.50" dia.) with 1/4" wide vanes (5)
3. Single internal mechanical seal: Carpenter 20 Cb-3 metal bellows, chemical grade carbon vs silicon carbide mating faces, EPDM elastomers. No stuffing box or chamber bore restrictions; no flush required
4. Deep-drawn and fabricated casing with self-venting top horizontal discharge and drain
5. Marine-duty chemical Processina hostile environment motor: 2 HP 3450 RPM, 230/460 VAC, TEFC, 1.15 SF, 145 TC frame with base, cast iron frame.

[Click here](#) to return to the Close Coupled Centrifugal Pumps page.

For Centrifugal Pumps, see:

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1. [Close Coupled Centrifugal Pumps](#)
2. [Pedestal Mount Centrifugal Pumps](#)
3. [Self Priming Centrifugal Pumps](#)
4. [Vertical Sump Pumps](#)
5. [Vertical Options](#)

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- [General Catalog of Corrosion-Resistant Pumps: Size and Styles](#)
- [Centrifugal Pumps](#)
- [Mag Drive Pumps](#)
- [Pump Questionnaire](#)

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 **R.S.  
CORCORAN  
COMPANY**  
P.O. BOX 429  
500 N. VINE STREET  
NEW LENOX, IL 60451-0429  
Phone: 815-485-2156  
Fax: 815-485-5840  
TOLL FREE: 800-637-1067

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Subject: Technical Question - Your E-mail, 28 Feb 00

Date: Wed, 1 Mar 2000 09:01:35 -0600

From: "Lewis, James" <JLewis@osmonics.com>

To: "'kasidit@seas.upenn.edu'" <kasidit@seas.upenn.edu>

Dear Mr. Nootong,

Thank you for inquiry regarding Osmonics' Pleated Filter Cartridges. For any of our cartridges, we recommend a maximum flow rate of 5 gpm/Ten Inch Equivalent (TIE) length. Thus, a 20" long cartridge would have a maximum flow rate of 10 gpm. The maximum can be reduced by other factors such as the micron rating of the cartridge, the solution viscosity and available pressure drop.

When we size a housing for a given filtration, typically we would include enough TIEs to keep the initial differential pressure at 2 psid or less. For the ~~Flotrex~~-AP021 cartridge this rate would be 5 gpm. For the ~~Mentrex~~-FE921, this rate would be about 3.5 gpm. In each case, a fluid with a viscosity of water was assumed. The data necessary for these calculations is available on our website, osmonics.com.

Please let us know if you have further questions. Good luck on your project.

Best regards,

Jim Lewis  
Application Engineer

Mentrex

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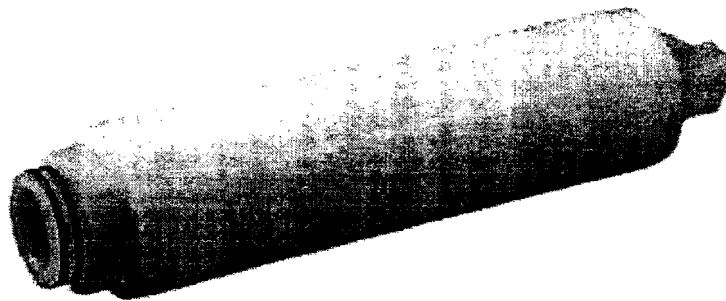


## Flotrex™-AP Filters Polypropylene Microfiber

### Product Information

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(0.65, 1, 2, 3, 5, 10, 20, and 40 µm Absolute Ratings)



Constructed of thermally-bonded polypropylene fiber media, absolute-rated Flotrex-AP (FAP) filters combine exceptional solids holding capacities with precise micron retention ratings. The FAP filters are constructed of high-purity polypropylene and are made with all FDA-acceptable materials.

FAP filters are absolute-rated for air, gas and liquid filtration with low pressure drop across the wide range of 0.65 to 40 micron. The graded sheets of melt-blown media are layered to provide absolute particle retention, high solids loading and long service life.

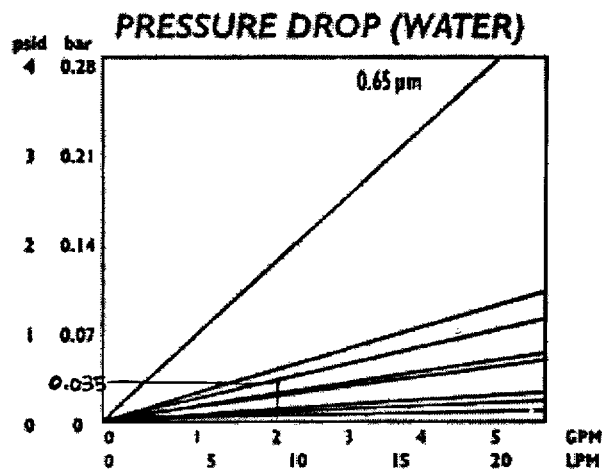
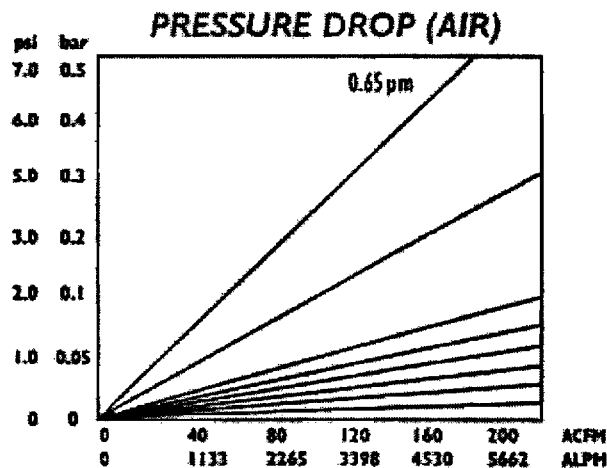
The FAP filter is just one example of our strong commitment to liquid, gas and air treatment. Our complete portfolio includes filters for every stage of processing, and we offer custom solutions for your unique applications. Osmonics is your complete source for filters, housings and other filtration equipment.

Whether you require an integrated solution or a single component for a specific application, look to Osmonics first. From one end of the filtration spectrum to the other, Osmonics has a total commitment to fluid purity.

### FAP Advantages

---

- Broad chemical compatibility
- Dependable protection for final filters
- Efficient removal of *Cryptosporidium* and *Giardia* cysts
- High throughput
- High efficiency
- Long service life
- Absolute rating (99.9%)
- Fast rinse up to 18 megohm
- Thermally-bonded polypropylene fiber media
- All FDA-acceptable materials



ACFM = Actual Cubic Feet per Min.

ACFM = SCFM at 70°F and 14.7 psia

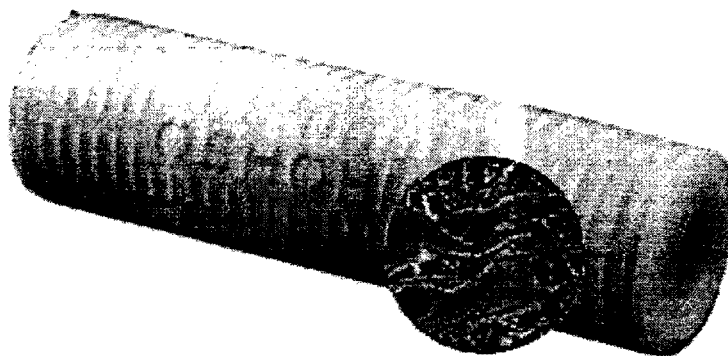
Note: For pressures and temperatures other than 14.7 psia (0 psig) and 70°F, ACFM can be approximated with the following formula:

$$\text{ACFM} = \text{SCFM} \left[ \frac{14.7 \text{ psia}}{(\text{psig} + 14.7)} \right] \left[ \frac{^{\circ}\text{F} + 460}{530} \right]$$

$$4^{\circ}\text{C} = 39.2^{\circ}\text{F}$$

$$\frac{(0.035)}{264} \left[ \frac{14.7}{0 + 14.7} \right] \left[ \frac{39.2 + 460}{530} \right] = 0.033 \text{ bar}$$

## Specifications



*Polypropylene Microfibers*

### **Effective Filtration Area for 10-inch Equivalent    Cartridge Dimensions (Nominal)**

0.65µm - 5.0 ft<sup>2</sup> (0.45 m<sup>2</sup>)

1µm - 5.0 ft<sup>2</sup> (0.45 m<sup>2</sup>)

2µm - 5.2 ft<sup>2</sup> (0.48 m<sup>2</sup>)

3µm - 5.2 ft<sup>2</sup> (0.48 m<sup>2</sup>)

5µm - 6.1 ft<sup>2</sup> (0.57 m<sup>2</sup>)

10µm - 6.7 ft<sup>2</sup> (0.62 m<sup>2</sup>)

20µm - 6.9 ft<sup>2</sup> (0.64 m<sup>2</sup>)

40µm - 7.2 ft<sup>2</sup> (0.67 m<sup>2</sup>)

Outside diameter -

2.75 inches (70 mm)

Inside diameter -

1.25 inch (31 mm)

### **Operational Data**

Maximum Rated Differential Pressure -

Forward flow - 60 psi (4.14 bar)

Reverse flow - 30 psi (2.07 bar)

Maximum Rated Operating Temperature -

180° F (82° C) at 10 psid

(0.69 bar) in water

Integrity test and particle retention data available on request.

## Applications

Flotrex-AP filters are specifically designed for pharmaceutical prefiltration. Typical

applications include:

**Prefiltration and Final Chemical Filtration**

- Broad chemical compatibility

**Bottled Water Final Filtration**

- Efficient removal of *Giardia* and *Cryptosporidium* cysts

**Prefiltration of Pharmaceuticals and Biological Fluids**

- Dependable protection for final filters

**High Throughput for Beer Filtration**

## Ordering Information

Type	Absolute Micron Rating	Cartridge Length	End #1 Adapter	End #2 Adapter	Elastomer Material
FAP	96 = 0.65µm 01 = 1.0µm 02 = 2.0µm 03 = 3.0µm 05 = 5.0µm 10 = 10.0µm 20 = 20.0µm 40 = 40.0µm	1 = 10 Inch 2 = 20 Inch 3 = 30 Inch 4 = 40 Inch	A = Open End Gasket B = 120 O-Ring C = 213 O-Ring E = 222 O-Ring F = 226 O-Ring J = 020 O-Ring Q = 222 O-Ring Stainless Steel Support Ring Z = 226 O-Ring Stainless Steel Support Ring	A = Open End Gasket B = 120 O-Ring C = 213 O-Ring G = Closed End Cap H = Fin Adapter	B = Buna-N E = EPDM S = Silicone T = Teflon* Encapsulated (C222 and 226 Sizes) V = Viton*

\* Viton and Teflon are registered trademarks of E.I. DuPont de Nemours and Company, Inc.

## Downloads



Flotrex-AP Filters Polypropylene Microfiber

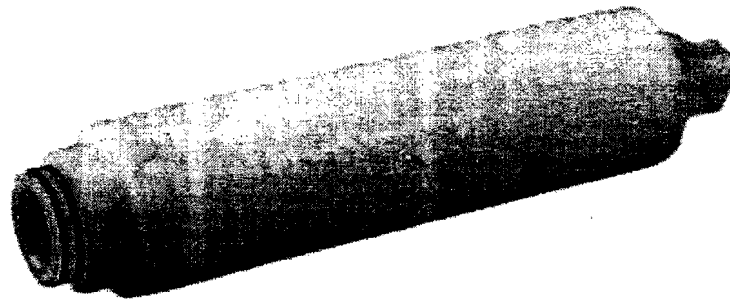


## Memtrex™-FE Filters PTFE Membrane

### Product Information

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(0.1, 0.2, 0.45, and 1µm Absolute Ratings)



Memtrex-FE (MFE) filters consistently achieve absolute filtration with low extractables. Constructed using a 100% PTFE membrane, they are especially designed for reliable general chemical and air filtration.

The PTFE membranes achieve absolute filtration and are ideal for aggressive chemicals. They are designed with all FDA-acceptable materials. MFE filters are ideal for air and process gas filtration and vent uses. These highly hydrophobic membranes from 0.1 to 1.0 micron provide critical control over your processes. MFE filters are integrity testable.

The MFE filter is just one example of our strong commitment to liquid, gas and air. Our complete portfolio includes filters for every stage of processing, and we offer custom solutions for your unique applications. Osmonics is your complete source for filters, housings and other filtration equipment.

Whether you require an integrated solution or a single component for a specific application, look to Osmonics first. From one end of the filtration spectrum to the other, Osmonics has a total commitment to fluid purity.

### MFE Advantages

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### Electronics Grade Chemical Filtration

- High purity

### Bulk Chemical Filtration

- Broad chemical compatibility

### Acids, Base, and Oxidant Filtration

- Highly hydrophobic membrane
- High air flow

### Process Air and Gas Filtration

- Excellent vent filter

## Ordering Information

Type	Absolute Micron Rating	Cartridge Length	End #1 Adapter	End #2 Adapter	Elastomer Material
MFE	91 = 0.1µm 92 = 0.2µm 94 = 0.45µm 01 = 1.0µm	1 = 10 Inch 2 = 20 Inch 3 = 30 Inch 4 = 40 Inch	A = Open End Gasket B = 120 O-Ring C = 213 O-Ring E = 222 O-Ring F = 226 O-Ring J = 020 O-Ring Q = 222 O-Ring Stainless Steel Support Ring Z = 226 O-Ring Stainless Steel Support Ring	A = Open End Gasket B = 120 O-Ring C = 213 O-Ring G = Closed End Cap H = Fin Adapter	B = Buna-N E = EPDM S = Silicone T = Teflon* Encapsulated (Only in 222 and 226 Sizes) V = Viton*

\* Viton and Teflon are registered trademarks of E.I. DuPont de Nemours and Company, Inc.

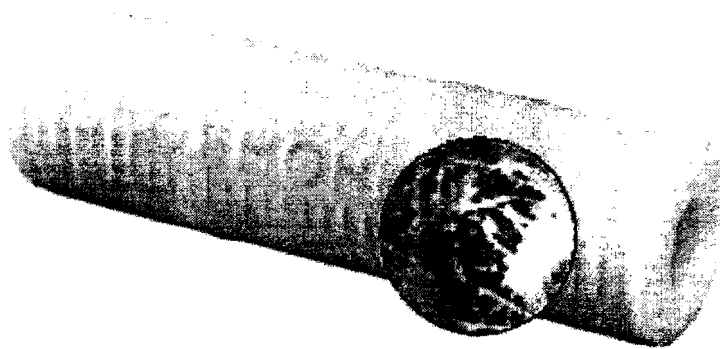
## Downloads



Memtrex™- FE Filters PTFE Membrane

## Associated Pages

## Specifications



*PTFE Membrane*

### **Effective Filtration Area for 10-inch Equivalent**

0.1, 0.2, 0.45, and 1.0 $\mu$ m - 6.6 ft<sup>2</sup> (0.52 m<sup>2</sup>)

### **Operational Data**

Maximum Rated Differential Pressure -

Forward flow - 60 psi (4.14 bar)

Reverse flow - 30 psi (2.07 bar)

Maximum Rated Operating Temperature -

180°F (82°C) at 10 psid  
(0.69 bar) in water

### **Cartridge Dimensions (Nominal)**

Outside diameter - 2.75 inches (70 mm)

Inside diameter - 1.25 inch (31 mm)

### **Sterilization**

Autoclave at 250°F (121°C) -Maximum  
10 hours

Steam-in-Place at 257°F (125°C) -  
Maximum 10 hours

### **Integrity Test: Diffusional Flow (60% IPA)**

0.1 $\mu$ m - <15 cc/min at 20 psi (1.38 bar)

0.2 $\mu$ m - <15 cc/min at 13 psi (0.90 bar)

0.45 $\mu$ m - <10 cc/min at 9 psi (0.62 bar)

1.0 $\mu$ m - <8 cc/min at 5 psi (0.34 bar)

Retention data available on request.

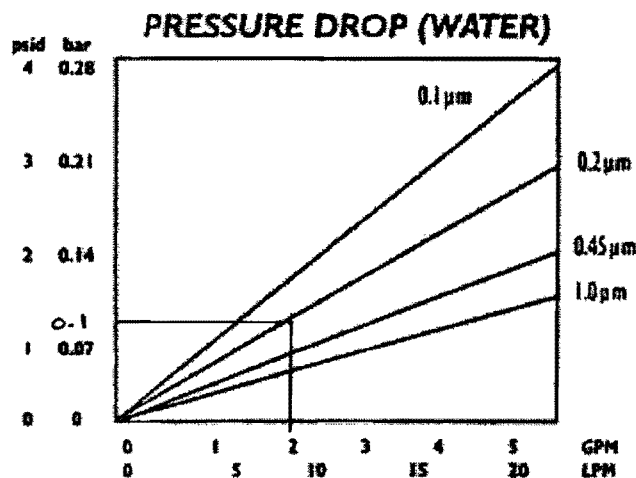
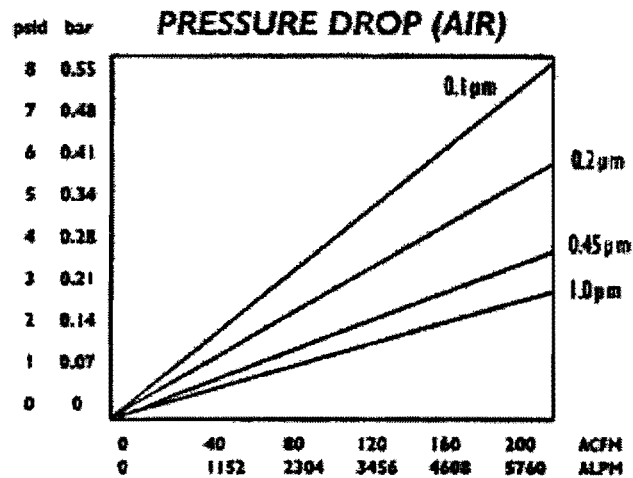
## Applications

Memtrex-FE filters are specifically designed for chemical and vent filtration. Typical applications include:

269

- PTFE membrane from Gore\*
- Low extractables
- Ideal for aggressive chemicals
- Ideal for vent filtration
- All FDA-acceptable materials
- Highly hydrophobic membrane

\* Gore is a registered trademark of W.L. Gore & Associates, Inc.



ACFM = Actual Cubic Feet per Min.

ACFM = SCFM at 70° and 14.7 psia

Note: For pressures and temperatures other than 14.7 psia (0 psig) and 70°, ACFM can be approximated with the following formula:

$$\text{ACFM} = \text{SCFM} \left[ \frac{14.7 \text{ psia}}{(\text{psig} + 14.7)} \right] \left[ \frac{^{\circ}\text{F} + 460}{530} \right]$$

$$0.1 \left[ \frac{14.7}{0 + 14.7} \right] \left[ \frac{39.2 + 460}{530} \right] =$$

$$4^{\circ}\text{C} = 39.2^{\circ}\text{F}$$

\*\*\*Correspondence with Hyclone Media Company. All other correspondence occurred over the phone with Neil Collins.\*\*\*

From: mark.wight@perbio.com  
X-Lotus-FromDomain: PPUS22@PPUS35@PIERCE  
To: maudette@seas.upenn.edu  
Date: Mon, 6 Mar 2000 09:12:30 -0700  
Subject: Request for information  
Content-Disposition: inline  
X-Status:  
X-Keywords:  
X-UID: 33

Melissa,

Thanks for visiting the HyClone website and for your request for information. Neil Collins would best address your questions about the mixing tank and I will give you his email and contact information below. Another option for you to consider would be to allow HyClone to produce the media for you and deliver it in BioProcess Containers up to 900L in volume. Either way we would be happy to work with you.

Here is Neil's contact information:  
Here is Neil's contact information:

Neil Collins  
neil.collins@perbio.com  
800-492-5663 ext. 7182

Please feel free to contact me as well:

Mark Wight  
Technical Services  
HyClone Labs  
mark.wight@perbio.com  
800-492-5663 ext. 7223

Thanks  
Mark

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HyQ PF-CHO is a protein-free medium designed for growing suspension cultures of Chinese Hamster Ovary (CHO-K1) cells as well as many derivatives of this parent line. HyQ PF-CHO has been specifically engineered for minimal adaptation, making it an excellent choice for producing and purifying recombinant proteins. Cell densities obtained using HyQ PF-CHO compare favorably to those of serum-free and serum-containing media. This product has been designed to decrease the cost of manufacturing and purifying recombinant proteins.



## ✓ HyQ PF-CHO MPS™

HyQ PF-CHO Multi-Powder System is a two-powder protein-free medium designed for the suspension growth of Chinese Hamster Ovary (CHO) cells. The medium is designed to maintain high cell densities and high protein output.



HyQ PF-CHO MPS is ideal for large scale recombinant protein production because it contains only minimal animal-derived material and plant hydrolysates with no peptides >10,000 Daltons MW.

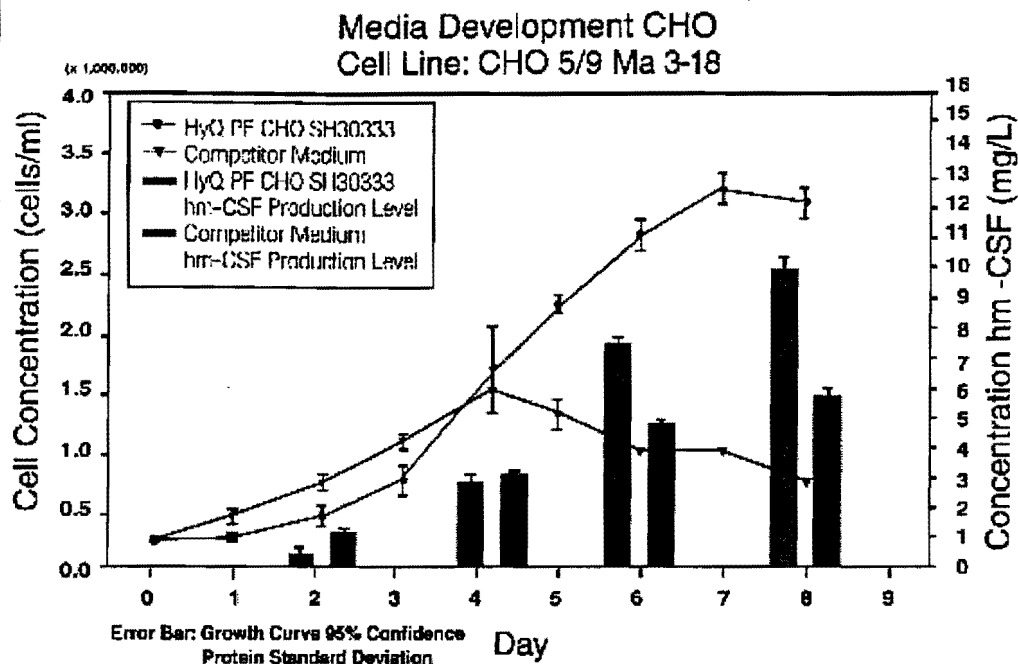
HyQ PF-CHO MPS gives you the convenience and stability of a dry powder medium. The two-powder system is economical for large volume users. Powders are packaged in matching aliquots so no measuring is required and hydration is easy.

HyQ PF-CHO MPS can be used with Dihydro Folate Reductase selection systems because HyQ PF-CHO MPS contains no glutamine and is deficient in hypoxanthine and thymidine.

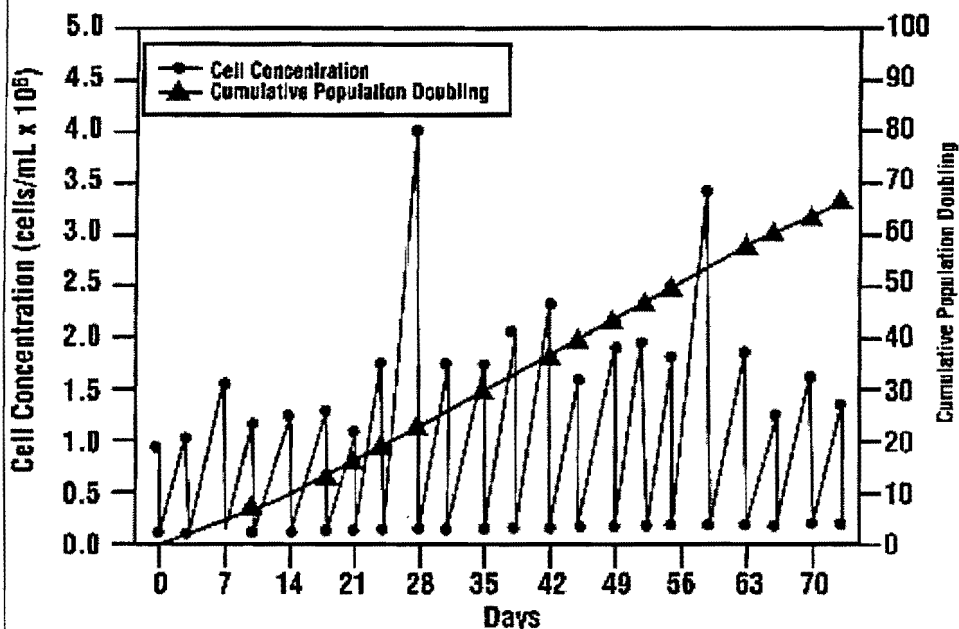
HyQ PF-CHO Multi-Powder System performs as well as HyClone's liquid medium (SH30220) and supports greater cell growth and sustained viability compared to competitive products. Cells maintained in HyQ PF-CHO MPS show consistent performance over the long term. Population doubling times were constant over more than 10 weeks with cell viability over 90%.

Product	PF-CHO MPS : Protein-free medium for CHO cells - Multi-powder system
Catalog Number	SH30333
Packaging	5L to 1000L
Animal Components	Cholesterol from sheep's wool, Cod liver oil
Cell Density	>3.0 x 10 <sup>6</sup> /ml in 5 days; viability >95%
Selection system	Dihydro Folate Reductase, Glutamine Synthetase
Nutrition	thymidine, hypoxanthine, deficient, <u>glutamine free</u>
Protein Expression	>5 mg/L
Longevity	cumulative population doubling >90 days
Adaptation	minimal

HyQ PF-CHO MPS supports higher cell density and longer sustained viability than the competitive product.

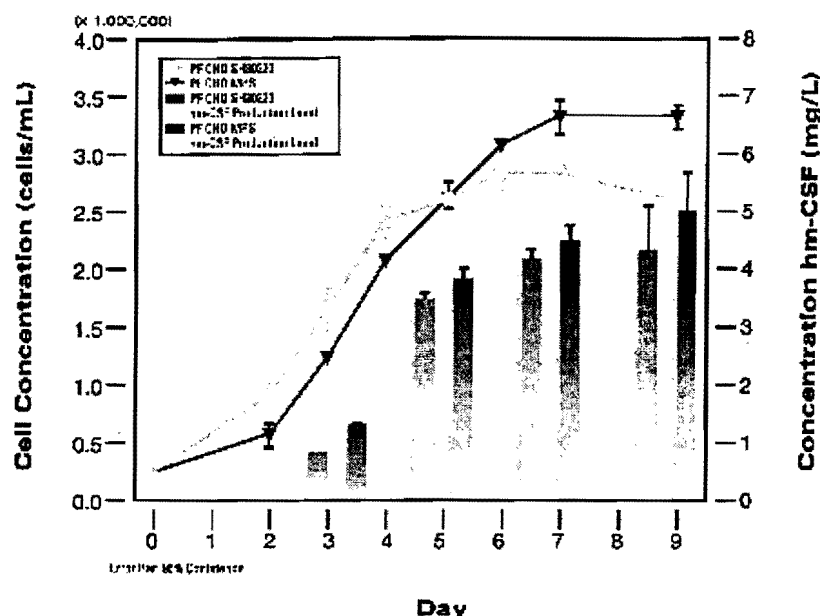


Cells maintained in HyQ PF-CHO show consistent performance over the long term. Population doubling times were constant over more than 10 weeks with cells viability over 90%



The multi-powder system is composed of two parts - main powder and base powder. The powders are packaged in matching aliquots so no measuring is required. Sodium bicarbon (2g/L) is added when the medium is hydrated to achieve a pH of 7.2-7.8. No other supplies are required, but some cell clones may show improved growth with addition of glutamine

+Pluronic F-68®. For culture systems which require more buffering capacity, HEPES may be added (up to 25 mM).



Comparison of cell growth and protein (hm-CSF) production in HyQ PF-CHO MPS and HyQ PF-liquid medium (Catalog No. SH30220)

### Features and Benefits of HyQ PF-CHO Multi-Powder System

Dry powder system	Stability, economy, and convenience of a powder
Multi-powder system	Two powder components combined without the need to measure makes hydration easy.
Proprietary lipid blend	Powder is stable and the requirement to add supplements is limited.
HyClone powdered medium	Lots of up to 400,000 L-eq. can be produced.
No glutamine	Medium can be used with glutamine synthetase (GS) selection. Glutamine levels can be adjusted for individual cell line requirements.
Deficient in hypoxanthine and thymidine	Medium may be used with dihydrofolate reductase (DHFR) selection.
No Pluronic F-68	Pluronic may be added for suspension cultures that require extra surfactant.
Metabolically designed	All components of the medium are identified for simplified protein purification.
Simplified medium composition	Supplements may be added to meet requirements of particular cell lines.
Protein-free	Medium components will not interfere with recombinant protein purification.

## Pure Gases



To Order Call:

**1-800-BOC-GASES**

## Carbon Dioxide CO<sub>2</sub>

A colorless, odorless, liquefied gas. Also available in bulk quantities and tube trailers.

	Purity	Cylinder Size*		Contents @ NTP		Pressure (psig)	Valve Outlet	Equipment Recommendations
		U.S.	Can.	lb	kg	@70 °F		
<b>Spectra-Clean® Grade 5<sup>TM</sup></b>	99.999%	152	152	40	18.14	838	CGA 320	<b>Regulator</b> 1 Stage Brass, Model BHS 500 1 Stage High Flow Model BSR 310
<b>Research Grade 5<sup>TM</sup></b>	99.999%	152	152	40	18.14	838	CGA 320	
<b>Pure-Clean Grade 4.5</b>	99.995%	200	200	60	27.22	838	CGA 320	
<b>Lasershield</b>	99.99%	See Lasershield Gases Section						<b>Purge</b> Brass Deep Purge Model 4810
	99.995%							
<b>Coleman Grade 4<sup>TM</sup></b>	99.99%	200	K	60***	27.22	838	CGA 320	<b>Filter</b> Brass 10 micron, Model 7510
<b>Anaerobic Grade 4<sup>TM</sup></b>	99.99%	200	K	60***	27.22	838	CGA 320	
<b>Bone Dry Grade 2.8<sup>TM</sup></b>	99.8%	200	K	60***	27.22	838	CGA 320	<b>Flowmeter</b> Model B7920 (choose flowtube for required flowrate)
		L.B.		0.5	0.23	838	CGA 170	
<b>Liquid</b>	99.8%	Various sizes and volumes available upon request.						

\* Other sizes available upon request.



<b>Purity Specifications (ppm unless otherwise noted)</b>	<b>Grade</b>	<b>Minimum Purity</b>	<b>Maximum Impurities (ppm)</b>	<b>CO</b>	<b>N<sub>2</sub></b>	<b>O<sub>2</sub></b>	<b>THC</b>	<b>H<sub>2</sub>O</b>
	Spectra-Clean®	99.999%	10		4	1	0.5	2
	Research	99.999%	10	1	8	2	0.5	2
	Pure-Clean	99.995%	50				5	5
	Coleman**	99.99%			70	20	10	10
	Anaerobic	99.99%				10		
	2.8**	99.8%						

**Technical Data:**

<b>Mol. Wt.</b>	44.01
<b>Boiling Point</b>	-109.3 °F (-78.4 °C)
<b>Sp. Volume</b>	8.74 cf/lb (0.547 m3/kg)
<b>Critical Temp.</b>	87.8 °F (31 °C)
<b>Critical Press</b>	1071 psia (7381.5 kPa)
<b>Flammable Limits</b>	Nonflammable
<b>Toxicity</b>	TLV 5000 ppm
<b>Compatibility</b>	Moisture promotes corrosion

**Shipping Information:**

<b>DOT/TDG Name</b>	Carbon Dioxide
<b>Hazard/TDG Class</b>	2.2
<b>ID No.</b>	UN 1013
<b>DOT/TDG Label</b>	Nonflammable Gas
<b>WHMIS</b>	Class A, D2B
<b>CAS No.</b>	124-38-9
<b>MSDS No.</b>	G-8

\* Available with a dip tube upon request.

\*\* Liquid phase.

\*\*\* In Canada volume is 50 lbs (22.68 kg).



To Order Call:

**1-800-BOC-GASES**

**Air** Also available in bulk quantities, tube trailers and aluminum cylinders.

	Cylinder Size*		Contents @ NTP		Pressure (psig)	Valve Outlet***	Equipment Recommendations
	U.S.	Can.	cf	m3	@70 °F		
Zero 0.1™	300	T	310	8.70	2640	CGA 590	<b>Regulator</b> 2 Stage Brass Model BHT 500 1 Stage Brass Model BHS 500
	200	K	230	6.38	2200	CGA 590	
		Q	86	2.38	2200	CGA 590	
TOC Grade*	300	T	310	8.70	2640	CGA 590	<b>High Pressure</b> 1 Stage Brass, Model BHS 4 Lecture Bottle, Model 325
	200	K	230	6.38	2200	CGA 590	
		Q	86	2.38	2200	CGA 590	
Zero Ambient	See Environmental Section						
Vehicle Emission**Zero	300	T	310	8.70	2640	CGA 590	<b>Filter</b> 10 micron, Model 7520
	200	K	230	6.38	2200	CGA 590	
		Q	86	2.38	2200	CGA 590	
Zero 1.0™	300	T	310	8.70	2640	CGA 590	<b>Flowmeter</b> Model B7920 (choose flowtube for required flowrate)
	200	K	230	6.38	2200	CGA 590	
		Q	86	2.38	2200	CGA 590	
CO <sub>2</sub> Free	300	T	310	8.70	2640	CGA 590	
	200	K	230	6.38	2200	CGA 590	
		Q	86	2.38	2200	CGA 590	
Zero 2.0™	300	T	310	8.70	2640	CGA 590	
	200	K	230	6.38	2200	CGA 590	
		Q	86	2.38	2200	CGA 590	
Dry	L.B.		2	.06	1775	CGA 170	
	300	T	310	8.70	2640	CGA 590	
	200	K	230	6.38	2200	CGA 590	
		Q	86	2.38	2200	CGA 590	
High Pressure	500		2	.06	1775	CGA 170	
			502	13.92	6000	CGA 702	

\*\*\* Other sizes available upon request.

<b>Purity Specifications</b> (ppm unless otherwise noted)	<b>Grade</b>	<b>CO</b>	<b>CO<sub>2</sub></b>	<b>NOX</b>	<b>THC</b>	<b>H<sub>2</sub>O</b>
	Zero 0.1				0.1	3
	TOC Grade	0.5	0.5		0.1	
	Vehicle Emission	1	400	0.1	1	
	Zero 1.0				1	3
	CO <sub>2</sub> Free		1			
	Zero 2.0				2	
	Dry					10

**Technical Data:**

<b>Mol. Wt.</b>	28.97
<b>Sp. Volume</b>	13.3 cf/lb (0.833 m <sup>3</sup> /kg)
<b>Critical Temp</b>	-221.1 °F (140.6 °C)
<b>Critical Press</b>	546.8 psia (3774 kPa)
<b>Flammable Limits</b>	Supports Combustion
<b>Toxicity</b>	Nontoxic
<b>Compatibility</b>	Noncorrosive

**Shipping Information:**

<b>DOT/TDG Name</b>	Air, Compressed
<b>Hazard/TDG Class</b>	2.2
<b>ID No.</b>	UN 1002
<b>DOT/TDG Label</b>	Nonflammable Gas
<b>WHMIS Class</b>	A
<b>CAS No.</b>	(O <sub>2</sub> ) 7782-44-7 (N <sub>2</sub> ) 7727-37-9
<b>MSDS No.</b>	G-113

\* TOC Grade is used on a Total Organic Carbon analyzer which measures carbon compounds in water.

\*\* Prepared and certified to meet Federal Register Specifications, Title 40 CFR 86.114-78.

\*\*\* The Valve Outlet for Canada is CGA 346.

**BOILER.ICS (Project Summary)**

ITEM	UNITS	VALUE
PROJECT INFORMATION		
Project Name		TPA-2
Project Description		
Analysis Date and Time		Thu Apr 20 14:08:39 2000
Simulator Type		AspenTech
Simulator Version		10.1-0 Build 25--
Simulator Report File		C:\boiler.rep
Simulator Report Date		Sunday, April 09, 2000
Economic Analysis Type		IPE
IPE Version		5
Project Directory		C:\PROJECTS.IPE\Projects\TPA-2
Scenario Name		
Scenario Description		

**CAPITAL COST EVALUATION BASIS**

Date		20-Apr-00
Country		US
Units of Measure		I-P
Currency (Cost) Symbol		U.S. DOLLAR
Currency Conversion Rate	USD/U.S. DOLLAR	1
System Cost Base Date		1Q 98
Project Type		Grass roots/Clear field
Design code		ASME
Prepared By		IPE 5.0 User
Plant Location		North America
Capacity		1.#INF
Time Difference Between System Cost Base Date and Start Date for Engineering	Days	276
User Currency Name		USD
User Currency Description		US Dollar
User Currency Symbol		USD

**PROJECT RESULTS SUMMARY**

Total Project Capital Cost	Cost	1.12E+05
Total Raw Materials Cost	Cost/period	0
Total Products Sales	Cost/period	0
Total Operating Labor and Maintenance Cost	Cost/period	440000
Total Utilities Cost	Cost/period	10682.3
Total Operating Cost	Cost/period	843137
Operating Labor Cost	Cost/period	440000
Maintenance Cost	Cost/period	0
Operating Charges	Cost/period	110000
Plant Overhead	Cost/period	220000
Subtotal Operating Cost	Cost/period	780682
G and A Cost		62454.6

~AP3F.tmp

Aspen stream report for the design of the plant steam boiler:

1 2

---

STREAM ID	1	2
FROM :	----	B1
TO :	B1	----
SUBSTREAM: MIXED		
PHASE:	LIQUID	VAPOR
COMPONENTS: LBMOL/HR		
WATER	61.0849	61.0849
TOTAL FLOW:		
LBMOL/HR	61.0849	61.0849
LB/HR	1100.4617	1100.4617
CUFT/HR	17.6573	1.2148+04
STATE VARIABLES:		
TEMP F	68.0000	266.0000
PRES PSI	14.7000	39.1600
VFRAC	0.0	1.0000
LFRAC	1.0000	0.0
SFRAC	0.0	0.0
ENTHALPY:		
BTU/LBMOL	-1.2297+05	-1.0243+05
BTU/LB	-6825.8988	-5685.8179
BTU/HR	-7.5116+06	-6.2570+06
ENTROPY:		
BTU/LBMOL-R	-39.1336	-10.0992
BTU/LB-R	-2.1722	-0.5605
DENSITY:		
LBMOL/CUFT	3.4594	5.0286-03
LB/CUFT	62.3232	9.0592-02
AVG MW	18.0152	18.0152

~AP3D.tmp

Aspen Block Report for the plant wide steam boiler:

BLOCK: B1            MODEL: HEATER

-----  
 INLET STREAM:            1  
 OUTLET STREAM:           2  
 PROPERTY OPTION SET:    IDEAL        IDEAL LIQUID / IDEAL GAS

	***	MASS AND ENERGY BALANCE	***	
		IN	OUT	RELATIV
E DIFF.				
TOTAL BALANCE				
MOLE(LBMOL/HR)		61.0849	61.0849	0.0000
00E+00				
MASS(LB/HR )		1100.46	1100.46	0.0000
00E+00				
ENTHALPY(BTU/HR )		-0.751164E+07	-0.625703E+07	-0.1670
23				

	***	INPUT DATA	***	
ONE PHASE TP FLASH		SPECIFIED PHASE IS	VAPOR	
SPECIFIED TEMPERATURE		F		266.00
0				
SPECIFIED PRESSURE		PSI		39.16
00				
MAXIMUM NO. ITERATIONS				30
CONVERGENCE TOLERANCE				0.00
010000				

	***	RESULTS	***	
OUTLET TEMPERATURE		F		266.00
OUTLET PRESSURE		PSI		39.160
HEAT DUTY		BTU/HR		0.12546E+
07				



THE FOLLOWING IS A PRINTOUT OF THE SUPERPRO DESIGNER STREAM REPORT.  
IT PROVIDES COMPONENT FLOWS FOR STREAMS THROUGHOUT THE SEPARATION SECTION.

BULK RAW MATERIAL REQUIREMENTS PER SECTION

SECTIONS IN: Main Branch

Separation Section

Raw Material	kg/Year	kg/Batch	kg/kg MP
Arginine	72491.27	1066.048	N/A
WFI	20811.12	306.046	N/A
Glycine	540.29	7.945	N/A
NaOH (1 M)	13106.55	192.743	N/A
Media	242386.00	3564.500	N/A
Biomass	31094.70	457.275	N/A
tPA	146.88	2.160	N/A
Sodium Chloride	4500.97	66.191	N/A
PBS	116234.76	1709.335	N/A
Sucrose	1.75	0.026	N/A
Section Total	501314.31	7372.269	N/A

SUMMARY (Entire Flowsheet)

Raw Material	kg/Year	kg/Batch	kg/kg MP
Arginine	72491.27	1066.048	N/A
WFI	20811.12	306.046	N/A
Glycine	540.29	7.945	N/A
NaOH (1 M)	13106.55	192.743	N/A
Media	242386.00	3564.500	N/A
Biomass	31094.70	457.275	N/A
tPA	146.88	2.160	N/A
Sodium Chloride	4500.97	66.191	N/A
PBS	116234.76	1709.335	N/A
Sucrose	1.75	0.026	N/A
Flowsheet Total	501314.31	7372.269	N/A



## BREAKDOWN PER RAW MATERIAL AND SECTION (kg/batch)

Raw Material	Separation Section	Subtotal
Arginine	1066.048	1066.048
WFI	306.046	306.046
Glycine	7.945	7.945
NaOH (1 M)	192.743	192.743
Media	3564.500	3564.500
Biomass	457.275	457.275
tPA	2.160	2.160
Sodium Chloride	66.191	66.191
PBS	1709.335	1709.335
Sucrose	0.026	0.026
TOTAL	7372.269	7372.269

## BREAKDOWN PER RAW MATERIAL AND SECTION (kg/year)

Raw Material	Separation Section	Subtotal
Arginine	72491.3	72491.3
WFI	20811.1	20811.1
Glycine	540.3	540.3
NaOH (1 M)	13106.6	13106.6
Media	242386.0	242386.0
Biomass	31094.7	31094.7
tPA	146.9	146.9
Sodium Chloride	4501.0	4501.0
PBS	116234.8	116234.8
Sucrose	1.8	1.8
TOTAL	501314.3	501314.3

## COMPONENT BALANCE AND STREAM REPORT

STREAM NAME	P-2	P-11	P-16	P-19	P-20
SOURCE	Centrifuge	UF2 Pump	INPUT Affinity Co	ER Tank	INPUT
DESTINATION	OUTPUT	UF2 Affinity Co			ER Tank

## STREAM PROPERTIES

ACTIVITY	U/ml	0.0	0.0	0.0	0.0	0.0
TEMP	°C	4.0	5.7	4.0	4.0	25.0
PRES	bar	1.0	3.0	1.0	1.0	1.0
DENSITY	g/l	1050.0	1150.5	1187.7	1186.8	1043.0

## COMPONENT FLOWRATES (kg/Batch)

Arginine	0.0000	115.1645	148.6561	114.3509	0.0000
Biomass	457.2750	0.0000	0.0000	0.0000	0.0000
Glycine	0.0000	0.0000	7.9455	6.1119	0.0000
Media	0.3565	200.3153	0.0000	0.0000	0.0000
Sodium Hydroxid	0.0000	0.0000	0.0000	0.0000	0.0126
tPA	0.1080	1.9375	0.0000	1.6068	0.0000
Water	0.0000	0.0000	0.0000	0.0000	0.3154
WFI	0.0000	0.0000	199.9221	153.7862	0.0000

TOTAL (kg/batch)	457.7394	317.4173	356.5237	275.8558	0.3280
TOTAL (m3/batch)	0.4360	0.2759	0.3002	0.2324	0.0003

STREAM NAME	P-4	P-27	P-25	P-7	P-5
SOURCE	INPUT	INPUT	ER Column	UF1	UF1 Tank
DESTINATION	UF1 Tank	MF Tank	MF Tank	OUTPUT	UF1 Pump

## STREAM PROPERTIES

ACTIVITY	U/ml	0.0	0.0	0.0	0.0	0.0
TEMP	°C	4.0	25.0	25.0	4.0	30.6
PRES	bar	1.0	1.0	1.0	3.0	1.0
DENSITY	g/l	1562.0	1000.0	1186.6	1074.4	1074.4

## COMPONENT FLOWRATES (kg/Batch)

Arginine	850.0000	0.0000	114.3509	802.2275	850.0000
Glycine	0.0000	0.0000	6.1119	0.0000	0.0000
Media	0.0000	0.0000	0.0000	3363.8282	3564.1436
Sodium Hydroxid	0.0000	0.0000	0.0126	0.0000	0.0000
Sucrose	0.0000	0.0000	0.0258	0.0000	0.0000
tPA	0.0000	0.0000	1.6028	0.1145	2.0520
Water	0.0000	0.0000	0.3154	0.0000	0.0000
WFI	0.0000	59.0000	153.7862	0.0000	0.0000

```
=====
TOTAL (kg/batch)      850.0000      59.0000      276.2056      4166.1702      4416.1956
TOTAL (m3/batch)      0.5442      0.0590      0.2328      3.8775      4.1103
=====
```

```
=====
STREAM NAME           P-8           P-10           P-13           P-14           P-22
SOURCE                UF1          UF2 Tank       UF2 Affinity Ta  INPUT
DESTINATION           UF2 Tank     UF2 Pump Affinity Ta Affinity Co  ER Column
=====
```

## STREAM PROPERTIES

```
ACTIVITY  U/ml      0.0      0.0      0.0      0.0      0.0
TEMP      °C        4.0      4.0      4.0      4.0      4.0
PRES      bar        3.0      1.0      3.0      1.0      1.0
DENSITY   g/l       1074.2   1150.5   1150.5   1150.5   1043.0
```

## COMPONENT FLOWRATES (kg/Batch)

```
Arginine      47.7725   115.1645   112.8646   112.8646   0.0000
Media         200.3153   200.3153   196.3148   196.3148   0.0000
Sodium Hydroxid 0.0000     0.0000     0.0000     0.0000     7.3888
tPA           1.9375     1.9375     1.8904     1.8904     0.0000
Water         0.0000     0.0000     0.0000     0.0000    185.0267
```

```
=====
TOTAL (kg/batch)      250.0253   317.4173   311.0698   311.0698   192.4154
TOTAL (m3/batch)      0.2327     0.2759     0.2704     0.2704     0.1845
=====
```

```
=====
STREAM NAME           P-23           P-32           P-31           P-1           P-18
SOURCE                INPUT Microfilter Microfilter  INPUT Affinity Co
DESTINATION           ER Column     FD Tank       OUTPUT Centrifuge  OUTPUT
=====
```

## STREAM PROPERTIES

```
ACTIVITY  U/ml      0.0      0.0      0.0      0.0      0.0
TEMP      °C        4.0      4.0      4.0      37.0     4.0
PRES      bar        1.0      2.3      2.3      1.0      1.0
DENSITY   g/l       1000.0   1148.9   1148.8   1005.5   1047.0
```

## COMPONENT FLOWRATES (kg/Batch)

```
Arginine      0.0000   114.3497   0.0011   0.0000   147.1698
Biomass        0.0000     0.0000     0.0000   457.2750   0.0000
Glycine        0.0000     6.1119     0.0001   0.0000   1.8336
KCl            0.0000     0.0000     0.0000   0.0000   0.0034
KH2PO4         0.0000     0.0000     0.0000   0.0000   0.0034
Media          0.0000     0.0000     0.0000  3564.5000  196.3148
Na2HPO4        0.0000     0.0000     0.0000   0.0000   1.8803
Sodium Chloride 0.0000     0.0000     0.0000   0.0000   79.8655
Sodium Hydroxid 0.0000     0.0126     0.0000   0.0000   0.0000
Sucrose        0.0000     0.0258     0.0000   0.0000   0.0000
tPA            0.0000     1.6028     0.0000   2.1600   0.2836
Water          0.0000     0.3154     0.0000   0.0000   0.0000
WFI            47.1239   212.7841   0.0021   0.0000  1739.9087
```

TOTAL (kg/batch)	47.1239	335.2022	0.0034	4023.9350	2167.2631
TOTAL (m3/batch)	0.0471	0.2918	0.0000	4.0021	2.0699

STREAM NAME	P-24	P-15	P-17	P-12	P-3
SOURCE	ER Column	INPUT	INPUT	UF2	Centrifuge
DESTINATION	OUTPUT	Affinity Co	Affinity Co	OUTPUT	UF1 Tank

## STREAM PROPERTIES

ACTIVITY	U/ml	0.0	0.0	0.0	0.0
TEMP	°C	4.0	4.0	4.0	37.0
PRES	bar	1.0	1.0	1.0	1.0
DENSITY	g/l	1016.6	1020.5	1020.5	1150.4

## COMPONENT FLOWRATES (kg/Batch)

Arginine	0.0000	0.0000	0.0000	2.2999	0.0000
KCl	0.0000	0.0011	0.0023	0.0000	0.0000
KH2PO4	0.0000	0.0011	0.0023	0.0000	0.0000
Media	0.0000	0.0000	0.0000	4.0005	3564.1436
Na2HPO4	0.0000	0.6268	1.2535	0.0000	0.0000
Sodium Chloride	0.0000	26.6218	53.2436	0.0000	0.0000
Sodium Hydroxid	7.3888	0.0000	0.0000	0.0000	0.0000
tPA	0.0040	0.0000	0.0000	0.0471	2.0520
Water	185.0267	0.0000	0.0000	0.0000	0.0000
WFI	47.1239	564.5910	1129.1819	0.0000	0.0000

TOTAL (kg/batch)	239.5433	591.8418	1183.6836	6.3475	3566.1956
TOTAL (m3/batch)	0.2356	0.5800	1.1599	0.0055	3.5661

STREAM NAME	P-6	P-21	P-30	P-33	P-35
SOURCE	UF1 Pump	ER Tank	MF Pump	FD Tank	Freeze Drye
DESTINATION	UF1	ER Column	Microfilter	Freeze Drye	OUTPUT

## STREAM PROPERTIES

ACTIVITY	U/ml	0.0	0.0	0.0	0.0
TEMP	°C	32.2	4.0	5.1	4.0
PRES	bar	3.0	1.0	2.3	1.0
DENSITY	g/l	1074.4	1186.6	1148.9	1148.9

## COMPONENT FLOWRATES (kg/Batch)

Arginine	850.0000	114.3509	114.3509	114.3497	0.0000
Glycine	0.0000	6.1119	6.1119	6.1119	0.0000
Media	3564.1436	0.0000	0.0000	0.0000	0.0000
Sodium Hydroxid	0.0000	0.0126	0.0126	0.0126	0.0000
Sucrose	0.0000	0.0258	0.0258	0.0258	0.0000
tPA	2.0520	1.6068	1.6028	1.6028	0.0000
Water	0.0000	0.3154	0.3154	0.3154	0.3154
WFI	0.0000	153.7862	212.7862	212.7841	212.7841

TOTAL (kg/batch)	4416.1956	276.2096	335.2056	335.2022	213.0995
TOTAL (m3/batch)	4.1103	0.2328	0.2918	0.2918	0.2131

STREAM NAME	P-29	P-36	P-9	P-26
SOURCE	MF Tank	Freeze Drye	INPUT	INPUT
DESTINATION	MF Pump	OUTPUT	UF2 Tank	ER Tank

## STREAM PROPERTIES

ACTIVITY	U/ml	0.0	0.0	0.0	0.0
TEMP	°C	4.0	12.0	4.0	4.0
PRES	bar	1.0	1.0	1.0	1.0
DENSITY	g/l	1148.9	1552.1	1562.0	1581.0

## COMPONENT FLOWRATES (kg/Batch)

Arginine	114.3509	114.3497	67.3920	0.0000
Glycine	6.1119	6.1119	0.0000	0.0000
Sodium Hydroxid	0.0126	0.0126	0.0000	0.0000
Sucrose	0.0258	0.0258	0.0000	0.0258
tPA	1.6028	1.6028	0.0000	0.0000
Water	0.3154	0.0000	0.0000	0.0000
WFI	212.7862	0.0000	0.0000	0.0000

TOTAL (kg/batch)	335.2056	122.1027	67.3920	0.0258
TOTAL (m3/batch)	0.2918	0.0787	0.0431	0.0000

## OVERALL COMPONENT BALANCE (kg/Batch)

COMPONENT	IN	OUT	(OUT-IN)
Arginine	1066.048129	1066.048129	0.000000
Biomass	457.275000	457.275000	0.000000
Glycine	7.945491	7.945491	0.000000
KCl	0.003419	0.003419	0.000000
KH2PO4	0.003419	0.003419	0.000000
Media	3564.500000	3564.500000	0.000000
Na2HPO4	1.880268	1.880268	0.000000
Sodium Chloride	79.865456	79.865456	0.000000
Sodium Hydroxid	7.401347	7.401347	0.000000
Sucrose	0.025800	0.025800	-0.000000
tPA	2.160000	2.160000	0.000000
Water	185.342066	185.342066	0.000000
WFI	1999.818810	1999.818810	0.000000
TOTAL	7372.269205	7372.269205	0.000000

**Subject: Re: Senior Design Project**

**Date:** Thu, 02 Mar 2000 13:55:03 +0000

**From:** Scott Diamond <sld@seas.upenn.edu>

**To:** Christian M Metallo <cmetallo@seas.upenn.edu>

tPA is shipped at 100-retPA per bottle. When the tPA is dissolved in 100 of sterile water, the result is 2 M arginine, I think. I do not know the sucrose level but I suspect that it is between 10 uM and 200 uM sucrose.

As long as the final salt concentration after dissolving the tPA in 100 m of sterile water is less than 150 uM it will look like a balanced saline solution suitable for injection.

corrected to 150mM in personal communication w/ Prof. Diamond.

Dr. Diamond

Christian M Metallo wrote:

> Prof. Diamond,  
> Do you know where I can find info on the exact packing "recipe" for tPA  
> I need to know how much tPA per dose. I'm assuming the rest would be  
> sucrose and arginine. There would also be some salts left from adjusti  
> the pH after the anion exchange. Will that be a problem?  
>  
> Please get back to me when you get a chance.  
> Thanks  
>  
> Chris



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Product Number: **A6969**Product Name: **L-Arginine Hydrochloride****Product Information**

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Select Items	Quantity	Package Sizes	US \$
<input type="checkbox"/>	1	25G	10.30
<input type="checkbox"/>	1	100G	25.35
<input type="checkbox"/>	1	1KG	139.95 (0.85) = \$119

**Add To Basket****Ordering FAQ****Molecular Formula:**  $C_6H_{14}N_4O_2 \cdot HCl$ **Molecular Weight:** 210.6**CAS:** 1119-34-2**Purity Grade:** Biotechnology Performance Certified, EP, JP, USP,  $\geq 98.5\%$ **Quality/Application:** Cell culture tested**Form/Aspect:** Powder**Assay:**  $\geq 98.5\%$ **Comments: Source:** Non-animal source

Soluble in water (100 mg/ml).

Endotoxin tested



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Product Number: **G8790**Product Name: **Glycine****Product Information**

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Select Items	Quantity	Package Sizes	US \$
<input type="checkbox"/>	1	100G	19.35
<input type="checkbox"/>	1	1KG	50.25 (.55) = \$42.71
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**Synonyms:** Aminoacetic acid

Aminoethanoic acid

Glycocoli

**Molecular Formula:** C<sub>2</sub>H<sub>5</sub>NO<sub>2</sub>**Molecular Weight:** 75.07**CAS:** 56-40-6**Purity Grade:** Biotechnology Performance Certified, >=98.5%**Quality/Application:** Cell culture tested**Form/Aspect:** Powder**Assay:** >=98.5%**Comments:** **Source:** Non-animal source

Soluble in water (100 mg/ml).

Endotoxin tested





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Product Number: **84105**Product Name: **D(+)-Sucrose****Product Information**

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Select Items	Quantity	Package Sizes	US \$
<input type="checkbox"/>	1	1KG	9.50
<input type="checkbox"/>	1	5KG	28.50
<a href="#">Add To Basket</a>		<a href="#">Ordering FAQ</a>	

**Synonyms:** Beet sugar; Cane sugar; $\beta$ -D-Fructofuranosyl- $\alpha$ -D-glucopyranoside; D(+)-Saccharose; Sugar**Molecular Formula:** C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>**Molecular Weight:** 342.30**CAS:** 57-50-1**Purity Grade:** BioChemika for biotechnological purposes;  $\geq 98.0\%$  (HPLC)**Comments:** $\alpha^{20}_{546}$  ... +78.0 $\pm$  1° (c= 26 in H<sub>2</sub>O) $\alpha^{20}_D$  ... +66.5 $\pm$  1° (c= 26 in H<sub>2</sub>O)Solubility (0.1 g/ml H<sub>2</sub>O) ... soluble

mp ... ~185°C (dec.) (Lit.)

**ELINCS/EINECS Number:** 2003349**BRN:** 90825**Merck Index:** 12, 9051**Beilstein Index:** 17, 8, V, 399**R&S:** F: 3**Compliance:** RTECS WN6500000 • WGK 0**Miscellaneous:** Mono- and oligosaccharides; Culture media, additives for~~28.50~~ = ~~24.23~~~~24.546/kg~~

↓



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Product Number: **D7030**Product Name: **Dulbecco's Phosphate Buffered Saline Hybri-Max®****Product Information**

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Select Items	Quantity	Package Sizes	US \$
<input type="checkbox"/>	1	10L	9.40 (0.85) = \$ 7.99
<input type="checkbox"/>	1	10X1L	13.10
<b>Add To Basket</b>		<b>Ordering FAQ</b>	

**Synonyms:** D-PBS**Quality/Application:** Hybridoma tested.**Form/Aspect:** Powder**Comments:** Without calcium chloride and magnesium chloride. Endotoxin tested.

Formulated to contain 9.6 grams of powder per liter of medium.

**Storage Temp:** Store at 2-8°C**R&S:** R: 36/37/38, S: 26,36

\$0.799/L



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Product Number: **S5881**Product Name: **Sodium hydroxide****Product Information**

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Select Items	Quantity	Package Sizes	US \$
<input type="checkbox"/>	<input type="text" value="1"/>	500G	19.70
<input type="checkbox"/>	<input type="text" value="1"/>	1KG	35.45
<input type="checkbox"/>	<input type="text" value="1"/>	5KG	103.20

**Add To Basket****Ordering FAQ**

**Molecular Formula:** NaOH  
**Molecular Weight:** 40  
**CAS:** 1310-73-2  
**Purity Grade:** Minimum 98%  
**Form/Aspect:** Pellets  
**Assay:** Minimum 98%  
**Comments:** Bulk packages  
**Storage Temp:** Store at RT.  
**R&S:** R: 35, S: 26,37/39,45

$$103.20 \text{ (US)} \sim \$87.72$$

$$\$17.544/\text{kg}$$



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Product Number: **71376**Product Name: **Sodium chloride****Product Information**

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Select Items	Quantity	Package Sizes	US \$
<input type="checkbox"/>	1	1KG	35.30
<input type="checkbox"/>	1	5KG	106.15
<b>Add To Basket</b>		<b>Ordering FAQ</b>	

**Molecular Formula:** ClNa**Molecular Weight:** 58.44**CAS:** 7647-14-5**Purity Grade:** BioChemika MicroSelect for molecular biology; ≥ 99.5% (AT)**Comments:** Sales restrictions may apply**pH** (1 M in H<sub>2</sub>O, 25°C) ... 5.0-8.0**Solubility** (1 M in H<sub>2</sub>O, 20°C) ... complete, colorless**Insoluble matter:** ... passes filter test**DNases, RNases:** ... none detected**Proteases, phosphatases:** ... none detected**Total nitrogen (N)** ... ≤ 0.001%

<b>Bromide (Br)</b>	≤ 0.005%	<b>Fe</b>	≤ 0.0001%
---------------------	----------	-----------	-----------

<b>Iodide (I)</b>	≤ 0.001%	<b>K</b>	≤ 0.005%
-------------------	----------	----------	----------

<b>Phosph. (PO<sub>4</sub>)</b>	≤ 0.0005%	<b>Li</b>	≤ 0.0005%
---------------------------------	-----------	-----------	-----------

<b>Sulfate (SO<sub>4</sub>)</b>	≤ 0.01%	<b>Mg</b>	≤ 0.0005%
---------------------------------	---------	-----------	-----------

<b>Al</b>	≤ 0.0005%	<b>Mn</b>	≤ 0.0005%
-----------	-----------	-----------	-----------

<b>As</b>	≤ 0.00001%	<b>Mo</b>	≤ 0.0005%
-----------	------------	-----------	-----------

<b>Ba</b>	≤ 0.0005%	<b>Ni</b>	≤ 0.0005%
-----------	-----------	-----------	-----------

<b>Bi</b>	≤ 0.0005%	<b>Pb</b>	≤ 0.0005%
-----------	-----------	-----------	-----------

<b>Ca</b>	≤ 0.001%	<b>Sr</b>	≤ 0.0005%
-----------	----------	-----------	-----------

<b>Cd</b>	≤ 0.0005%	<b>Zn</b>	≤ 0.0005%
-----------	-----------	-----------	-----------

<b>Co</b>	≤ 0.0005%	<b>Absorption</b>	1 M in H <sub>2</sub> O
-----------	-----------	-------------------	-------------------------

<b>Cr</b>	≤ 0.0005%	<b>λ(nm): 260</b>	<b>A<sub>max</sub>: 0.01</b>
-----------	-----------	-------------------	------------------------------

<b>Cu</b>	≤ 0.0005%	<b>λ(nm): 280</b>	<b>A<sub>max</sub>: 0.01</b>
-----------	-----------	-------------------	------------------------------

**ELINCS/EINECS Number:** 2315983**Merck Index:** 12, 8742

**Literature References:** Component of lysis buffer and extraction buffer in the large-scale phenol extraction of RNA: D.M. Wallace, Meths. Enzymol. 152, 39 (1987); Simple salting out procedure for extracting DNA from human nucleated cells: S.A. Miller, et al., Nucleic Acids Res. 16, 1215 (1988)

**Compliance:** RTECS VZ4725000 • CH-Giftkl. free • WGK 0

**Miscellaneous:** Density gradient centrifugation; Crystallization of biopolymers; Sodium salts (MicroSelect); Molecular biology, common reagents

18.046/kg

**Westfalia Separator,**

# Fax

**To:** Christian Metallo **From:** Derek Ettie 201-784-6477  
**Fax:** 215-573-7601 **Pages:** 1  
**Phone:** **Date:** 03/29/00  
**Re:** CHO cells **CC:**

☐ Urgent ☐ For Review ☐ Please Comment ☐ Please Reply ☐ Please Recycle

• **Comments:**

PRICE FOR CSC 4 IS \$355,000 SKID MOUNTED , MOTOR AND CONTROLS. CAPACITY OF THIS UNIT IS 400-500 L/HR.

ANY QUESTIONS PLEASE CALL ME

DEE1@WSUS.COM

Design CalculationsSeparation Section  
Centrifuge

~ 4000 L cell suspension enters holding tank  
approx. cell diameter =  $40 \times 10^{-4}$  cm (40 microns)

volume of one cell:  $\frac{4}{3} \pi (20 \times 10^{-4})^3 = 3.35 \times 10^{-8} \text{ cm}^3 \approx 3.35 \times 10^{-8} \text{ mL}$   
(assuming spherical)

$$4 \times 10^6 \text{ mL cell susp.} \times \frac{3.25 \times 10^6 \text{ cells}}{\text{mL cell susp.}} \times \frac{3.35 \times 10^{-8} \text{ mL}}{\text{cell}} \times \frac{\text{L}}{1000 \text{ mL}} = 435.5 \text{ L of cells}$$

435.5 L of cell waste

Volume of Effluent

$$4000 - 435.5 = 3564.5 \text{ L of media}$$

~~centrifuge capacity (w/ 3-channel centripetal pump):  
Westfalia quotes it at ~ 300 L/hr~~

~~I will use a value of 200 L/hr.~~

This was for inclusion bodies. For CHO cells,  
Westfalia quoted 400-500 L/hr

$$\sim \frac{4000 \text{ L}}{400 \text{ L}} \times \frac{\text{hr}}{1} \approx 10 \text{ hrs of centrifugation}$$

### Data Sheet

# CSC 4 Clarifier

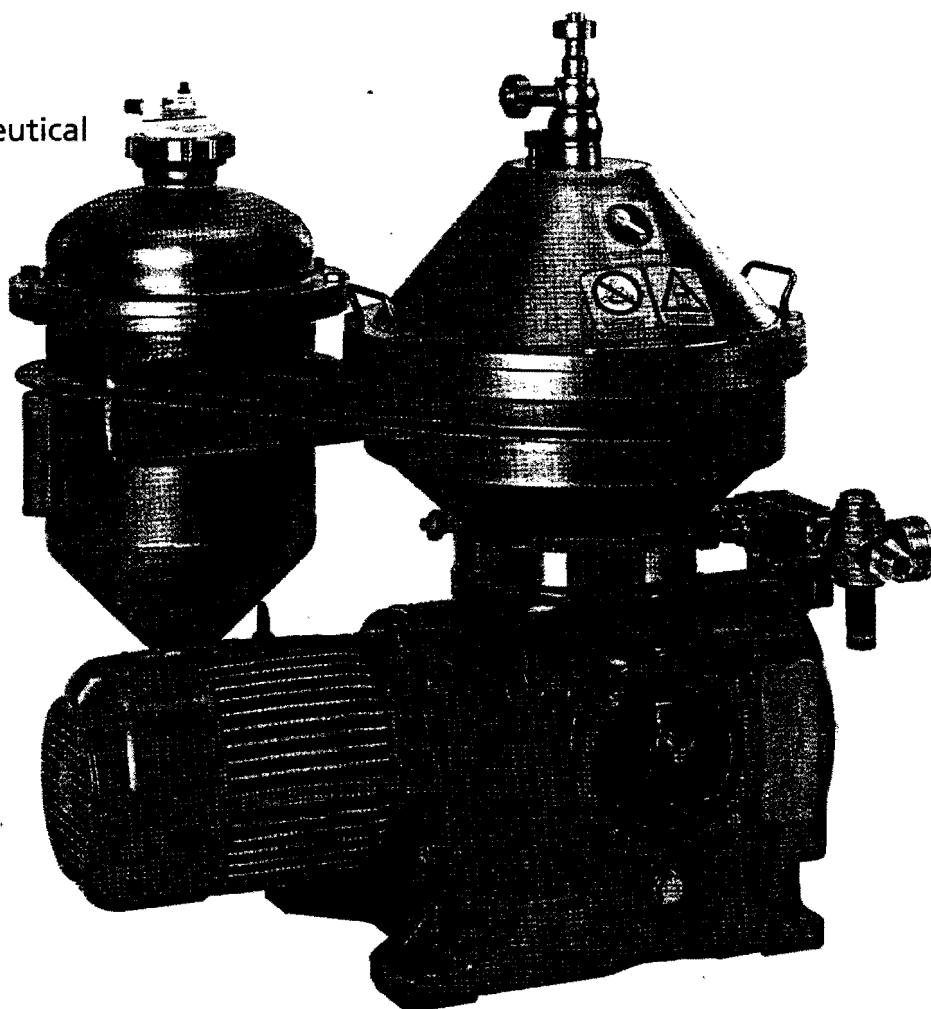
## In steam-sterilizable design

CSC 4-06-476

Complete plant systems  
with automatic or manual SIP  
for down-stream processing  
of fermentation broths under  
sterile conditions

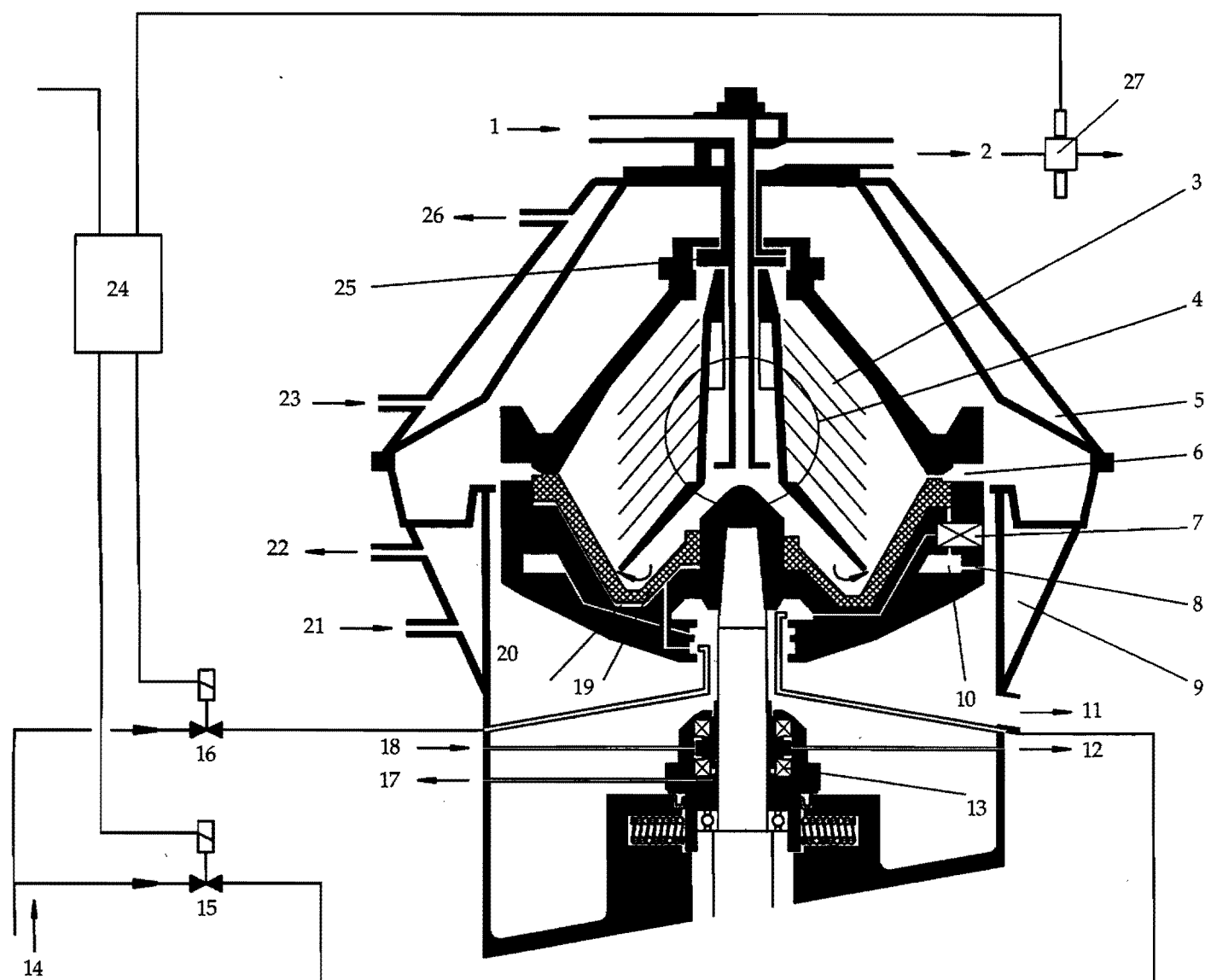
### Fields of application

Biotechnology, pharmaceutical  
and food industry



# Operating principles and constructional features

## CSC 4-06-476 Bowl with turbidity measurements



- |                              |  |
|------------------------------|--|
| 1 Feed                       | 15 Valve (closing and pre-filling)     |
| 2 Discharge                  | 16 Valve (Opening)                     |
| 3 Disc stack                 | 17 Leakage outlet                      |
| 4 Hydrohermetic feed system  | 18 Sealing liquid feed                 |
| 5 Cooling, hood              | 19 Sliding piston                      |
| 6 Sediment ejection ports    | 20 Closing chamber                     |
| 7 Annular valve              | 21 Feed, solids collector cooling      |
| 8 Discharge nozzle           | 22 Discharge, solids collector cooling |
| 9 Cooling, solids collector  | 23 Feed, hood cooling                  |
| 10 Storage chamber           | 24 Control unit                        |
| 11 Operating water discharge | 25 Centripetal pump                    |
| 12 Sealing liquid discharge  | 26 Discharge, hood cooling             |
| 13 Slide-ring packing        | 27 Turbidity meter                     |
| 14 Operating-water feed      |  |



## Typical product applications

- Starter cultures for the food and pharmaceutical sectors (e.g. lactic acid bacteria).
- Recovery of biomass for pharmaceutical products (e.g. E-Coli).
- Treatment of genetically engineered micro-organisms and vaccines, made possible by the closed machine and installation concept (biocontainment).
- Gentle discharge of biomass with product-damaging cell constituents.

This clarifier incorporates the very latest developments in centrifuge construction.

The basic features of this new development are:

- Clarifier bowl in sanitary design.
- Cleaning-in-place - capacity of the clarifier.
- Steam-sterilizable (SIP) up to 1.5 bar steam pressure (127 °C).
- Double-acting slide-ring packing as interface to the drive chamber.
- Closed product feed and gentle treatment of shear-sensitive micro-organisms due to the hydrohermetic feed system.
- No product contamination due to wear of lip seals and mechanical seals as is the case with hermetically sealed machines.
- Low noise pollution due to double-walled coolable hood and solids collector suitable for connection to a closed sealing system up to 1 bar overpressure.
- "Hydrostop" system for controlled partial solids ejections.

## Bowl

The product enters the bowl via the inlet (1) and is clarified in this disc stack (3). Centripetal pump (25) then conveys the clarified liquid under pressure to outlet (2), where it is discharged without foam.

The separated solids collect in sediment holding space and are ejected periodically via ports (6).

Operating water is used only during the actual process of ejection.

## Automatic solids ejection

Bowl ejections are controlled automatically by control unit (24)

The following operations are possible:

- partial ejections
- a combination of short and long partial ejections
- Displacement of the liquid phase from the bowl before a total ejection
- Flush ejection after every total ejection

## Control systems

The following systems are available for controlling the automatic bowl ejections:

- Time-dependent control to suit the particular operation. Recommended for use with products in which the solids content remains constant.

- Photoelectric control using a turbidity measurement (21) to monitor the clarified liquid. If a pre-set turbidity level is exceeded, a signal is passed to the control unit (24) which then initiates the solids ejection process. Recommended for use with translucent liquids in which the solids content is not constant or if the throughput capacity varies. This monitoring system can be installed on every standard centrifuge.

## The "hydrostop" system for controlled partial solids ejections

When product is processed, only partial solids ejections are performed.

The solids ejections is initiated by the control unit.

In the case of partial solids ejection, the hydraulically operated sliding piston (19) must be opened within as short a time as possible so as to ensure that the ejection ports are opened wide enough to allow unimpeded solids ejection.

The sliding piston (19) is in closed position (left side of figure) when the closing chamber (20) is full. The annular valve (7) is hydraulically opened via valve (16) (right side of fig.). The operating-water flows from the closing chamber (20) into the storage chamber (10). When the storage chamber (10) is full, the flow of liquid from the closing chamber (20) will automatically stop, although the annular piston (7) is still open ("hydrostop" system). The bowl then opens and the solids are discharged rapidly through the ejection ports (6).

The amount of solids ejected depends on the liquid level in the storage chamber (10) (controlled partial solids ejection). The amount of solids to be ejected can be pre-selected by partially filling the storage chamber (10) before the solids ejection is initiated. This is done by opening valve (15).

After the ejection process, the closing chamber (20) is topped up via valve (15). The annular valve (7) then closes. The storage chamber empties through discharge nozzle (8). This new "hydrostop" system reduces the actual solids ejection time to less than 1/10 second. In the case of solids which are difficult to eject, a longer solids ejection cycle is initiated after several partial solids ejections, which has the effect of flushing out remaining solids while the product feed is still open.

## Total solids ejection sequence

For total solids ejection, the closing chamber (20) is emptied via the annular valve (7) and the nozzle (8) by the addition of operating-water via valve (15). The sliding piston (19) remains open until the whole bowl contents have been ejected. The sliding piston (19) is then closed by supplying closing water via valve (15).

## Feed and discharge

The product is fed into the centrifuge by means of a closed system of pipes. The clarified liquid is discharged foam-free and under pressure via a centripetal pump.

## Hydrohermetic product feed

In this design the centrifuge is equipped with a hydrohermetic product feed (4). This new inlet system prevents shearing forces from acting on the product when entering the bowl. Similar as with the fully hermetic design, the product stream is accelerated by the product itself in the filled bowl. This makes for gentle treatment and optimum clarifying efficiency, especially in the case of sensitive products and low throughput capacities.

## Cooling

The solids collector and the hood are of double-wall design for cooling the discharged solids (5, 9).

## Cleaning-in-place (CIP)

Once the centrifugation process has been completed, the machine can be cleaned-in-place. The cleaning solution is circulated round the centrifuge and the connected system. Total ejections are initiated during CIP manually or automatically on the control unit via an external CIP control.

## Sterilization

The stationary installation is sterilized with hot steam (127 °C) under an excess pressure of 1.5 bar. The duration of sterilization is dependent on the nature of the bacteria and the number of germs. Experience suggests a time between 60 minutes.

The condensate is conveyed from the lowest point of the system to the killing line.

Following sterilization, the whole system is blanketed with sterile air until the next product run.

## Frame and drive

The cast-iron frame is equipped with brakes, a rev indicator and an oil level sight glass.

The machine is driven by a three-phase AC motor with frequency converter for smooth starting. Power is transferred to the bowl spindle via a flexible coupling and worm wheel gear. All bearings and the gear are splashlubricated from a central oil bath.

## Monitoring

- Lube oil sight glass
- Temperature feeler and overcurrent release for motor protection in case of overload \*
- Speed measuring device
- Vibration monitoring system \*
- Turbidity meter for monitoring the discharge \*
- Level probes in the solids tank in case of enclosed solids discharge

\*Additional parts available at extra charge

## Materials

All parts coming into contact with the product are made of austenitic Cr-Ni-Mo stainless steel or, in the case of highly stressed bowl parts, of soft martensitic Cr-Ni-Mo steel or duplex alloys.

The gaskets are made of ethylene-propylene-diene-caoutchouc (EPDM) and Polyamid.

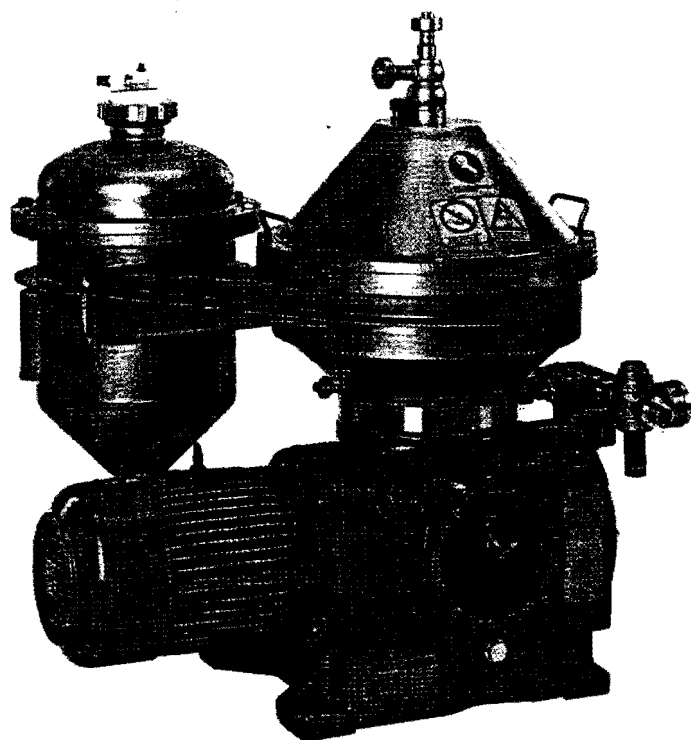
## Assembly and dismantling

Special tools for installing and removing the bowl are included in the supply schedule.

## Additional equipment (available at extra charge)

Complete plant systems with automatic or manual SIP

# Technical data



Height 900 mm  
Width 1000 mm  
Depth 750 mm

## Technical data

Bowl	
Speed	up to 12000 min <sup>-1</sup>
Total bowl volume	1.8 l
Volumen of sediment holding space	0.7 l
Max. pressure produced by centripetal pump	approx. 5 bar
Three-phase AC motor	
Motor power	up to 7.5 kW
Speed at 87 Hz	3000 min <sup>-1</sup>
Type	IM B5
Type of protection	IP 55

## Weights

Separator complete	approx. 230 kg
Bowl	approx. 50 kg

## Capacity

Rated capacity	
with one channel centripetal pump	approx. 100 l/h
with three channel centripetal pump	approx. 300 l/h

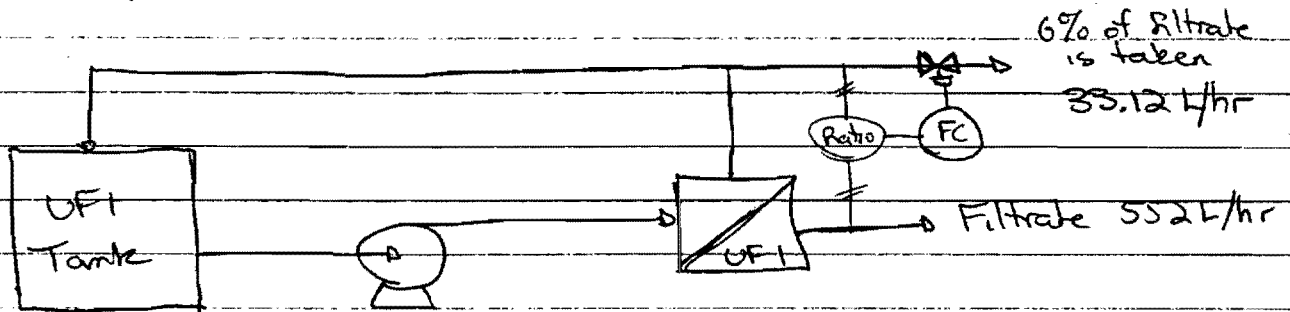
The rated capacity indicates the maximum throughput rate of the bowl. The actual operating capacity is usually lower. It depends on the particular product and on the required level of clarification.

Subject to modification

UF1 Calcs

Filtrate flux  $\geq 250 \frac{\text{gal}}{\text{ft}^2 \cdot \text{day}}$  given  $14 \text{ ft}^2$  membrane  
 $\text{Flux} = 552 \text{ L/hr}$

Unit-op looks like:



33.12 L/hr is taken off as retentate  
 The rest is recycled  
 total outflow is 585.12 L/hr

$$3564.5 \text{ L} \times \frac{\text{hr}}{585.12 \text{ L}} = \underline{6.09 \text{ hrs per batch}}$$

$$(6.09 \text{ hr}) (33.12 \frac{\text{L}}{\text{hr}}) = \underline{201.7 \text{ L to UF2 Tank}}$$

UF2

Filtrate is collected

Flux rated at  $\geq 400 \frac{\text{gal}}{\text{ft}^2 \cdot \text{day}}$

$14 \text{ ft}^2$  membrane yields a flux = 883.3 L/hr

Retentate is completely recycled

$$201.7 \text{ L} \times \frac{\text{hr}}{883.3 \text{ L}} = \underline{0.228 \text{ hrs per batch}}$$

## KOCH PRODUCT SPECIFICATIONS

PRODUCT TYPE: HF 14-43-PM50

PART NUMBER: 0720082

DATE: 7/18/95

REVISION: 1

CONFIGURATION: 3" X 25" HOLLOW FIBER CARTRIDGE

### COMPONENTS:

MEMBRANE:	POLYSULFONE
HOUSING SHELL/END CAPS:	POLYSULFONE
CONWED:	YELLOW
FDA STATUS:	APPROVED MATERIALS OF CONSTRUCTION

### DIMENSIONS/PROPERTIES:

MODULE DIAMETER:	3.0"	(7.6 cm)
MODULE LENGTH:	25"	(63.5 cm)
MEMBRANE AREA (NOMINAL):	<u>14 ft<sup>2</sup></u>	(1.3 m <sup>2</sup> )
FIBER COUNT	MAXIMUM	660
	MINIMUM	625

MEAN WATER FLUX @ 25/15 psi, 45°C:

≥ 250 GFD Gal/ft/day

TYPICAL RANGE:

(Based on New Membrane Using RO Water)

permeate

FEED FLOW VS. PRESSURE DROP:

$$Q = 12.1261 \times \ln(\Delta P) - 5.1685 \quad \}$$

RECOMMENDED OPERATING LIMITS (PROCESS TECHNOLOGY MUST APPROVE/DEFINE OPERATING AND CLEANING CONDITIONS FOR ALL NEW PLANTS AS WELL AS ANY CHANGES TO EXISTING PLANTS):

MAXIMUM INLET PRESSURE @ 25°C:	40 psi	2.43 gal/min
MAXIMUM TRANSMEMBRANE PRESSURE @ 25°C:	35 psi	
MAXIMUM BACKFLUSH PRESSURE @ 25°C:	20 psi	
MAXIMUM TEMPERATURE AT pH 6:	140°F (60°C)	
MAXIMUM pH RANGE AT 130°F (54°C):	1.5-13.0	
MAXIMUM CHLORINE (CLEANING):	200 ppm @ pH 10-10.5, 130°F (54°C)	
	0 ppm @ pH less than 9.5	

$$\frac{250}{140} \times \frac{1440 \times}{14 \text{ ft}^2} = 250$$

304

$$\lambda = \frac{\text{gal}}{\text{min}}$$

## KOCH PRODUCT SPECIFICATIONS

PRODUCT TYPE: HF 14-43-PM500

PART NUMBER: 0720083

DATE: 7/18/95

REVISION: 1

CONFIGURATION: 3" X 25" HOLLOW FIBER CARTRIDGE

### COMPONENTS:

MEMBRANE:	POLYSULFONE
HOUSING SHELL/END CAPS:	POLYSULFONE
CONWED:	YELLOW
FDA STATUS:	APPROVED MATERIALS OF CONSTRUCTION

### DIMENSIONS/PROPERTIES:

MODULE DIAMETER:	3.0"	(7.6 cm)
MODULE LENGTH:	25"	(63.5 cm)
MEMBRANE AREA (NOMINAL):	14 ft <sup>2</sup>	(1.3 m <sup>2</sup> )
FIBER COUNT	MAXIMUM	690
	MINIMUM	650

MEAN WATER FLUX @ 25/15 psi, 45°C:  $\geq 400$  GFD

TYPICAL RANGE:  
(Based on New Membrane Using RO Water)

FEED FLOW VS. PRESSURE DROP:  $Q = 12.6419 \times \ln(\Delta P) - 5.3779$

RECOMMENDED OPERATING LIMITS (PROCESS TECHNOLOGY MUST APPROVE/DEFINE OPERATING AND CLEANING CONDITIONS FOR ALL NEW PLANTS AS WELL AS ANY CHANGES TO EXISTING PLANTS):

MAXIMUM INLET PRESSURE @ 25°C:	40 psi
MAXIMUM TRANSMEMBRANE PRESSURE @ 25°C:	35 psi
MAXIMUM BACKFLUSH PRESSURE @ 25°C:	20 psi
MAXIMUM TEMPERATURE AT pH 6:	140°F (60°C)
MAXIMUM pH RANGE AT 130°F (54°C):	1.5-13.0
MAXIMUM CHLORINE (CLEANING):	200 ppm @ pH 10-10.5, 130°F (54°C) 0 ppm @ pH less than 9.5

# SYNERGIES BETWEEN ULTRAFILTRATION AND ION EXCHANGE, or

## WHY UF SHOULD BE USED AS IX PRE- AND POST-TREATMENT

by Dr. Jamie P. Monat

Ion Exchange (IX) is a unit operation widely used for water softening, boiler feedwater conditioning, ultrapure water generation for electronics manufacture, pharmaceutical-grade water, and other applications. Ultrafiltration (UF) is another unit operation often used to remove suspended solids, colloids, and macromolecules from liquid streams. There is a natural synergy between these two unit operations, which has largely been unexploited. As pre-treatment to IX, UF can significantly increase resin life and time between cleanings, thereby reducing operating expense. As post-treatment to IX, UF can eliminate resin fines, bacteria, organics, and colloids, thus improving product water quality.

### Overview of Ultrafiltration

Ultrafiltration (UF) is a pressure-driven unit operation in which particulates, colloids, emulsified oils, and macromolecules are separated from a liquid feed stream upon passage through a porous semi-permeable membrane (Figures 1 and 2).

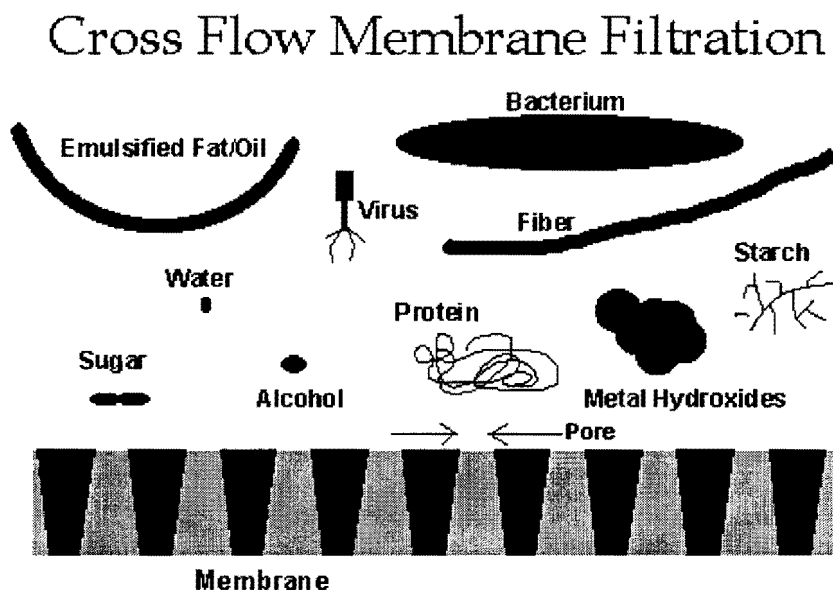


Figure 1

## Basic Membrane Concepts

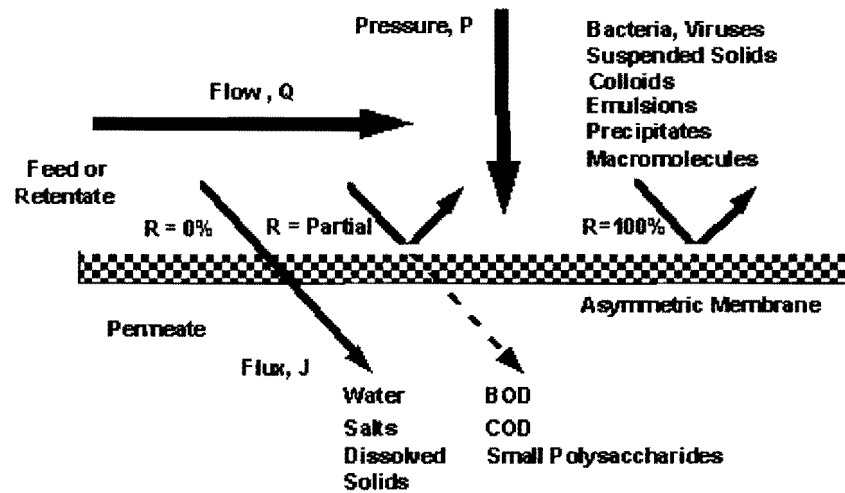


Figure 2

The separation is based primarily on the size of the species in the liquid relative to the size of the membrane pores (i.e. a simple sieving process) although geometry of the pores, geometry of the species to be separated, electric charge, and membrane surface chemistry may also play a part. On the separation size spectrum, UF falls between Nanofiltration or NF (membrane pore sizes below approximately 0.01 micrometer) and Microfiltration or MF (pore sizes greater than 1.0 micrometer; see Figure 3).

## Tangential Flow Filtration

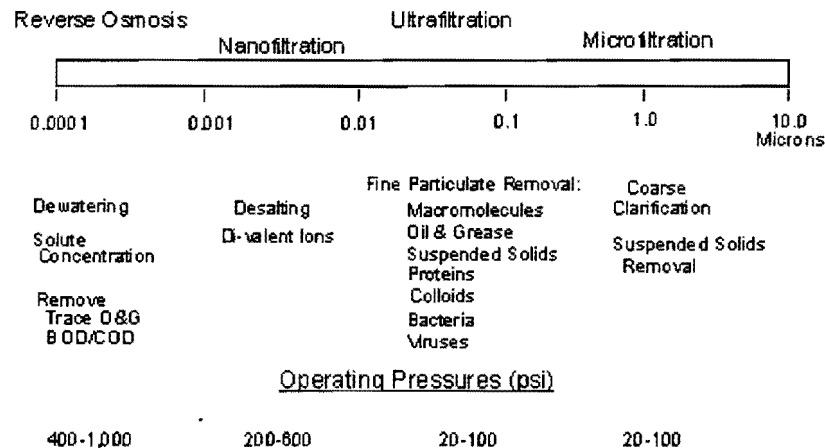


Figure 3

In ultrafiltration, small molecules such as water, monosaccharides, simple alcohols, and all ionic species pass through the membrane while larger molecules, particulates, bacteria, and emulsified oils and fats are retained.

The UF membrane is usually a polymer such as polyethersulfone, polysulfone, polyvinylidene fluoride, or polyamide and is typically cast on either a flat sheet or on the inside diameter of a tube. It may also be extruded as a hollow fiber. Tubular membrane products are excellent for high solids loadings as may be found in wastewater streams, but their low surface area-to-volume ratio makes them too expensive for pure water applications (Figure 4).



# Membrane Configurations

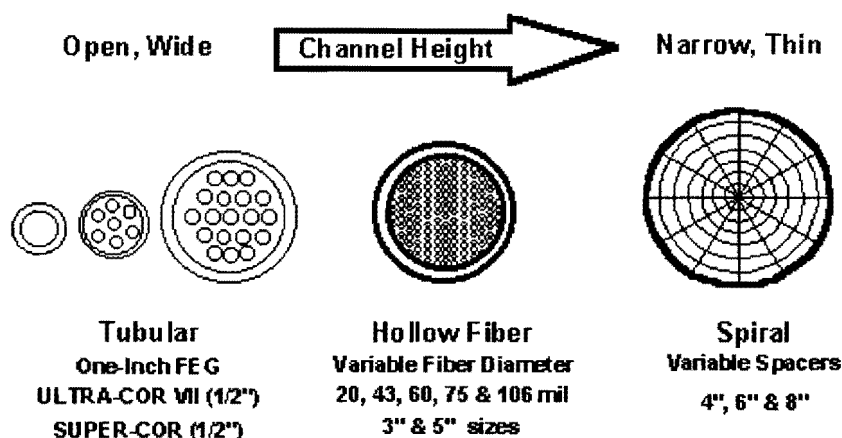


Figure 4

Spirals, on the other hand, have a high surface area-to-volume ratio, but their narrow flow channel renders them susceptible to plugging. Hollow Fibers strike an excellent balance between high surface area-to-volume (and hence reasonable cost) and resistance to plugging, and are typically the configuration of choice for IX pre- and post-treatment. In addition, because hollow fibers are not cast on a backing they are not subject to delamination; this allows hollow fibers to be back-pulsed which is crucial in cleaning foulants from the membrane surface.

Ultrafiltration is a well-known, proven unit operation that has historically been widely used in the food, dairy, juice, pharmaceutical, biotech, automotive paint, environmental control, textile, and potable water industries. Typical applications include the concentration of whey protein during cheese-making, clarification of fruit juices, removal of emulsified oils and particulates from wastewaters, separation of water from pigment in electrocoat paint, and removal of microorganisms from potable water. There are dozens of other applications as well. Removal and concentration capabilities of UF vs reverse osmosis (RO), nanofiltration (NF), and microfiltration (MF) are shown in Figure 3.

Either batch or continuous process designs are available with UF. Batch processing is preferred when the feed stream is available intermittently in batches (as opposed to continuously) as may be the case when a small volume of pharmaceutical liquid needs to be clarified or concentrated, or when larger volumes of wastewater are processed once per week. Continuous designs are preferred when the feed stream is continuous as in a potable water filtration application or in the continual filtration of an automotive electrocoat paint bath; or when a biologically active feed must be processed quickly as in food and dairy streams.

Hollow fiber systems are constructed with from 1 to 80 hollow-fiber cartridges on a single "stage" which is comprised of the membrane cartridges, a pump, feed and permeate manifolds, and appropriate valves, pipe, and fittings. Up to 6 stages may be ganged together for increased capacity. Hollow fiber UF membranes come with membrane pore sizes from 10,000 to over 2,000,000 daltons and with fiber inside diameters between 10 mils and 106 mils, while hollow fiber cartridges come in diameters of 1, 2, 3, 5, and 8 inches and in lengths ranging from 6 to 72 inches. Several hundred to many thousand hollow fibers are potted together in a single hollow fiber cartridge. Typical cartridge membrane areas are between 10 and 132 square feet per cartridge.

Hollow fiber water ultrafiltration systems typically operate at feed pressures of about 25 psig and with capacities between 50 and 2 million gallons per day. On each hollow fiber system stage, for a typical 5" diameter x 43" long cartridge, the pump must have the capacity to output approximately 20 gpm per hollow fiber cartridge at 25 psig. Thus, a 50-cartridge skid would require a 1000 gpm pump at 25-30 psig.

As an adjunct to IX, UF is commonly used in three places: Before the IX system as pre-treatment, after the IX system as post-treatment, and between the cation exchange column and the anion exchange column in multiple-bed systems (Figure 5).

## Enhancing Water Purification with Ultrafiltration Technology

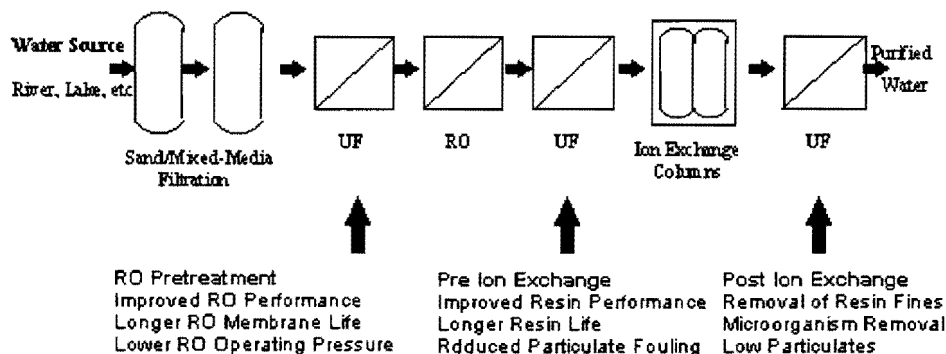


Figure 5

### UF As Pre-Treatment to IX

Resin beds are often fouled by particulates deriving from upstream processes, bacteria, RO membrane particle shedding, or suspended matter in the feed stream such as colloidal or "non-reactive" silica. Colloidal silica is often naturally present in well waters and river waters. Polymerization (the linking together of silica molecules in long chains) of silica may occur if the pH is maintained below 7.0. This polymerized silica is extremely difficult to remove from the resin. The typical result is long bed rinses and ineffective resin regeneration. A UF system upstream of the IX column typically removes more than 95% of the colloidal silica (and often more than 98%) before it reaches the IX resin, preventing irreversible silica fouling.

In addition, in those instances where an IX column follows a reverse osmosis systems, bacteria growth in the upstream RO system can result in resin bed fouling. Many RO systems and RO membrane cartridges for industrial use are not designed to high sanitary standards and contain "dead zones" or spaces that are not adequately flushed by cleaning solutions, as well as non-sanitary valves and seals and unpolished wetted surfaces. If conditions of temperature and pH favor their growth, bacteria may build up in these regions. A well-designed sanitary-standard ultrafiltration system, however, will reduce the bacterial concentration in the RO effluent by 4-6 orders of magnitude. Note that in this scenario, the UF system would be located between the RO system and the IX system. Of course, in those instances where there is no upstream RO system, bacteria may still be present and present a fouling problem for the IX column. A UF system also obviates this problem.

Many liquid streams contain particulates that are too small to be removed by bag filters, strainers, or conventional dead-end filters, or even by microfiltration upstream of the IX system. Particulates in the size range 0.01-0.1 micrometers would fit into this category. Unfortunately, IX resin beds tend to remove these particulates quite effectively, but the resin becomes fouled in the process. Reducing the turbidity of the influent to less than 3 Nephelometric Turbidity Units (NTU's) is usually sufficient to avoid particulate fouling; UF typically addresses this problem fully and can often achieve NTU's less than 1.

For some water types such as those found in chemical processes, petrochemical recycle, or wastewater recycle, oil contamination is another problem affecting resin performance. Oil is often present naturally or it may be present as a result of lubricating or cooling fluids having made their way into the feed stream. While free oil is easily removed via settling, coalescence, and skimming, this is not true for naturally, chemically, or mechanically emulsified oil. In severe cases, resin fouling by emulsified oil has been so serious and difficult to correct that it has proven more economical to replace the resin bed than to attempt to clean it of oil! Fortunately, UF is extremely effective (>99% removal) at removing emulsified oil prior to the resin bed.

UF is also very good at removing large molecular weight dissolved organics from process streams. These organics may be present in the feed water or may be leached from wetted surfaces upstream of the IX column. Organics can seriously foul resin beds, significantly increasing operating expense due to cleaning chemicals, downtime, and ineffective regeneration. The organics can foul both the surface of the resin beads and the pores. Although activated carbon is often used to address this problem, it is typically not effective at removing high molecular weight organics, and it is often selective in the organics it removes. Activated carbon also tends to grow microorganisms, which are removed by UF. UF, on the other hand, is selective only based on molecular weight and can remove organics with molecular weights as low as 10,000 daltons, provided that the correct UF membrane is selected. The optimum UF membrane will be one whose pore size is sufficiently small to remove the organics, whose surface charge is the same as that of the organics (typically negative, but not always) so that membrane fouling is minimized, and whose chemistry will resist the temperature, pH, and chemicals that may be present in the feed *and* during cleaning so that an adequate membrane life is achieved.

### **UF As Post-Treatment to IX**

A common problem with IX effluent is the presence of resin fines due to bead breakage and oxidation. A certain amount of resin attrition is inescapable due to attack by chlorine and other oxidizers, and without post-treatment, the fines contaminate the effluent. The oxidizing agents attack the bonds of the plastic from which the IX resin beads are made. Over time, a sufficient number of these bonds break, yielding fracturing of the resin bead. Thermal shock due to cleaning and regeneration chemicals also contributes to resin bead breakage, as do friction and abrasion as the beads scrape against each other and against the IX vessel walls. Finally, shrinking and swelling of resin beads due to drying and re-wetting causes mechanical stresses which can fracture the beads. Resin attrition rates of 3-5% per year due to bead breakage are not uncommon; these fractured beads contaminate the effluent. UF is very effective at removing these fragments.

Another problem is the growth of bacteria within the resin bed or within the RO system upstream of the resin bed. As for RO systems, many industrial-grade IX systems are not designed to high sanitary standards and contain "dead zones" or spaces, unpolished wetted surfaces, and non-sanitary valves and seals that are not adequately flushed by cleaning solutions. If conditions of temperature and pH are favorable, bacteria may build up in these regions. A well-designed sanitary-standard ultrafiltration system, however, will reduce the bacterial concentration in the IX effluent by 4-6 orders of magnitude, eliminating this as an effluent quality problem.

Still another problem with IX effluent is the leaching of high molecular weight organics from the resin itself. UF effectively removes these contaminants, as well as any other high molecular weight organics that were not removed by pre-treatment or by the IX process itself.

To quantify the improvements in post-IX water that can be afforded by ultrafiltration, Kunin (3) has developed the following data:

Water Property	Mixed-Bed De-Ionized Water	Mixed-Bed De-Ionized Water After Ultrafiltration
Turbidity	0.50 JTU*	0.21 JTU*
Non-reactive SiO <sub>2</sub>	0.18	0.01
Bacterial Count	10,000/mL	1/mL
Electrical resistivity	12,000,000 ohm-cm	18,000,000 ohm-cm

\* JTU= Jackson Turbidity Units

These data show that in this example, ultrafiltration reduced the water turbidity by a factor of 2.5; removed 95% of the non-reactive silica, reduced the bacteria count by four orders of magnitude, and increased the electrical resistivity of the water to near its theoretical limit.

### **Economics**

Ultrafiltration as pre-treatment to IX reduces down-time, cleaning frequency, and regeneration frequency, and increases resin life. There are clear direct economic benefits of this. UF as post-treatment to IX yields higher quality de-ionized water that is *free of particulates*, which also has direct economic and performance benefits.

The data below from Owens (4) show that for a 500 gpm demineralization system, ion exchange annual operating costs can be reduced by \$361,000 if the influent water is upgraded from "poor" to "excellent".

### **Ion Exchange Annual Operating Costs as a Function of Feedwater Quality**

(500 gpm system)

Influent Water Quality	Excellent	Poor
Resin Cleaning		
Frequency:	2 years	1 month
Annual Cost:	\$2,000	\$24,000
Cation Replacement		
Frequency:	8-10 years	3 years
Annual Cost:	\$2,200	\$6,700
Anion Replacement		
Frequency:	5-6 years	1.5 years
Annual Cost:	\$4,600	\$18,400
Cation Regenerations		
Frequency:	1 per day	2 per day
Annual Acid:	\$35,000	\$70,000
Annual Water	\$63,000	\$126,000
Anion Regenerations		
Frequency:	1 per day	2 per day
Annual Acid:	\$173,000	\$346,000
Annual Water:	\$60,000	\$120,000
<b>Annual Totals</b>	<b>\$340,000</b>	<b>\$701,000</b>

Upgrading the influent water to this degree should be achieved by a well-designed hollow fiber ultrafilter positioned upstream of the IX system.

The operating costs of the UF system must be subtracted from the IX system cost savings. Annual operating costs for the UF system are estimated to be \$104,000, broken down as follows:

Membrane Replacement	\$50,000
Electricity	\$33,000
Cleaning Chemicals	\$10,000
Operating Labor	\$11,000
<b>TOTAL</b>	<b>\$104,000</b>

Thus the NET ANNUAL BENEFIT of the UF system is \$361,000-\$104,000, or \$257,000 per year.

To determine return on investment or simple payback, the capital cost of the UF system must next be considered. A hollow-fiber ultrafiltration system to accommodate 500 gpm of water would cost approximately \$650,000 installed, broken down as follows:

Hollow Fiber UF System, complete with (100) 5"-diameter hollow fiber cartridges	\$500,000
Concrete Pad, Equipment Setting, Utility Connections, Piping, and Transportation to site	\$100,000
Start-Up and Operator Training	\$25,000
Miscellaneous and contingency	\$25,000
<b>TOTAL</b>	<b>\$650,000</b>

The investment of \$650,000 in this UF system would result in an annual net savings in IX operating costs of \$257,000, resulting in a **simple payback** for the operation of the IX system of  $(\$650,000)/(\$257,000/\text{year}) = 2.5$  **years**. Or, looked at another way, the Return on Investment for this project would be  $(\$257,000)/(\$650,000) = 40\%$ , which is an excellent investment. (Please note that these simple calculations account for neither depreciation nor taxes, both of which must be considered for an accurate analysis. They have not been included here because they are highly variable and dependent upon the location and business practices of each individual firm.) And these values take -0- credit for improved IX effluent quality or for the economic and process benefits associated with no suspended solids, low NTU's, and no bacteria in the effluent. Installation of a UF system in this case would be a clear money-saver.

Pre-treatment options other than UF should also be considered. Nanofiltration and Reverse Osmosis would clarify the feedwater as effectively as UF. However, the lower flux rates inherent in these unit operations together with much higher-pressure pumping energy requirements would yield a larger system with substantially more membrane area and pump horsepower and hence substantially higher first costs and operating costs. Microfiltration, on the other hand, (as well as sand filtration, cartridge filtration, and diatomaceous earth filtration) would not be effective in removing colloidal silica, some bacteria, smaller particulates, and large molecular-weight organics.

### Sources of UF Equipment

The thirteen largest North American vendors of Ultrafiltration membranes and equipment are:

VENDOR	MEMBRANE CONFIGURATIONS
Koch Membrane Systems, Inc. Wilmington, MA	S, T, HF*
Osmonics/DeSal Minnetonka, MN	S
AMT San Diego, CA	S, T
UOP/Fluid Systems San Diego, CA	S
PCI Eden Prairie, MN	T
Synder Vacaville, CA	S
Hoechst-Celanese Charlotte, NC	S
Zenon Burlington, Ontario, Canada	T
AG Technology Needham, MA	HF
Millipore Bedford, MA	S
Pall/Asahi-Kasei East Hills, NY	HF
Hydranautics San Diego, CA	T, S
Membrex Fairfield, NJ	S

\*S=Spirals, T=Tubes, HF=Hollow Fibers

### **Conclusion**

Ultrafiltration is an under-utilized unit operation that can significantly enhance both the economics and product water quality deriving from IX systems for water purification. As both pre-treatment and post-treatment, UF can reduce chemical consumption, increase resin life, reduce down time, eliminate microorganisms, and scavenge resin fines from product water resulting in higher produced water quality at lower net operating cost.

### **References:**

1. "Ion Exchange System Troubleshooting", Ken Frederick, ION EXCHANGE ASSOCIATES, P.O. Box 6167, Reading, PA 19610, WaterTech '96 Conference, November 1996.
2. "Ion Exchange for Industrial Systems", Ken Frederick, ION EXCHANGE ASSOCIATES, P.O. Box 6167, Reading, PA 19610, WaterTech '96 Conference, November 1996.
3. Kunin, Robert, Amber Hi-Lites, Tall Oaks Publishing, Littleton, CO, 1996, Nos. 146 and 165
4. Owens, Al, "Without Proper Pretreatment You May Be on the Losing Side", WaterTech '96 Conference, Houston, TX, November 1996

### **Bibliography:**

1. Cheryan, M., Ultrafiltration Handbook, Technomic Publishing Co., Inc., Lancaster, PA, 1986
2. Murkes, J., and Carlsson, C.G., Crossflow Filtration, John Wiley & Sons, New York, 1988
3. Brock, T., Membrane Filtration, Science Tech, Inc., Madison, WI, 1983



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## Affinity Chromatography Calcs

Antibody (AB) binding to column  $\frac{12.3 \text{ mg AB}}{\text{mL resin}}$

assume 1 mg tPA binds 1 mg ~~resin~~ AB  
 " resin is only 85% utilized

$$\frac{12.3 \text{ mg AB}}{\text{mL resin}} \times \frac{1 \text{ mg tPA}}{\text{mg AB}} \times \frac{0.85 \text{ mL resin used}}{\text{mL resin}} = \frac{10.455 \text{ mg tPA}}{\text{mL resin}} \quad \frac{10.455 \text{ g tPA}}{\text{L resin}}$$

Stream P-14 contains 1,890.4 kg tPA

assume 15% of tPA is lost

will have enough binding for 100% of it

$$\frac{1,890.4 \text{ g tPA}}{10.455 \text{ g tPA}} \times \frac{\text{L resin}}{\text{L resin}} = 180.8 \text{ L resin required}$$

$$\text{in 4 cycles: } \frac{180.8}{4} = 45.2 \text{ L resin}$$

Column specs: Bioprocess stainless steel columns are custom made

$$\text{Diam.} = 70 \text{ cm} \quad V = (15 \text{ cm}) \pi (35 \text{ cm})^2 = 57.7 \text{ L}$$

$$\text{Height} = 15 \text{ cm}$$

this is more than enough resin, ensuring estimates of 85% utilization + 85% yield, which are ~~generous~~ though recommended by Prof. Diamond

## Required Antibody

$$\frac{12.3 \text{ mg}}{\text{mL}} = \frac{12.3 \text{ g}}{\text{L}} \times 57.7 \text{ L} = 709.7 \text{ g}$$

We account for 750 g/column

# Pumps for chromatography columns Affinity Column Pump

$$v_o := 100 \cdot \frac{\text{cm}}{\text{hr}} \quad l := 15 \cdot \text{cm} \quad \mu := .015 \cdot \text{poise} \quad D_p := 105 \cdot 10^{-4} \cdot \text{cm}$$

$$\epsilon := 0.35 \quad \rho := 1000 \cdot \frac{\text{kg}}{\text{m}^3}$$

$$\Delta P := \left[ 150 \cdot \mu \cdot v_o \cdot \frac{(1 - \epsilon)^2}{D_p^2 \cdot \epsilon^3} + 1.75 \cdot \rho \cdot v_o^2 \cdot \frac{(1 - \epsilon)}{D_p \cdot \epsilon^3} \right] \cdot l \quad \Delta P = 1.216 \text{ psi}$$

Assume pressure drop across column is 10 psi  $\Delta P := 10 \cdot \text{psi}$

$$F := 134.6 \cdot \frac{\text{kg}}{\text{hr}} \quad \text{Flow through Pump} \quad \text{Size} := F \cdot \frac{\Delta P}{\rho}$$

$$\rho := 63.72 \cdot \frac{\text{lb}}{\text{ft}^3} \quad \text{Density of Water} \quad \text{Power} := \frac{\text{Size}}{\eta}$$

$$\eta := 0.80 \quad \text{Pump efficiency} \quad \text{Head} := \frac{\Delta P}{\rho \cdot g}$$

Size =  $3.457 \cdot 10^{-3}$  hp Hours per batch:  $t := 7920 \cdot \text{hr}$

Power = 3.222 W

Electricity := Power · t

Head = 22.599 ft

Electricity = 25.521 kW · hr



Next  
column pricing  
" availability



amersham pharmacia biotech

TO: Chris Metallo

FAX: 215 573 7601

PHONE: 215 898 2180

PAGES: Cover + 2

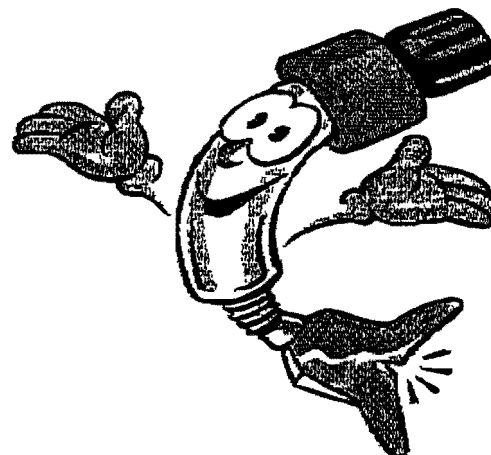
FROM: Andy Mitchell Ph.D.

DEPT: Technical Support

PHONE: 800-526-3593

FAX 732-457-8643

DATE: Monday, February 21, 2000



TAKE A LOOK AT OUR NEW PRODUCTS  
Purify GST fusion proteins in one step  
High binding capacity

- GSTrap™  
1 ml and 5 ml prepacked HiTrap™ columns
  - Glutathione Sepharose™ 4 Fast Flow 25 ml
- For more information and to place your order, call Amersham Pharmacia Biotech today.

Dear Chris,

Here is the information that you requested on CNBr Sepharose & Superdex 200 pg.

CNBr 4 FF

250g \$3514

2Kg \$28096

How to determine volume from mass?  
4-5 mL/g

Superdex 200 pg

1L \$1703

5L \$8499

1-800-526-3593

Regards,

Andy Mitchell

Technical Support

③ - tech support

① extension, sales or service rep

② customer service

P H A R M A C I A   B I O T E C H

## **CNBr-activated Sepharose® 4 Fast Flow**

### **INSTRUCTIONS**

---

#### **Important user information**

Please read these instructions carefully before using CNBr-activated Sepharose 4 Fast Flow media. Should you have any comments on this instruction manual, we will be pleased to receive them at:

Pharmacia Biotech AB  
S-751 82 Uppsala  
Sweden

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## 1. Introduction

The preparation and use of affinity chromatography media by coupling biospecific ligands to CNBr-activated matrices is a widely used, successful and well-documented technique.

CNBr-activated Sepharose 4 Fast Flow is a new pre-activated affinity matrix that combines the advantages of CNBr coupling with the high flow and stability characteristics of Sepharose 4 Fast Flow. In our experience, the CNBr coupling technique has a well-proven track record for the purification of therapeutic proteins. This, plus the performance of the matrix at large scale, makes the use of CNBr-activated Sepharose 4 Fast Flow particularly attractive for manufacturing applications in the biopharmaceutical industry. Furthermore, the medium is a member of the BioProcess media family and carries comprehensive technical and regulatory support for production applications.

To ensure best performance and trouble-free operation, please read these instructions before using CNBr-activated Sepharose 4 Fast Flow.

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## 2. Media characteristics

CNBr-activated Sepharose 4 Fast Flow is a bead-formed, highly cross-linked pre-activated matrix produced by reacting Sepharose 4 Fast Flow with cyanogen bromide (CNBr). This coupling makes the medium more rigid which in turn improves the pressure/flow characteristics, see Fig. 2. Proteins and other molecules containing primary amino groups can be coupled directly to the pre-activated medium. Multi-point attachment of proteins provides the immobilized product with good chemical stability. The resulting affinity medium can isolate a specific substance from a complex mixture, often achieving very high yield and purity in a single step. Many references demonstrate that binding affinity is frequently well maintained after CNBr coupling.

A typical application of pre-activated affinity media like CNBr-activated Sepharose 4 Fast Flow is based on antigen-antibody reactions with immobilized monoclonal antibodies as ligands. In such cases, purification factors of 2 000–20 000 can be obtained.

Table 1 summarizes the main characteristics of CNBr-activated Sepharose 4 Fast Flow.

### Sepharose 4 Fast Flow Matrix

Mean particle size	90 µm
Particle size range	45–165 µm
Bead structure	Highly cross-linked 4% agarose, spherical

### CNBr-activated Sepharose 4 Fast Flow

Swelling factor	4–5 ml drained medium/mg
Coupling capacity	13–26 mg α-chymotrypsinogen/ml
pH stability*	
long term	3–11
short term (CIP)	3–11

\* Depends on ligand stability. Test results are with Protein A as ligand

### Sepharose 4 Fast Flow matrix

Sepharose 4 Fast Flow is a highly cross-linked agarose matrix. In its pre-activated CNBr form, it offers much improved performance when compared with the well established CNBr-activated Sepharose 4B. The Sepharose 4 Fast Flow matrix has higher rigidity and can thus be run at high flow rates (see Table 1). As the available capacities for proteins are similar in both cases, the Fast Flow matrix offers greater productivity.

The higher mechanical strength of the cross-linked matrix makes it well-suited for use in large columns. Scaling up a purification developed on CNBr-activated Sepharose 4 Fast Flow is therefore simple and more predictable. The coupled product is stable at low pH, which is often required for elution from some immunoadsorbents.

(For applications that require operation at high pH, note that the amide bond formed when using the companion product NHS-activated Sepharose 4 Fast Flow is stable up to pH 13 for normal use).

## 3. Coupling

CNBr-activated Sepharose 4 Fast Flow is supplied freeze-dried in the presence of additives. Instructions for swelling, the medium and preparation for coupling the ligand are given below. In order to retain maximum binding capacity of CNBr-activated Sepharose 4 Fast Flow prior to coupling the ligand, use cold (0–4 °C) solutions. The time interval between washing and coupling must be minimised; therefore preparations of all required solutions prior to coupling is recommended.

1. Prepare the coupling solution, i.e. dissolve the ligand to be coupled in a suitable coupling buffer. For good coupling efficiency avoid unnecessarily dilute solutions (Recommended ratio of volumes, coupling solution/medium is 0.5:1). The coupling pH depends on the ligand. Normally pH in the range 7–9 is used.
2. CNBr-activated Sepharose 4 Fast Flow is supplied freeze-dried with sugar additives and is washed initially with 10–15

4

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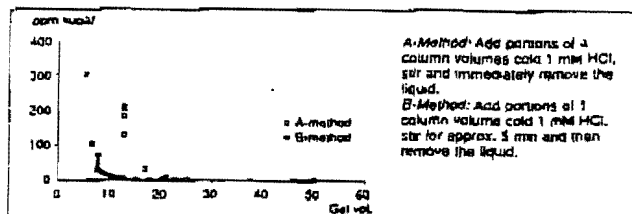


Fig. 1. The content of sugar in the filtrate after washing with different medium volumes of cold 1 mM HCl.

medium volumes of cold 1 mM HCl, see Fig 1. Use small wash portions (e.g. 1 medium volume) and let the mixture equilibrate a few minutes during each washing step. After washing, determine the exact medium volume obtained using e.g. centrifugation or PD-10 column (the medium volume may vary between experiments).

- Mix the washed medium and coupling solution. Adjust pH to the desired value. To obtain good reproducibility it is wise to adjust total reaction volume to a fixed value with coupling buffer.
- Coupling is normally very fast. At room temperature the reaction is usually completed after 2-4 hours. If coupling is performed at 4 °C, it can be performed overnight. It may be practical to follow the reaction using UV-absorbance measurements.
- After coupling, non-reacted groups on the medium should be blocked by keeping the coupled medium in Tris buffer or ethanol amine for a few hours.
- Wash the coupled medium using alternate low and high pH. Recommended buffers are acetate pH 3-4 and Tris HCl pH 8-9. A suitable procedure could be 3x1 medium volume Tris HCl buffer followed by 3x1 volumes acetate buffer. Repeat this cycle 3-6 times.
- The coupled medium is now ready for use. To prevent microbial growth, store in 20% ethanol for example.

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#### 4.2 Packing laboratory scale columns

- Equilibrate all material to the temperature at which the chromatography will be performed.
  - Degas the slurry.
  - Eliminate air from the column dead spaces by flushing the end-pieces with binding buffer. Make sure that no air has been trapped under the column net. Close the column outlet leaving a few centimetres of binding buffer remaining in the column.
  - Pour the slurry into the column in one continuous motion. Pouring the slurry down a glass rod held against the wall of the column will minimize the introduction of air bubbles.
  - Immediately fill the remainder of the column with binding buffer, mount the column top-piece onto the column and connect the column to a pump.
  - Open the outlet of the column and set the pump to run at the desired flow rate. Ideally, Fast Flow media are packed at constant pressure not exceeding 1 bar (0.1 MPa) in XK columns. If the packing equipment does not include a pressure gauge, use a packing flow rate of at least 400 cm/h (1.5 cm bed height, 25 °C, low viscosity buffer). If the recommended pressure or flow rate cannot be obtained, use the maximum flow rate the pump can deliver. This should also give a reasonably well-packed bed.
- Note:** Do not exceed 75% of the packing flow rate in subsequent chromatographic procedures.
- Maintain the packing flow rate for 3 bed volumes after a constant bed height is obtained.

#### Using an adaptor

Adaptors should be fitted as follows:

- After the medium has been packed as described above, close the column outlet and remove the top-piece from the column. Carefully fill the rest of the column with binding buffer to form an upward meniscus at the top.

#### 4. Column packing guidelines

General column packing guidelines for Sepharose 4 Fast Flow based media.

##### 4.1 Recommended columns

###### Laboratory scale

- HR10/10 (10 mm i.d.) for bed volumes up to 8 ml at bed height up to 10 cm.
- XK 16/20 (16 mm i.d.) for bed volumes up to 50 ml at bed height up to 15 cm.
- XK 26/20 (26 mm i.d.) for bed volumes up to 80 ml at bed height up to 15 cm.

###### Large scale

- BPG 100/500 (100 mm i.d.) for bed volumes up to 2.4 L at a maximum bed height of 30 cm.
- BPG 200/500 (200 mm i.d.) for bed volumes up to 9.4 L at a maximum bed height of 30 cm.
- BPG 300/500 (300 mm i.d.) for bed volumes up to 21 L at a maximum bed height of 30 cm.
- BPG 450/500 (450 mm i.d.) for bed volumes up to 43 L at a maximum bed height of 27 cm.
- PS 370 (370 mm i.d.) with a fixed bed volume of 16 L at a fixed bed height of 15 cm.
- BioProcess Stainless Steel 400/150, 600/150, 800/150, 1000/150, 1200/150 and 1400/150 (400-1400 mm i.d.). With fixed bed volumes ranging from 19 to 230 L at a fixed bed height of 15 cm.
- INDEX 100/500 (100 mm i.d.) for bed volumes up to 2.4 L at a maximum bed height of 30 cm.
- INDEX 200/500 (200 mm i.d.) for bed volumes up to 9.4 L at a maximum bed height of 30 cm.

- Insert the adaptor at an angle into the column, ensuring that no air is trapped under the net.
- Make all tubing connections at this stage. There must be a bubble-free liquid connection between the column and the pump and the sample application system (LV-3 or LV-4 valves).
- Slide the plunger slowly down the column so that the air above the net and in the capillary tubing is displaced by the eluent. Valves on the inlet side of the column should be turned in all directions during this process to ensure that air is removed.
- Lock the adaptor in position, open the column outlet and start the eluent flow. Pass eluent through the column at the packing flow rate until the bed is stable. Re-position the adaptor on the medium surface as necessary.

##### 4.3 Packing large scale columns

###### General packing procedures

Columns can be packed in different ways depending on the type of column and equipment used. Always read and follow the relevant column instruction manual carefully.

Sepharose 4 Fast Flow based media are easy to pack since their rigidity allows the use of high flow rates, see Fig. 2. Four suitable types of packing methods are given:

- Pressure packing (for columns with adaptors)
- Combined pressure/suction packing (for medium sized columns with fixed bed heights)
- Suction packing (for large columns with fixed bed heights)
- Hydraulic pressure packing

How well the column is packed will have a major effect on the result of the separation. It is therefore very important to pack and test the column according to the following recommendations.

Begin the packing procedure by determining the optimal packing flow rate. Guidelines are given for determining the optimal packing flow rates for columns with adaptors and fixed bed heights.

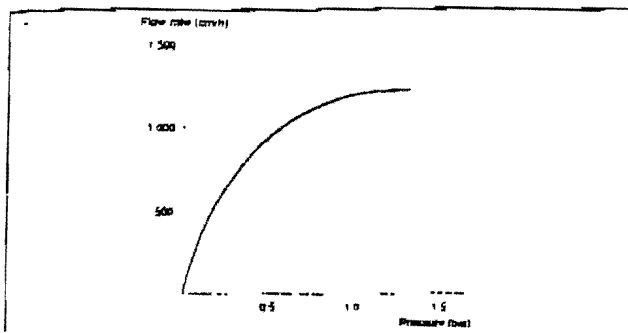


Fig. 2. Pressure/flow rate curve for Protein A Sepharose 4 Fast Flow media (BPG 100; 1 bar; 5.1 cm bed height).

#### 4.4 Determining optimal packing flow rates

The optimal packing flow rate is dependent on column size and type, medium volume, packing solution and temperature. The optimal packing flow rate must therefore be determined empirically for each individual system.

To determine the optimal packing flow rate, proceed as follows:

1. Calculate the amount of medium needed for the slurry (this is especially important for columns with fixed bed heights). The quantity of medium required per litre packed volume is approximately 1.15 litres sedimented medium.
2. Prepare the column exactly as for column packing.
3. Begin packing the medium at a low flow rate (30 cm/h).
4. Increase the pressure in increments and record the flow rate when the pressure has stabilized. Do not exceed the maximum pressure of the column, or the maximum flow rate for the medium.

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5. The maximum flow rate is reached when the pressure/flow curve levels off or the maximum pressure of the column is reached. Stop the packing and do not exceed this flow rate. The optimal packing flow rate/pressure is 70–100% of the maximum flow rate/pressure.

6. Plot the pressure/flow rate curve as in Fig. 2 and determine the optimal packing flow rate.

The operational flow rate/pressure should be <70% of the packing flow rate/pressure.

Note: For BPSS columns, first pack the column by suction packing at a low flow rate then determine the flow/pressure characteristics as above by pumping with downward flow through the column.

#### 4.5 Pressure packing

##### BPG Columns

BPG Columns are supplied with a movable adaptor. They are packed by conventional pressure packing by pumping the packing solution through the chromatographic bed at constant flow rate (or back pressure).

1. Pour some water (or packing buffer) into the column. Make sure that there is no air trapped under the bottom net. Leave about 2 cm of liquid in the column.
2. Mix the packing buffer with the medium to form a 50–70% slurry. (Sedimented bed volume/slurry volume = 0.5–0.7.) Pour the slurry into the column. Insert the adaptor and lower it to the surface of the slurry, making sure no air is trapped under the adaptor. Secure the adaptor in place.
3. Seal the adaptor O-ring and lower the adaptor a little into the slurry, enough to fill the adaptor inlet with packing solution.
4. Connect a pump and a pressure meter and start packing at the predetermined packing flow rate (or pressure). Keep the flow rate (or pressure) constant during packing and check the pressure at the column inlet. Never exceed the pressure limit for column or medium.

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5. When the medium has settled, close the bottom valve and stop the pump. The bed starts rising in the column. Loosen the O-ring and lower the adaptor to about 0.5–1.0 cm from the bed surface.
6. Seal the O-ring, start the pump and continue packing. Repeat steps 5 and 6 until there is a maximum of 1 cm between bed surface and adaptor when the bed has stabilized. Mark the bed height on the column tube.
7. Close the bottom valve, stop the pump, disconnect the column inlet and push the adaptor down to approximately 3 mm below the mark on the column tube, without loosening the adaptor O-ring. The packing solution will flush the adaptor inlet. Remove any trapped air by pumping liquid from the bottom (after the inlet tubing and the bottom valve have been properly filled).

#### 4.6 Combined pressure/suction packing

##### Process Stack (PS 370) Column

The Process Stack Column is supplied with fixed end-pieces and a fixed bed height of 15 cm. It is packed by a combined pressure/suction technique.

1. Fit an extra column section on top of the column tube, for use as a packing device.
2. Pour some water (or packing buffer) into the column. Make sure that there is no trapped air under the bottom net. Leave about 2 cm of liquid in the column.
3. Pour the slurry into the column. Add buffer to within 1–2 cm of the rim of the upper section. Stir gently to give a homogeneous slurry. Add buffer until level with the upper rim and secure the lid in place.
4. Connect a pump and a pressure meter and start packing at the predetermined packing flow rate (or pressure). Keep the flow rate (or pressure) constant during packing and check the pressure at the column inlet. Never exceed the pressure limit for column or medium.

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5. When the bed has stabilized, the top of the bed should be exactly level with the top of the column tube. At this point, exclude the buffer tank from the system by simultaneously switching the valve at the column outlet and the valve on the suction side of the pump, as shown in Fig. 3. Packing buffer is now recirculated through the system. If, when stabilized, the packed bed is not exactly level with the top of the column, add or remove slurry. Always stir the slurry thoroughly before packing.
6. Keeping the pump running, disconnect the column inlet from the lid and direct it to waste. The packing solution in the packing section can be removed by suction through the bed.
7. While the packing section is being emptied, loosen the bolts holding the column and the packing section together so that the packing section can be removed. During this operation, manually press down on the packing section to prevent leakage between the two sections.

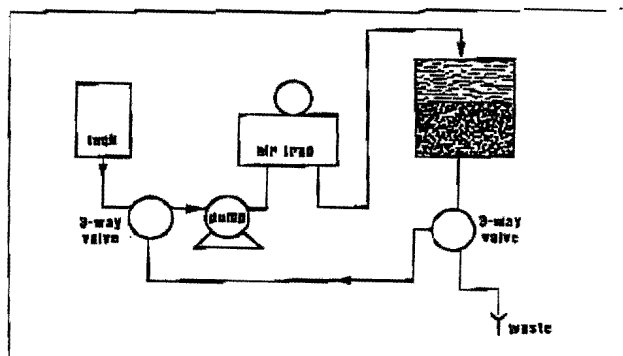


Fig. 3. Equipment set up for pressure/suction packing.

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8. When the packing solution is within 5–8 mm of the bed surface, close the valve at the column outlet, stop the pump, quickly remove the packing section and replace it with the lid. Manually press down on the lid while it is secured in place. This final operation should be completed as quickly as possible because the bed will expand when the flow stops.
9. Start pumping buffer with upward flow through the column to remove any air bubbles trapped under the lid.

#### 4.7 Suction packing

##### BioProcess Stainless Steel (BPSS) Columns

BioProcess Stainless Steel Columns are supplied with fixed end-pieces. They are packed by suction, i.e. by sucking packing solution through the chromatographic bed at a constant flow rate.

1. Fit a packing device on top of the column tube.
2. Pour some water (or packing solution) into the column. Make sure that there is no air trapped under the bottom net. Leave about 2–3 cm of liquid in the column.
3. Mix the packing buffer with the medium to form a 50% slurry (sedimented bed volume/slurry = 0.5). Pour the slurry into the column. Stir gently to give a homogeneous slurry.
4. Connect the column outlet valve to the suction side of a pump and start packing the bed by suction through the bed at the predetermined flow rate, see Fig. 3. Keep the flow rate constant during packing.
5. When the bed has stabilized, the top of the bed should be just below the junction of the column and the packing device. If, when stabilized, the level of the bed is incorrect, add or remove slurry. Always stir the slurry thoroughly before packing.
6. Just before the last of the solution has entered the packed bed (before the surface starts to dry), close the valve at the column outlet, stop the pump, quickly remove the packing device and replace it with the lid. This final operation should be completed

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as quickly as possible because the bed will expand when the flow stops.

7. Start pumping buffer with upward flow through the column to remove any air bubbles trapped under the lid.

#### 4.8 Hydraulic packing

##### INDEX Columns

INDEX Columns are supplied with a hydraulic function which allows an extremely simple, rapid and reproducible packing procedure. The medium is packed at the same time as the adaptor is lowered into position at the correct pressure.

The adaptor is pushed down by a constant hydraulic pressure, forcing water through the slurry and compressing it so that a packed bed is gradually built up. The hydraulic pressure can be generated using a pump and a pressure relief valve.

When the adaptor reaches the surface of the settled medium, it continues downwards under hydraulic pressure compressing the medium. The extent to which the medium is compressed depends upon the pressure from the adaptor and the elasticity of the medium. The quantity of medium required when packing Sepharose 4 Fast Flow media by hydraulic pressure is approximately 1.2 litre sedimented medium per litre packed bed.

1. Pour some water (or packing solution) into the column. Make sure that there is no air trapped under the bottom net. Leave about 2 cm of liquid in the column.
2. Pour the slurry into the column. Fill the column with packing solution up to the top of the glass tube and mix the slurry. Allow the medium to sediment to just below the bevel of the glass tube (G), see Fig. 4.
3. Put the adaptor in a resting position against the bevel of the glass tube. Avoid trapping air bubbles under the adaptor by slightly tilting the adaptor while mounting.
4. Lower the lid and secure it in place.

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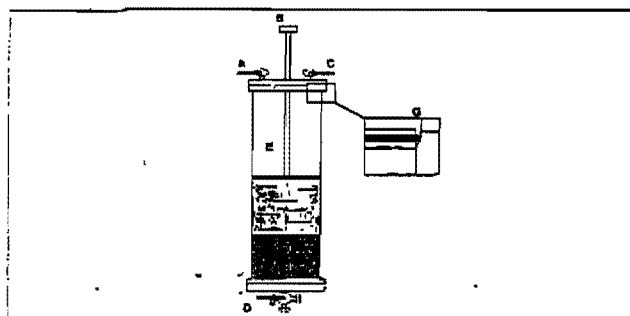


Fig. 4. Schematic representation of INDEX column with a 4-port (2-way) valve mounted at the bottom outlet.

5. Connect a pump to the inlet of the hydraulic chamber (A), with a manometer and a pressure relief valve in-line between the pump and the hydraulic chamber. The manometer should be placed after the valve in the direction of the flow.
6. Open the hydraulic inlet (A), and the hydraulic outlet (C). Start the pump and flush the hydraulic chamber (E) free of air and any residual medium.
7. Close (C) and open the elution inlet/outlet (B) to allow trapped air in the adaptor not to escape.
8. Close (B) and open the elution inlet/outlet (D) to start the packing, applying a predefined constant hydraulic packing pressure. When packing Sepharose 4 Fast Flow media in an INDEX column to a bed height of 15 cm, the recommended hydraulic packing pressure is 1.5 bar for INDEX 100 and 0.9 bar for INDEX 200.
9. When the adaptor has reached the surface of the settled bed, continue to run the pump until the adaptor has been lowered 5 mm into the packed bed.

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10. Close (A) and (D) and stop the pump.

11. Run the column with upward flow for a few minutes to remove residual air trapped in the adaptor. The column is now ready for use.

12. To unpack the column, connect the outlet from the pump to (B) and open (C) while keeping (D) closed. This will cause the adaptor to rise from the bed surface.

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## 5. Evaluation of packing

To check the quality of the packing and to monitor this during the working life of the column, column efficiency should be tested directly after packing, at regular intervals afterwards, and when separation performance is seen to deteriorate.

The recommended method of expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate, HETP, and the asymmetry factor,  $A_s$ . These values are easily determined by applying a sample such as 1% acetone solution to the column. Sodium chloride can also be used as a test substance. Use a concentration of 2.0 M NaCl in water with 0.5 M NaCl in water as eluent.

It is of utmost importance to realize that the calculated plate number will vary depending on the test conditions and it should therefore be used as a reference value only. It is also important that conditions and equipment are kept constant so that results are comparable. Changes in solute, solvent, eluent, sample volume, flow rate, liquid pathway, temperature, etc. will influence the results.

For optimal results, the sample volume should be at maximum 2.5% of the column volume and the flow rate between 15 and 30 cm/h.

If an acceptance limit is defined in relation to column performance, the column plate number can be used as part of the acceptance criteria for column use.

### Method for measuring HETP and $A_s$

To avoid dilution of the sample, apply it as close to the column inlet as possible.

Conditions	
sample volume:	2.5% of the bed volume
sample conc.:	1.0% v/v acetone
flow rate:	15 cm/h
UV:	280 nm, 1 cm, 0.1 AU

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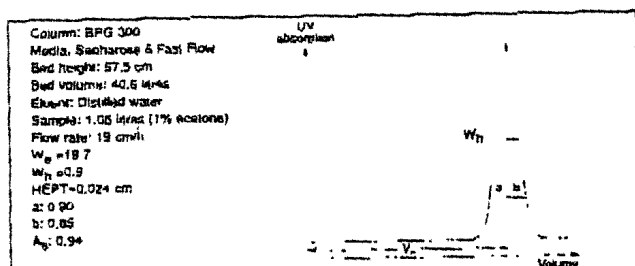


Fig. 5. UV trace for acetone in a typical test chromatogram showing the HETP and  $A_s$  value calculations.

## 6. Maintenance

For best performance of coupled CNBr-activated Sepharose 4 Fast Flow over a long working life, follow the general procedures described below. In all cases, we recommend testing the procedures at small scale first.

### Equilibration

After packing, and before a chromatographic run, equilibrate with working buffer by washing with at least 5 bed volumes.

### Cleaning-In-Place

Cleaning-in-place, (CIP), is a cleaning procedure which removes contaminants such as lipids, precipitates or denatured proteins that may remain in the packed column after regeneration. Such contamination is especially likely when working with crude materials. Regular CIP prevents the build-up of these contaminants in the packed bed, and helps to maintain the capacity, flow properties and general performance of the medium.

A specific CIP protocol should be designed for each process according to the type of contaminants present and stability of coupled ligand. The frequency of CIP depends on the nature and

Calculate HETP and  $A_s$  from the UV curve (or conductivity curve) as follows:

$$HETP = L/N$$

and

$$N = 5.54(V_e/W_h)^2$$

where L = Bed height (cm)

N = Number of theoretical plates

$V_e$  = Peak elution distance

$W_h$  = Peak width at half peak height

$V_e$  and  $W_h$  are in the same units

To facilitate comparison of column performance the concept of reduced plate height is often used.

The reduced plate height is calculated as

$$\frac{HETP}{d}$$

where d is the diameter of the bead. As a guideline, a value of  $\leq 3$  is normally acceptable.

The peak should be symmetrical, and the asymmetry factor as close as possible to 1 (values between 0.8-1.5 are usually acceptable). A change in the shape of the peak is usually the first indication of bed deterioration due to use.

Peak asymmetry factor calculation:

$$A_s = b/a$$

where

a = 1st half peak width at 10% of peak height

b = 2nd half peak width at 10% of peak height

Figure 5 shows a UV trace for acetone in a typical test chromatogram in which the HETP and  $A_s$  values are calculated.

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the condition of the starting material and other process requirements, but one CIP cycle is generally recommended every 1-5 separation cycles. Following are generally recommended procedures.

### CIP protocol

Precipitated or denatured substances

Wash with 2 column volumes of 6 M guanidine hydrochloride. Wash substances immediately with at least 5 column volumes of sterile filtered binding buffer.

Hydrophobically bound substances

Wash the column with 2 column volumes of a non-ionic detergent (conc. 0.1-0.5%). Wash immediately with at least 5 column volumes of sterile filtered binding buffer.  
or  
Wash the column with 3-4 column volumes of 70% ethanol. Wash immediately with at least 5 column volumes of sterile filtered binding buffer.

### Sanitization

Sanitization inactivates microbial contaminants in the packed column and related equipment. A specific sanitization protocol should be designed for each process according to the type of contaminants present and stability of coupled ligand. Following are generally recommended procedures.

Equilibrate with a buffer consisting of 2% hibitane digluconate and 20% ethanol. Allow to stand for 6 hours, then wash with at least 5 column volumes of sterile binding buffer.

or

Equilibrate with 70% ethanol. Allow to stand for 12 hours, then wash with at least 5 column volumes of sterile binding buffer.

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Note: Specific regulations may apply when using 70% ethanol since it can require the use of explosion-proof areas and equipment. Consult your local safety regulations for more information.

#### Storage

CNBr-activated Sepharose 4 Fast Flow is supplied freeze dried. Additives are included to preserve the bead form of the medium. When stored below 8 °C, the shelf life is at least 18 months. Packed columns should preferably be equilibrated in binding buffer containing 20% ethanol (ligand dependent) to prevent microbial growth.

## 7. Trouble-shooting

#### High back pressure

1. Check that all valves between the pump and the collection vessel are fully open.
2. Check that all valves are clean and free from blockage.
3. Check if equipment in use up to and after the column is generating any back-pressure. (For example valves and flow cells of incorrect dimensions.)
4. Perform CLP to remove tightly bound material from the media.
5. Check column parts such as filters, nets etc., according to the column instruction manual.

#### Unexpected chromatographic results

1. Check the recorder speed/signal.
2. Check the flow rate.
3. Check the buffers.
4. Check that there are no gaps between the adaptor and the medium bed, or back mixing of the sample before application.
5. Check the efficiency of the column packing, see page 18.
6. Check if there have been any changes in the pretreatment of the sample.

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#### Infections

1. Check the connections and prefilters.
2. Check the in-going components such as buffers, sample components, etc.
3. Check that the column has been properly sanitized.

#### Trapped air

1. Check that the buffers are equilibrated to the same temperature as the packed column.
2. Check that there are no loose connections or leaking valves.

If air has entered the column, the column should be repacked. However, if only a small amount of air has been trapped on top of the bed, or between the adaptor net and head, it can be removed by pumping eluent in the opposite direction. After this, check the efficiency of the packed bed, see page 18, and compare the result with the original efficiency values.

## 8. Ordering information

Product	Pack size	Code No
CNBr-activated Sepharose 4 Fast Flow	10 g	17-0981-01
	250 g	17-0981-03
	2 kg	17-0981-05

All products are supplied freeze dried in the presence of additives.

For additional information, including Data File, application references and Regulatory Support File, please contact your local Pharmacia Biotech representative.

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amersham pharmacia biotech

## Telefax

To: Chris Metallo

U. Penn

215-573-7601

Date: 2/23/00

From: Anne Barry

Separations Marketing

Amersham Pharmacia Biotech

Tel: 1-800-526-3593 X8224

Fax: 908-457-0557

No. pages (incl. cover) 9

Chris -

Our technical support rep requested this datafile on our steel production columns be sent to you. The file is a bit out of date, but the basic info is still correct.

Hope it suits your needs.

Regards,

Anne

Amersham Pharmacia Biotech  
800 Centennial Avenue  
PO Box 1327  
Piscataway  
New Jersey  
0885-1327

tel 800-526-3593  
fax 732-457-0557

web-site  
<http://www.apbiotech.com>

## BioProcess™ Stainless Steel Columns

## Data File *BioProcess Columns*

BioProcess Stainless Steel Columns are specially constructed fixed bed height columns intended for routine production. The range of columns has been constructed from component materials of the highest quality to meet the most stringent demands of production chromatography, and to resist sanitizing and cleaning agents such as sodium hydroxide and ethanol. Furthermore, the design of the columns with fixed end-pieces provides the highest standards of hygiene.

- Hygienic design and operation
- 300 kPa (3 bar) operating pressure
- High throughput
- Materials include high grade electropolished stainless steel
- Standard bed heights of 150, 300, 600 and 1 000 mm
- Supported with procedures for packing, sanitizing, cleaning and trouble-shooting

BioProcess Stainless Steel Columns, BPSS, are derived from the range of Sephamatic Gel Filter columns with improvements made in both column construction and design of the accessories. Columns are available in standard heights and both 10 mm and 22 mm internal diameter tubing can be obtained for the 400, 600 and 800 mm diameter dimensions. Sanitary tri-clamps facilitate quick and hygienic connection of all tubing.

High pressure specification and low flow resistance make these columns suitable for use in ion exchange, gel filtration, affinity, and hydrophobic interaction chromatography. They are compatible with all Pharmacia Biotech gel media and permit flow rates of up to 150 l/min, enabling kilograms of product to be processed.

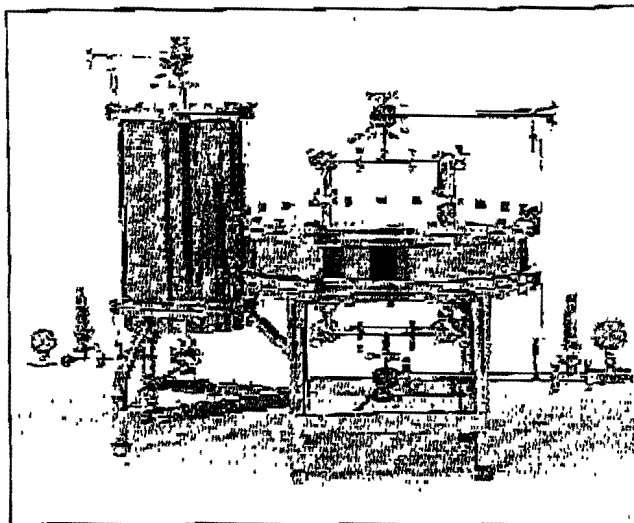


Fig. 1. A selection of BioProcess™ Stainless Steel Columns.

All gaskets are made of recognized EPDM rubber, and electropolished stainless steel to ASTM 316L is now a standard material. Each column is issued with a pressure test certificate and a material certificate for the steel. BPSS columns can also be made to specific dimensions and custom requirements.

The range of columns is fully supported with an Instruction Manual which gives details of packing, sanitizing and cleaning procedures, trouble-shooting and lists all spare parts and accessories.

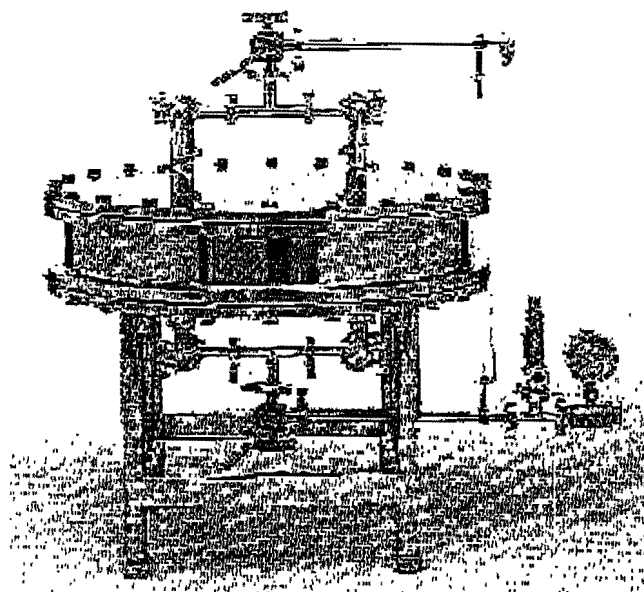


Fig. 2. BPSS 800/150 column showing main assemblies.

## Column Description

BioProcess Stainless Steel Columns comprise three major assemblies: column components; inlet and outlet valve assemblies, safety valve and pressure sensor; end-piece and stand. These are illustrated in Figure 6. As an option a complete BioProcess Stainless Steel Column can be fluoroplastic coated, in which case 22  $\mu$ m polyester nets are required.

## Column components

The column components are the stainless steel column tube, lid, 2 filter nets which are ordered separately, and 2 support nets with jet stream stoppers. Lids and column tubes are constructed of electropolished stainless steel; tubes are available in diameters of 400, 600, 800, 1000 and 1200 mm. Depending on diameter, different standard tube heights are available, refer to Table 1. Other tube heights can be constructed to order. Contact your Pharmacia Biotech representative for details.

## Valve assemblies

The inlet and outlet valve assembly configurations differ for the diameter of column in use. Two sizes of tubing are available for the 400, 600 and 800 mm diameter columns, either 10 mm or 22 mm internal diameter. The 22 mm i.d. tubing is standard for the 1000 and 1200 mm diameter columns. All gaskets and tubing used in the inlet and outlet assemblies are made from EPDM rubber. The columns are supplied for upward flow with the flow-through pressure sensor and safety valve fitted to the bottom of the column. Reversed flow kits are available as an accessory.

Table 1. Primary specifications and characteristics of the range of BPSS columns.

Column	Tube diameter (mm)	Tube height (mm)	Tube weight (kg)	Tube volume (litres)	Tube capacity (litres)	Tube pressure (bar)
BPSS 400 /150	40	1256	15	15	18.8	300
/300			30	30	37.7	300
/600			60	60	75.4	300
/1000			100	100	126.0	300
BPSS 600 /150	60	2827	15	15	42.4	300
/300			30	30	84.8	300
/600			60	60	169.6	300
/1000			100	100	282.7	300
BPSS 800 /150	80	5026	15	15	75.4	300
/300			30	30	150.8	300
/600			60	60	301.6	300
/1000			100	100	502.6	300
BPSS 1000 /150	100	7853	15	15	117.8	300
/300			30	30	235.6	300
/600			60	60	471.2	300
BPSS 1200 /150	120	11309	15	15	169.6	300
/300			30	30	339.3	300

## End-piece and stand

The combined end-piece and stand is supplied with adjustable feet and lifting bars as standard. The 400 and 600 mm diameter columns can be fitted with the optional wheel kit. The wheels incorporate foot operated brakes.



Fig. 3. Some components and accessories for BPSS columns: flow-through pressure sensor with manometer, safety valve, filter nets, support nets, valve, eye bolts, steering bolts, torque wrench, tubing connectors with clamps and gaskets.

## Standard components

BioProcess Stainless Steel Columns are supplied with a number of components, including a flow-through pressure sensor with manometer, a safety valve, 3-way manually operated valves, support nets, steering bolts, eye bolts, a box wrench and a torque wrench.

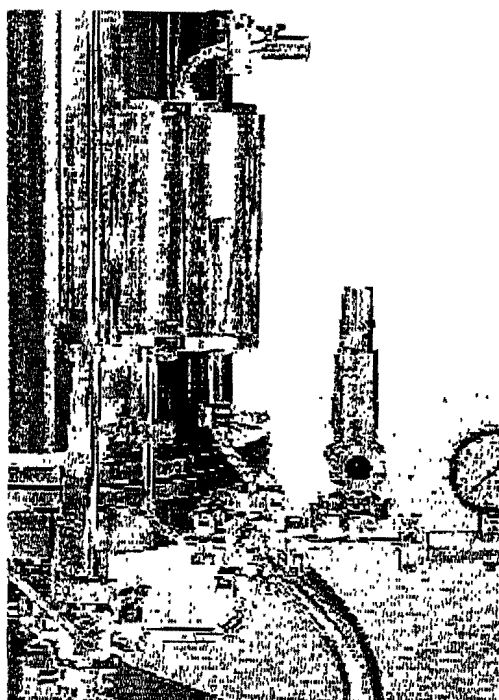


Fig. 4. The Air Trap is one of the accessories available for BPSS columns.

## Accessories

The accessories available for BioProcess Stainless Steel Columns include an Air Trap, Reversed Flow Kit, Packing Device, and connectors with clamps and gaskets.

The Packing Device facilitates the packing of media using the suction method. The packing device is supplied with a flat gasket and sufficient clamps (4, 6 or 8) to secure it to the column tube.

Hastelloy nets are an alternative to stainless steel nets when using fluoroplastic coated columns. They are available for all diameters.

## Column Performance

BioProcess Stainless Steel Columns are easy to pack, test, run and maintain. Their compatibility with BioProcess chromatography system and BioProcess Media (Sephadex G-25, Sepharose Fast Flow and Sephacryl High Resolution) greatly contributes to safe, effective and reliable bioprocessing, as the following example demonstrates.

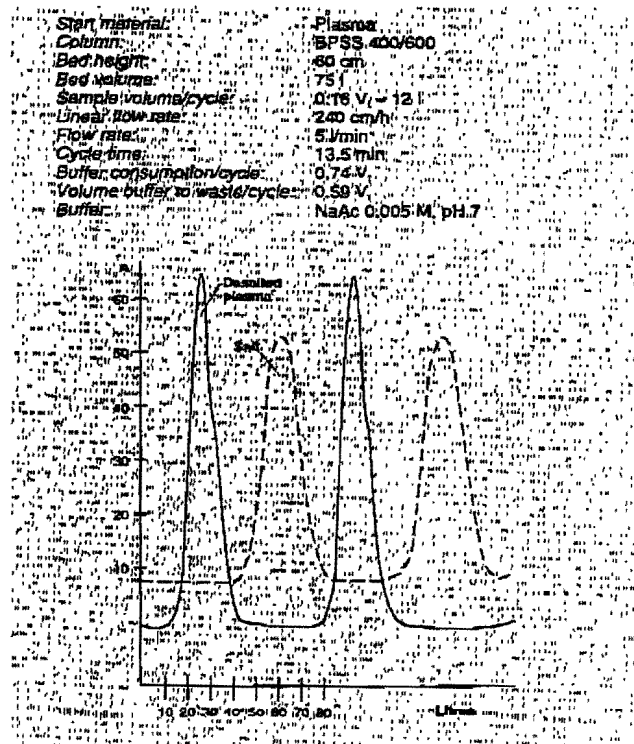


Fig. 5. Efficient desalting and removal of low molecular weight impurities on a BPSS 400/600 packed with Sephadex® G-25 Coarse.

## Large scale desalting

Figure 5 shows how a BPSS 400/600 packed with Sephadex G-25 Coarse to a bed height of 60 cm effectively desalts Factor IX depleted plasma, leaving it free of low molecular weight impurities. After centrifugation and pH adjustment, the plasma is loaded onto an ion exchange column for further purification. The productivity of this step was 53.3 litres of plasma per hour.

## Hygiene

BioProcess Stainless Steel Columns have been designed to give the highest level of hygiene. They contain the minimum of components to facilitate simple cleaning methods and reliable operation.

All the steel surfaces are electropolished to hinder microbial attachment and the clamp fittings used permit the use of cleaning-in-place (CIP) procedures. All column parts can be cleaned with the most commonly used agents, such as detergents, ethanol, weak acids, sodium hydroxide and high salt concentrations. Regular CIP of packed columns between production batches removes precipitated material, strongly bound substances and other contaminants from the column bed without dismantling the column.

## Challenge test procedure

To test the effectiveness of the hygienic design a BioProcess Stainless Steel Column was challenge tested with a combination of microbes (Table 2). The column, a BPSS 400/600, was packed with Sephacryl S-200 High Resolution and connected to an RRA 71B/2 pump. The column was first infected, then sanitized, and then tested to determine the number of viable organisms.

Table 2. Microbes used in the challenge tests are from ATCC.

Organism	ATCC	Gram
<i>Escherichia coli</i>	8739	Gram negative bacteria
<i>Pseudomonas aeruginosa</i>	9027	Gram negative bacteria
<i>Staphylococcus aureus</i>	6538	Gram positive bacteria
<i>Candida albicans</i>	10231	Yeast
<i>Aspergillus niger</i>	16404	Mould

\*ATCC = American Type Culture Collection

Contaminated Peptone Water was pumped into the column from the top at a flow rate of 36 l/h and a pressure of 80 kPa. The column was then left at room temperature for 18–20 hours with no pressure applied.

0.5 M NaOH was introduced into the column and circulated at a pressure of 80 kPa for 60 minutes. After this time interval 5 litres of sterile physiological saline were pumped through the column. The outlet sample was taken before the saline wash was completed.

The test sites were sampled following sanitization with NaOH and despite very high infection through the introduction of highly contaminated solutions no bacteria, yeast or fungi were detected.

## Challenge test results

The results confirm that NaOH is an effective anti-microbial agent, and that it is appropriate for use with BPSS columns. Pharmacia Biotech recommends, however, that this method be applied in conjunction with other carefully controlled hygiene routines and rigorous control of buffers, water and other materials.

## Materials

The materials used in the construction of BPSS columns have been carefully selected for their compatibility with the solvents most commonly used in liquid chromatography, column maintenance and cleaning procedures.

The components of the column may be considered to contain wet and dry parts. Table 3 shows the major components of the columns and identifies the materials from which the wet and dry parts are manufactured.

Table 4 is a guide to the resistance of the materials used in BPSS columns to chemicals used as solvents, and also to autoclaving.

ER column/resin

binding capacity of Aetideam Resin  
range from 200 - 20,000  $\frac{\text{EU}}{\text{mL resin}}$  (Endotoxin units)

Not many endotoxins in our product stream: no bacteria used

From Bioprocess Glass columns, use 8.2 L column  
200/500 - 26 cm bed height

Prof. Diamond recommended we assume 5 EU/mL in our stream

$$\frac{5 \text{ EU}}{\text{mL}} \times 232,000 \text{ mL} = 1.16 \times 10^6 \text{ EU}$$

$$\text{required [EU]} = 0.05 \frac{\text{EU}}{\text{mL}} \text{ (undetectable)}$$

$$0.05 \frac{\text{EU}}{\text{mL}} \times 232,000 = 11,600 \text{ EU}$$

$$\text{required removal} = 1.16 \times 10^6 - 11,600 = 1.15 \times 10^6 \text{ EU}$$

removal ability of 8.2 L column assuming only 200  $\frac{\text{EU}}{\text{mL}}$

$$200 \frac{\text{EU}}{\text{mL}} \times 8200 \text{ mL} = 1.64 \times 10^6 \text{ EU} > 1.15 \times 10^6 \text{ EU} \quad \text{lower bound}$$

8.2 L is okay      20 cm diameter  
26 cm bed height

## ER Column Pump

$$v_o := 100 \cdot \frac{\text{cm}}{\text{hr}} \quad l := 26 \cdot \text{cm} \quad \mu := .015 \cdot \text{poise} \quad D_p := 110 \cdot 10^{-4} \cdot \text{cm}$$

$$\varepsilon := 0.35 \quad \rho := 1000 \cdot \frac{\text{kg}}{\text{m}^3}$$

$$\Delta P := \left[ 150 \cdot \mu \cdot v_o \cdot \frac{(1 - \varepsilon)^2}{D_p^2 \cdot \varepsilon} + 1.75 \cdot \rho \cdot v_o^2 \cdot \frac{(1 - \varepsilon)}{D_p \cdot \varepsilon} \right] \cdot l \quad \Delta P = 1.92 \cdot \text{psi}$$

Assume pressure drop across column is 10 psi  $\Delta P := 10 \cdot \text{psi}$

$$F := 10.99 \cdot \frac{\text{kg}}{\text{hr}} \quad \text{Flow through Pump} \quad \text{Size} := F \cdot \frac{\Delta P}{\rho}$$

$$\rho := 63.72 \cdot \frac{\text{lb}}{\text{ft}^3} \quad \text{Density of Water} \quad \text{Power} := \frac{\text{Size}}{\eta}$$

$$\eta := 0.80 \quad \text{Pump efficiency} \quad \text{Head} := \frac{\Delta P}{\rho \cdot g}$$

$$\text{Size} = 2.823 \cdot 10^{-4} \cdot \text{hp}$$

$$\text{Power} = 0.263 \text{ W}$$

$$\text{Hours per batch: } t := 7920 \cdot \text{hr}$$

$$\text{Electricity} := \text{Power} \cdot t$$

$$\text{Head} = 22.599 \cdot \text{ft}$$

$$\text{Electricity} = 2.084 \cdot \text{kW} \cdot \text{hr}$$

$$b := 1.5 \cdot \text{hp}$$

$$b = 1.119 \cdot \text{kW}$$



March 28, 2000

Mr. Christian Metallo  
Univ of Pennsylvania

Dear Christian,

Thank you for your call earlier today. Enclosed please find the pricing information you requested on Acticlean Etox. The pricing info is as follows:

<u>Product</u>	<u>Cat#</u>	<u>Vol.</u>	<u>Price</u>
Acticlean Etox	2705-01	20ml	\$420.00
	2705-02	100ml	\$1575.00
	2705-04	1L	\$4800.00
	2705-04	10L	\$4320.00/L
	2705-05	25L	\$4080.00/L
	2705-05	50L	\$3840.00/L

The resin needs to be cleaned with 1N NaOH after each use. This allows the resin to be used between 50-75 times without loss of activity of the resin. Therefore, the resin is very efficient for long term use.

The resin is currently being used for the manufacture of therapeutics which are approved and still in clinical trials. In addition we have a Regulatory Support File and a Drug Master File on the resin to assist you with regulatory issues with the resin.

If you have further questions or need additional assistance, please give me a call. I can be reached at 800-535-0735 or through email at bshoffman@earthlink.net. Thank you for your interest in Sterogene Bioseparations, Inc. We look forward to working with you.

Sincerely,

Bruce Hoffman  
Director of Sales

331

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Sterogene Bioseparations, Inc.  
5922 Farnsworth Ct.  
Carlsbad, CA 92008  
Tel: 760 929-0455  
Fax: 760 929-4720

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Sterogene Bioseparations Europe  
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5032 Isnes - Belgium  
Tel: +32 81 56 99 11  
Fax: +32 81 56 99 19





## Easy, rapid endotoxin removal from biologics

- Removing endotoxins from proteins and other biologics can be difficult and expensive. The most commonly used methods include ultrafiltration, size exclusion and ion exchange chromatography. Recently, solid-phase endotoxin-adsorbing reagents have become available, however, their binding affinities are often too low to dissociate endotoxin-protein complexes effectively. Nonspecific binding of proteins to these reagents is another common problem.

## High Binding Capacity

In a comparative study, Acticlean Etox exhibited significantly higher endotoxin binding capacity than immobilized polymyxin:

## Stable Linkage Chemistry

## Column Chromatography

Acticlean Etox can be used in either a batch mode or a chromatography column. Columns are easily prepared by packing with the depyrogenated resin as a 50% slurry in sterile buffer. Contact time with the resin should be optimized for every application. To further increase endotoxin binding efficiency, the protein solution can be passed through more than one column. Column operation is generally more effective than batch processing.

## Batch Processing

For batch depyrogenation, the settled gel is simply added directly to the protein solution. Several contact times ranging from 3 to 20 min should be tested to determine the most complete removal of endotoxin.

## Nontoxic Ligand

The immobilized ligand is nontoxic, nonmutagenic and biodegradable. Both acute and chronic animal studies are completed.

## Specifications

Binding Capacity:	up to 20,000 EU/ml
Support Matrix:	Actigel ALD
Linkage:	Secondary amine
Leaching:	<0.1 ppm
Particle Size:	60-160 µm
Sanitization:	1 M NaOH for 2 h

## Sanitization

Acticlean Etox is the only endotoxin-removing resin that can be regenerated with 1 M NaOH. This is made possible by the stability of the ligand and matrix, as well as the ALD linkage chemistry. Acticlean Etox can be sterilized by autoclaving at pH 7.

## Drug Master File

Sterogene is a cGMP manufacturer of bioprocessing resins. A Drug Master File is on file with regulatory agencies in the US and Europe.

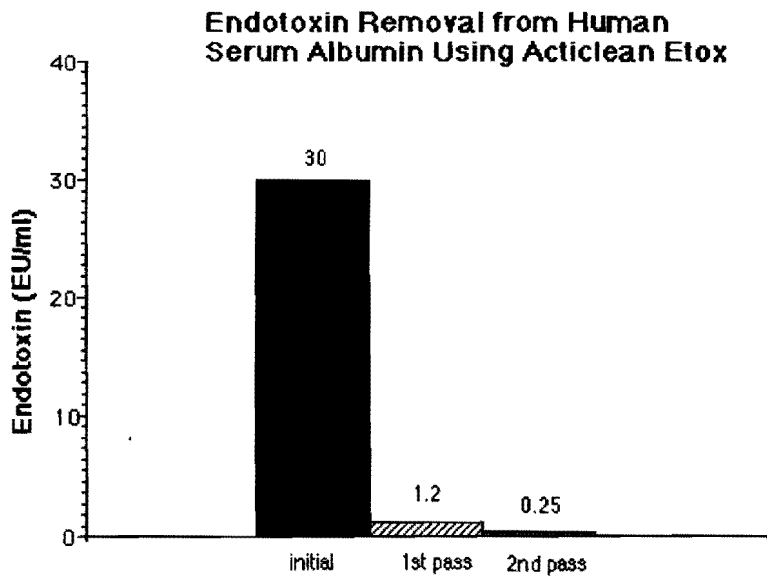
## Applications

The following applications were developed to demonstrate endotoxin removal from plasma derivatives. The final endotoxin levels reflect application needs. Lower final endotoxin levels can be achieved by increasing residence times on the column or increasing resin-to-sample ratios.

All solutions were prepared in endotoxin-free water (LAL Reagent Water, Bio-Whittaker Inc., Walkersville, MD). Endotoxin was measured using the Pyrogen Plus and chromogenic QCL-1000 LAL test kits from Bio-Whittaker. Sterile, disposable plasticware and glassware were used at all times to reduce the chance of endotoxin contamination. Acticlean Etox resin was packed into 2 ml bed volume columns. The columns were cleaned by perfusion with 30 ml of 1M NaOH, allowed to stand at 40C overnight, and washed with LAL water until neutrality. A sample of the final wash must test negative for endotoxin.

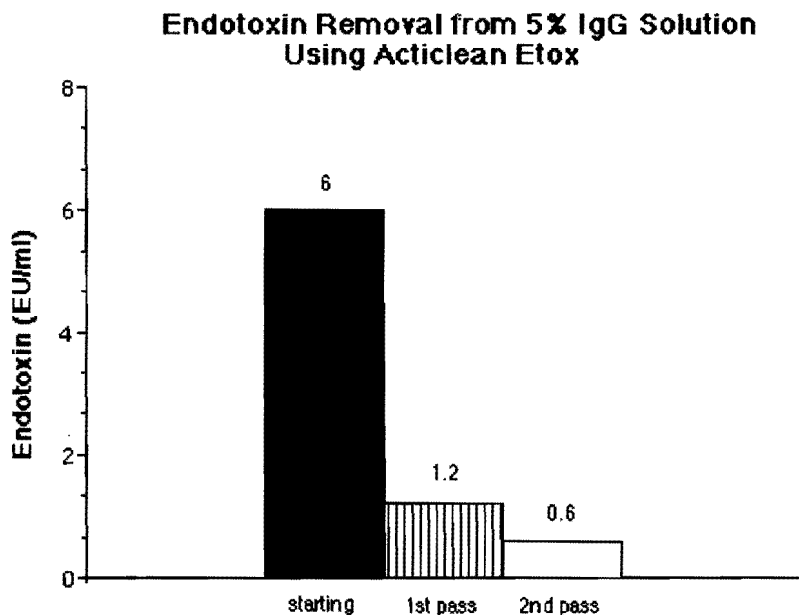
1. Sterile, 25% human serum albumin solution (HSA, Alpha Therapeutic Corp., Los Angeles, CA) was spiked with standard *E. coli* endotoxin to 30 EU/ml and adjusted to pH 7.0. Ten ml of the HSA solution were perfused through two columns set in series at approximately 0.3 ml/min. Five fractions were collected at 2.0 ml increments, then diluted 1 to 10 and heat inactivated (70 0C for 10 min) to prevent any enhancement or inhibition of the endotoxin assay. The fraction with the highest protein concentration was selected, and further dilutions were made to the maximum of expected lysate sensitivity.

A significant reduction in endotoxin was achieved while more than 99% of HSA was recovered.



2. A pyrogenic lot of human Intravenous Immunoglobulin solution (5%) was treated with Acticlean Etox. Ten ml of the IgG sample were perfused through the column at approximately 0.3 ml/min. Five fractions were collected at 2.0 ml increments, then diluted 1 to 3 and heat inactivated at 70°C for 10 min. The fraction with the highest protein concentration was selected, and further dilutions made to the maximum of expected lysate sensitivity.

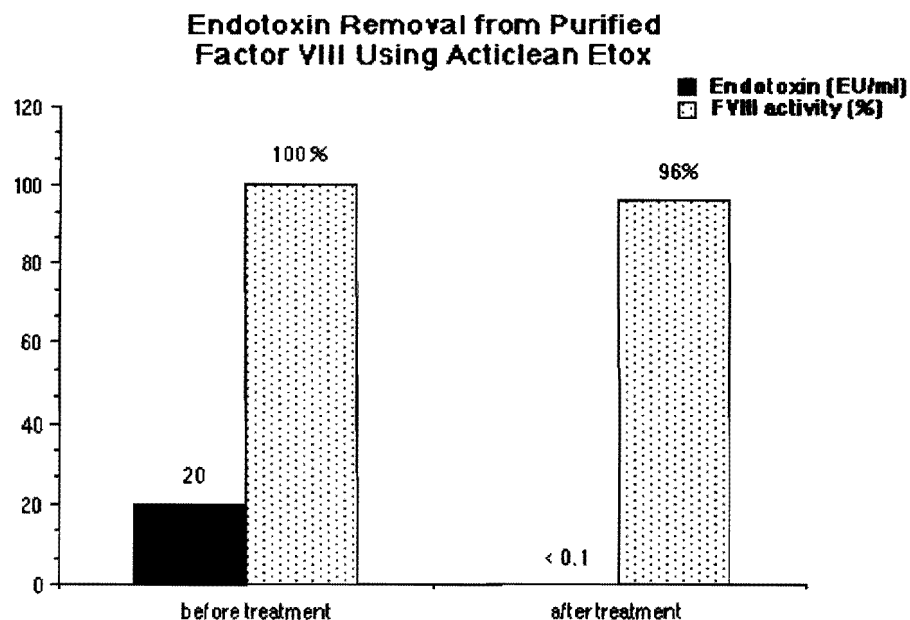
A 10-fold reduction in endotoxin was achieved while more than 98% of IgG was recovered.



3. A sterile, affinity-purified Factor VIII solution (Alpha Therapeutic Corp., Los Angeles, CA) containing 100 units Factor VIII/ml was spiked with *E. coli* endotoxin to 20 EU/ml final concentration and adjusted to pH 7.0. Ten ml of the Factor VIII solution were perfused through the column at 0.3 ml/min. Five fractions were collected at 2.0 ml increments, then diluted 1 to 3 and heat inactivated as above. The fraction with the highest protein concentration

was selected, and further dilutions were made to the maximum of expected lysate sensitivity.

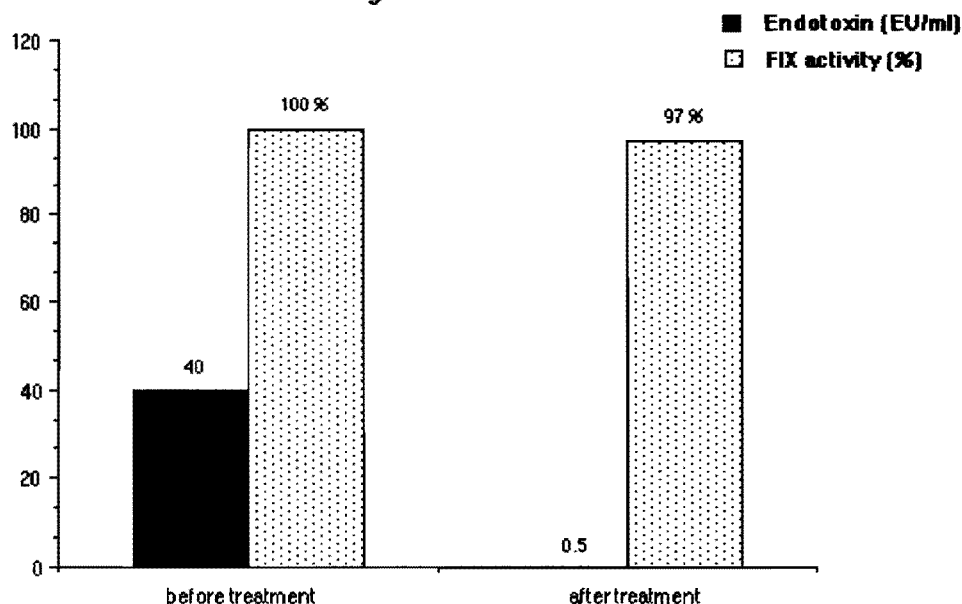
Endotoxin was reduced below 0.1 EU/ml while Factor VIII recovery, determined by a chromogenic assay, was found to be greater than 95%.



4. A sterile, affinity-purified Factor IX solution (Alpha Therapeutic Corp., Los Angeles, CA) containing 100 units Factor IX/ml was spiked with *E. coli* endotoxin to 40 EU/ml and adjusted to pH 7.0. Ten ml of the Factor IX solution were perfused through the column at 0.3 ml/min. Five fractions were collected at 2.0 ml increments, then diluted 1 to 3 and heat inactivated. The fraction with the highest protein concentration was selected, and further dilutions were made to the maximum of expected lysate sensitivity.

Endotoxin was reduced to 0.5 EU/ml while Factor IX recovery, determined by a chromogenic assay, was found to be greater than 98%.

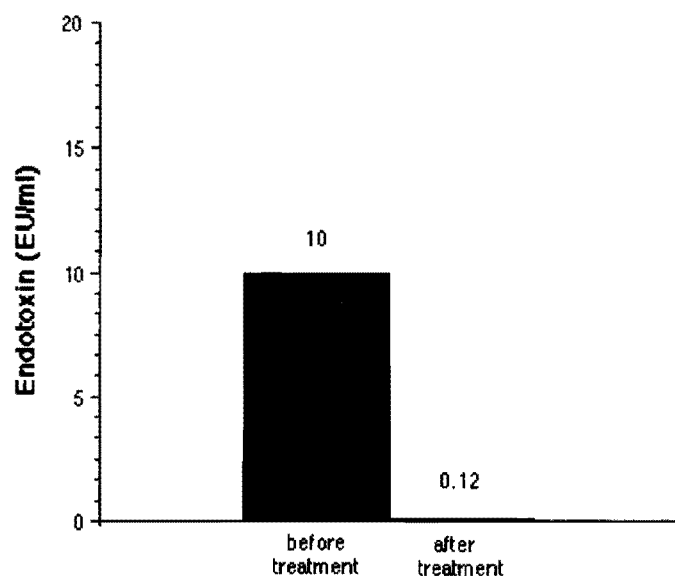
### Endotoxin removal from Purified Factor IX Using Acticlean Etox



5. Sterile fetal calf serum (Biocell Laboratories, Rancho Dominguez, CA) was spiked with *E. coli* endotoxin to 10 EU/ml final concentration and adjusted to pH 7.0. Ten ml of the serum were perfused through the Acticlean Etox column at 0.3ml/min. Five fractions were collected at 2.0 ml increments, and then diluted 1 to 3 and heat inactivated. The fraction with the highest protein concentration was selected, and further dilutions made to the maximum of expected lysate sensitivity.

The treatment reduced the endotoxin level in the serum down to 0.12 EU/ml.

### Endotoxin Removal from Serum (FBS) Using Acticlean Etox



### Ordering Information

	Catalog No.	Package Size
Acticlean Etox	2705-01	20 ml
	2705-03	500 ml
	2705-04	1 L
Acticlean Etox	2705-PP	9 ml (Prepacked column)

Acticlean Etox media is available in bulk pack sizes for industrial applications. Please inquire for pricing information.

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Sterogene Bioseparations Inc. 5922 Farnsworth Ct Carlsbad, CA 92008 USA Tel: 760-929-0455 Fax: 760-929-8720 email: [info@sterogene.com](mailto:info@sterogene.com) website: [www.sterogene.com](http://www.sterogene.com)

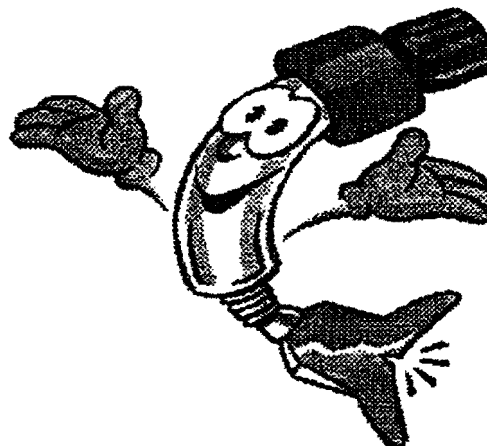
# TELEFAX



*amersham pharmacia biotech*

TO: Chris Metallo  
COMPANY: University of Pennsylvania  
DEPT:  
PHONE:  
FAX: 215-573-7601

FROM: Nancy Elser  
DEPT: Technical Support  
PHONE: 800-526-3593  
FAX: 415-695-1189  
DATE: February 22, 2000



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Purify GST fusion proteins in one step  
High binding capacity

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  - 1 ml and 5 ml prepacked HiTrap™ columns
  - Glutathione Sepharose™ 4 Fast Flow 25 ml
- For more information and to place your order, call  
Amersham Pharmacia Biotech today.

Dear Chris,

Please find enclosed the information you requested on BPG columns. I am still trying to find you more information on the BPSS columns. As soon as I find something appropriate, I will fax it to you.

Regards,  
Nancy Elser  
Tech Support

Amersham Pharmacia Biotech USA tel 800-526-3593  
800 Centennial Ave fax 415-695-1189  
P.O. Box 1327 www.apbiotech.com  
Piscataway, NJ 08855-1327

## BPG™ Columns 100, 140, 200, and 300 series

## Data File Columns

BPG™ columns are glass chromatography columns designed for industrial applications which demand high standards of hygiene. The columns are constructed from component materials of the highest quality and withstand the harsh conditions used for cleaning in place of packed separation media. They are characterized by:

- Hygienic design and operation. Microbial attachment and growth is hindered through the use of calibrated precision glass, high grade electropolished stainless steel and an absence of dead pockets.
- Easy, efficient packing and running with the single-screw adaptor.
- Operating pressures matching most BioProcess Media.
- Materials that conform to the requirements described in the US Pharmacopoeia (USP XXII).
- Comprehensive documentation.

### General description

BPG columns are designed to meet the needs of process development and biopharmaceutical manufacture:

- Inner diameters of 100, 140, 200 and 300 mm.
- Pressure specifications of 8, 6 and 4 bar.
- Low flow resistance.
- Single screw adaptor.
- Suitability for use in ion exchange, gel filtration, affinity and hydrophobic interaction chromatography and compatibility with BioProcess Media.
- Tubing connections made with hygienic sanitary clamp fittings.
- All gaskets recognized as suitable for use in biopharmaceutical production.
- Capacity to offer a wide range of bed heights and volumes.
- An instruction manual containing full details of components, packing, testing, procedures for cleaning and sanitizing, trouble-shooting, and spare parts lists.

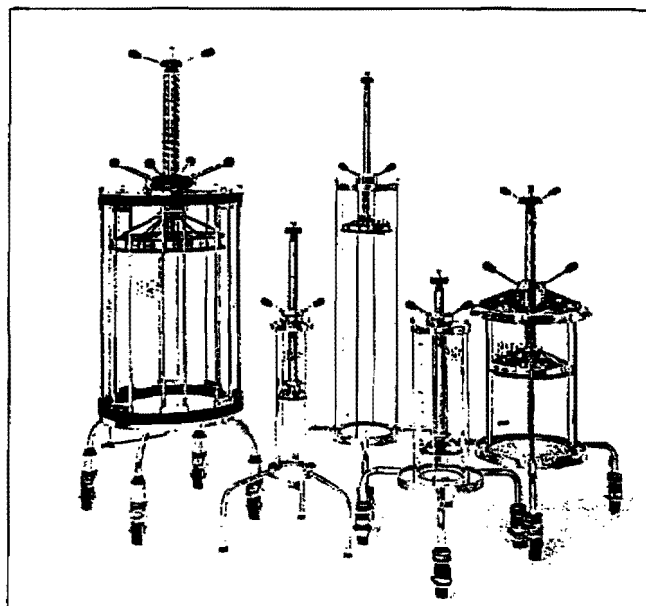


Fig. 1. BPG column family, including BPG 450.

### Design features *Column tube*

The columns are designed to very high standards and use high quality materials:

- Manufactured from calibrated precision borosilicate glass.
- Exact internal diameter tolerance of the glass tube.
- Thin O-ring between the column tube and the adaptor/end-piece forms a very tight seal.
- Minimum dead volume.
- Liquid distribution over a great surface area.



## Liquid distribution

Efficient liquid distribution is crucial for optimal column performance. In BPG columns this is assured through:

- Adaptors and end-pieces based on the well proven design of a single channel inlet/outlet.
- Support nets with a coarse, open structure to distribute liquid from the central inlet rapidly and uniformly over the entire surface area.
- Thin nets to maintain even pressure distribution over the bed surface and permit liquid to pass through quickly and evenly onto the bed, without creating extra back-pressure.
- Polypropylene distribution plates in the adaptor, and in the bottom plate in BPG 300 columns, which give uniform distribution/collection of liquid at the interface between the net and the packed bed. These plates are not necessary in narrower diameter columns.



Fig. 2. Adaptor end-piece and net. Flat surfaces give even spread of sample.

## Operation Hygiene

BPG column are intended for use in environments with some of the toughest regulatory controls:

- Design and materials of construction ensure hygienic operation.
- Little maintenance is required in routine use. The columns are easy to keep clean and free from microbial contaminations.
- Autoclavable when disassembled.
- All tubing connections are made with sanitary clamp fittings.

- Columns are easily sanitized. A packed BPG column was subjected to microbial challenge testing using five micro-organisms recommended by the United States Pharmacopoeia (USP XXI). Sodium hydroxide (NaOH) was the anti-microbial agent. The study showed that 0.5 M NaOH applied for 30-60 minutes is a good basis for developing an effective sanitization procedure. The study is presented in Technical Note 211.

## Easy to pack

The design of adaptor with a single-screw makes light work of all adaptor movement. It is easily adjusted during packing and operation, even on the largest of columns.

## Scalable

BPG columns are ideal for scaling up from smaller lab scale or method development columns. As the example shows here, Fig. 3, the chromatogram obtained with an XK 16/20 column, i.d. 16 mm, is consistent with the chromatogram obtained from the scaled up run on BPG 300/500 column, i.d. 300 mm. The scale up factor is 350 and no evidence of dilution or loss of recovery was detectable.

## Materials

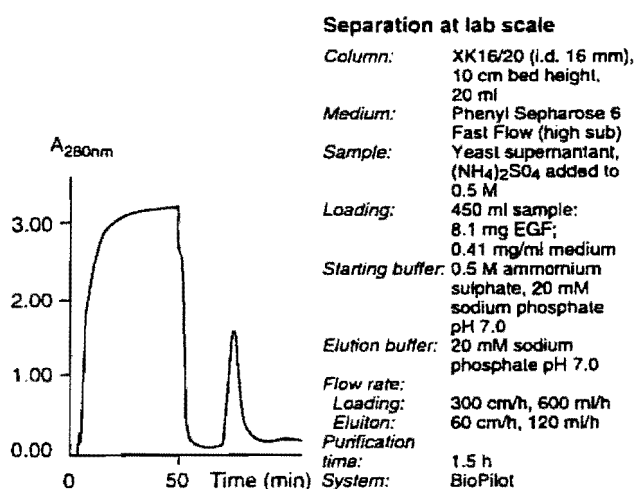
All the materials selected for the manufacture of BPG columns are of high quality, often with special properties, or specially treated, Table 1. All materials used in BPG columns meet the requirements described in the USP XXII:

- Materials are compatible with the liquids commonly used in process scale chromatography (including sanitization and cleaning agents such as NaOH and ethanol), see Table 2.
- Parts in contact with sample and process liquids are made of materials of very high quality and chemical resistance.
- All stainless steel components are electro-polished. This improves resistance to corrosion and reduces friction and contamination.
- Polymeric materials have been tested for their biological reactivity according to USP XXII.
- Documentation of all material testing is compiled in the Validation Support File.

Table 1. Column component materials. Components may be considered to contain "wet" parts, parts coming into contact with process liquids, and dry parts. The table identifies the materials from which the "wet" and dry parts are manufactured.

Material	Adaptor		Tube		Major Components		Stand	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Borosilicate glass	-	-	*	-	-	-	-	-
Ethylene Propylene rubber (EPDM)	*	-	-	*	*	-	-	-
Stainless steel: ASTM 316L	*	*	-	*	*	-	-	*
ASTM 304 <sup>1</sup>	-	-	-	-	-	-	-	-
Polypropylene (PP)	*	-	-	-	*	-	-	-
Polytetrafluoroethylene (PTFE) (Teflon)	-	-	-	-	*	*	-	-
Polyamide (PA) nylon (10 µm net)	*	-	-	-	*	-	-	-
Polyetheretherketone (PEEK) with 30 % carbon fibre	-	*	-	-	-	-	-	-
Acetal plastic (POM)	-	*	-	-	-	-	-	-
Fluoroethenepropene (FEP) <sup>2</sup>	*	-	-	-	*	-	-	-
Polyurethane	-	-	-	-	-	-	-	*
Polyvinylchloride (PVC) <sup>3</sup>	-	-	-	-	-	-	-	-

<sup>1</sup> The clamps are made of stainless steel. <sup>2</sup> Option to EPDM. <sup>3</sup> The tubing is made of PVC.



**Separation at process scale**

Column: BPG 300/500 (i.d. 300 mm), 10 cm bed height, 7.1 L  
Medium: Phenyl Sepharose 6 Fast Flow (high sub)  
Sample: Yeast supernatant, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> added to 0.5 M  
Loading: 80 L sample: 2.56 g EGF; 0.36 mg/ml medium  
Starting buffer: 0.5 M ammonium sulphate, 20 mM sodium phosphate pH 7.0  
Elution buffer: 20 mM sodium phosphate pH 7.0  
Flow rate: Loading: 300 cm/h, 2100 L/h  
Elution: 60 cm/h, 42 L/h  
Purification time: 1.5 h  
System: BioProcess controlled by UNICORN

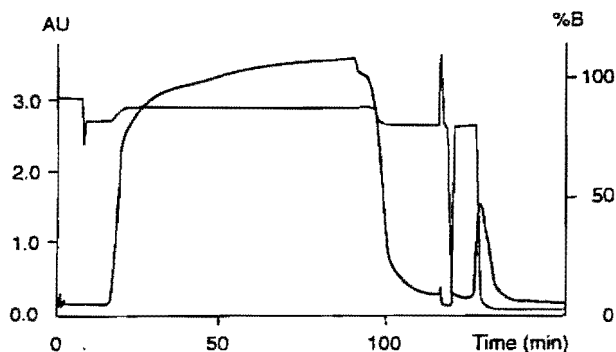


Fig. 3. Development and scale up of a chromatographic downstream process for the purification of recombinant EGF expressed as an extracellular protein from *S. cerevisiae*. The starting material was clarified supernatant. (Daniels, A.L., Petersson, N.T., Scandella, C. Poster presentation, Crystal City, USA, 1992.)

## Chemical resistance

Table 2 is a guide to the resistance of materials to chemical solvents. The information has been compiled from published material from several sources. Please note that the effects of a solvent will be more severe at higher temperatures and that combined effects have not been taken into consideration.

## Individual testing

As evidence of good manufacturing practice, all BPG columns are individually pressure tested and inspected. A test certificate accompanies each column delivery.

Table 2. Chemical resistance of materials of construction.

Substance	Concentration by volume	60-90 days <sup>1</sup>	Substance	Concentration by volume	60-90 days <sup>1</sup>
Acetic acid	1.7 M	see note 6	Hydrochloric acid	0.1 M	see note 6,7
Acetonitrile	5%	see note 2	Isopropyl alcohol	100%	see note 2
Acetonitrile	50%	see note 3	Methanol	100%	see note 2
Acetone	10%	see note 2	Nitric acid	0.1 M	see note 7
Cyclohexane	100%	see note 3	n-Propanol	100%	OK
Ethanol	100%	see note 2	Sodium chloride	2 M	see note 2,5
Ethyl acetate	100%	see note 4	Sodium hydroxide	2 M	OK
Ethylene glycol	50%	OK	Trifluoroacetic acid	0.1%	see note 7,8
Glycerol	100%	OK	Triton X-100	100%	OK
Hexane	100%	see note 2,7	Urea	8 M	OK

Note 1: The test does not include PVC tubing.

Note 2: EPDM rubber changes characteristics. For repetitive and long term use, use FEP O-rings.

Note 3: Change to FEP O-rings, polypropylene plastic resistance is fair.

Note 4: Polypropylene plastic resistance is good/fair.

Note 5: Can be used under normal running conditions. Do not use NaCl in storage solutions. Please note that NaCl can cause corrosion on stainless steel in acid solutions (pH below 4.0).

Note 6: Not longer than 4 hours.

Note 7: Not recommended to be used with PA nets.

Note 8: FEP changes characteristics. Use EPDM rubber.

## Overview of BPG columns

Column	Tube diam. (mm)	Tube height (mm)	Surface area (cm <sup>2</sup> )	Bed height min. (cm)	Bed height max. (cm)	Bed height with packing device (cm)	Bed volume min. (L)	Bed volume max. (L)	Bed volume with packing device (L)	Max. pressure (bar)	Total weight (kg)	Adaptor weight (kg)	Overall dimensions inc. stand & adaptor hxdxd (cm <sup>3</sup> )
100/500	100	500	79	0	26	48	0	2.0	3.8	8	15	7	127x48x48
100/750	100	750	79	25	41	66	2.0	3.2	5.2	8	16	7	152x48x48
100/950	100	950	79	45	54	83	3.5	4.2	6.6	8	17	7	172x48x48
140/500	140	500	154	0	26	48	0	4.0	7.4	6	25	11	127x59x59
140/950	140	950	154	45	54	83	6.9	8.3	12.8	6	27	11	172x59x59
200/500	200	500	314	0	26	48	0	8.2	15.1	6	34	13	127x59x59
200/750	200	750	314	25	41	66	7.3	12.9	20.7	6	36	13	152x59x59
200/950	200	950	314	45	54	83	14.1	16.9	26.1	6	39	13	172x59x59
300/500	296	500	688	0	26	48	0	17.9	33.0	4	68	29	133x69x69
300/750	296	750	688	25	41	66	17.2	28.2	45.4	4	73	29	158x69x69
300/950	296	950	688	45	54	83	31.0	37.2	57.1	4	78	29	178x69x69

## Ordering information

Column	Tube height 500 mm	Tube height 750 mm	Tube height 950 mm	Stand
BPG 100	18-1103-01	18-1103-02	18-1103-03	18-1031-10
BPG 140	18-1113-08	-	18-1113-09	18-1031-20
BPG 200	18-1103-11	18-1103-12	18-1103-13	18-1031-20
BPG 300	18-1103-21	18-1103-22	18-1103-23	included

Each column includes as standard: 23 µm polypropylene filter nets and polypropylene coarse nets, 2 clamps, 2 EPDM gaskets, 2 blank caps and O-rings in EPDM. All stands must be ordered separately except for BPG 300. BPG 100 stand has feet, while BPG 140, 200 and 300 stand has wheels.

Spare parts	BPG 100	BPG 140	BPG 200	BPG 300	Qty/pk
Flange O-ring	18-8494-01	18-1113-06	18-8489-01	18-1012-26	2
Adaptor O-ring	18-8475-01	18-1113-10	18-0275-01	18-1012-51	2
Support net, adaptor	18-1103-04	18-1112-99	18-0252-56	18-1012-53	2
Support net, end-piece	18-0251-55	18-1112-98	18-0252-55	18-1012-36	2
Net, 10 µm, adaptor	18-1103-05	18-1113-03	18-0252-76	18-1012-55	2
Net, 10 µm, end-piece	18-0251-77	18-1113-02	18-0252-77	18-1012-35	2
Net, 12 µm, adaptor	18-1103-06	18-1113-05	18-1104-42	18-1104-44	2
Net, 12 µm, end-piece	18-1104-41	18-1113-04	18-1104-43	18-1104-45	2
Net, 23 µm, adaptor	18-1103-08	18-1113-01	18-9253-01	18-1012-54	2
Net, 23 µm, end-piece	18-9252-01	18-1113-00	18-9254-01	18-1012-34	2

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343

## Preparing the gel

Protein G Sepharose 4 Fast Flow is supplied preswollen in 20% ethanol. Prepare a slurry by decanting the 20% ethanol solution and replace it with binding buffer in a ratio of 75% settled gel to 25% buffer. The binding buffer should not contain agents which significantly increase the viscosity. The column may be equilibrated with viscous buffers at reduced flow rate after packing is completed.

For batch procedures remove the ethanol by washing the gel on a medium porosity sintered glass funnel.

## Packing Sepharose Fast Flow gels

1. Equilibrate all material to the temperature at which the chromatography will be performed.
2. De-gas the gel slurry.
3. Eliminate air from the column dead spaces by flushing the end pieces with binding buffer. Make sure no air has been trapped under the column net. Close the column outlet with a few centimeters of binding buffer remaining in the column.
4. Pour the slurry into the column in one continuous motion. Pouring the slurry down a glass rod held against the wall of the column will minimize the introduction of air bubbles.
5. Immediately fill the remainder of the column with binding buffer, mount the column top piece onto the column and connect the column to a pump.
6. Open the bottom outlet of the column and set the pump to run at the desired flow rate. Ideally, Fast Flow gels are packed at a constant pressure not exceeding 1 bar (0.1 MPa) in XK columns. If the packing equipment does not include a pressure gauge, use a packing flow rate of at least 400 cm/h (15 cm bed height, 25°C, low viscosity buffer).  
If the recommended pressure or flow rate cannot be obtained, use the maximum flow rate the pump can deliver. This should also give a reasonably well-packed gel.

Note: Do not exceed 75% of the packing flow rate in subsequent chromatographic procedures.

7. Maintain the packing flow rate for 3 bed volumes after a constant bed height is reached.

## Using an adaptor

Adaptors should be fitted as follows:

1. After the gel has been packed as described above, close the column outlet and remove the top piece from the column. Carefully fill the rest of the column with binding buffer to form an upward meniscus at the top.
2. Insert the adaptor at an angle into the column, ensuring that no air is trapped under the net.
3. Make all tubing connections at this stage. There must be a bubble-free liquid connection between the column and the pump and column and the sample application system (LV-3 or LV-4 valves).
4. Slide the plunger slowly down the column so that the air above the net and in the capillary tubings is displaced by eluent. Valves on the inlet side of the column should be turned in all directions during this procedure to ensure that air is removed.
5. Lock the adaptor in position, open the column outlet and start the eluent flow. Pass eluent through the column at the packing flow rate until the gel bed is stable. Re-position the adaptor on the gel surface as necessary.

## Binding

IgG from most species binds Protein G Sepharose Fast Flow at neutral pH and physiological ionic strength.

As a general method we recommend 20 mM sodium phosphate, pH 7.0 as binding buffer.

The binding capacity of Protein G Sepharose 4 Fast Flow depends on the source of the particular immunoglobulin, see Table 3. However, the total capacity depends upon several factors, such as the flow rate during sample application, the sample concentration and binding buffer. Table 3 shows the total capacity under defined conditions for IgG from some species.

**Table 1.** The relative binding strength of polyclonal IgG from various species to Protein G and Protein A, as measured in a competitive ELISA test.

Species	Protein G	Protein A
Human		
IgG <sub>1</sub>	++	++
IgG <sub>2</sub>	++	++
IgG <sub>3</sub>	++	-
IgG <sub>4</sub>	++	++
Rabbit	++	++
Cow	++	+
Horse	++	-
Goat	++	+
Guinea pig	+	++
Sheep	++	-
Dog	+	++
Pig	++	++
Rat*	-	-
Mouse**	+	+
Chicken	-	-

++ = strong binding

+ = medium binding

- = weak or no binding

\* Note that IgG from rat binds to Protein G coupled to Sepharose 4 Fast Flow.

\*\* IgG<sub>1</sub> from mouse binds more strongly to Protein G than to Protein A.

Pharmacia LKB recombinant Protein G, MW 17 000 dalton, is produced in *E. coli* and contains two IgG binding regions. The albumin binding region of native Protein G has been genetically deleted, thereby avoiding undesirable cross-reactions with albumin.

**Table 2.** Gel characteristics

Ligand density:	~2 mg Protein G/ml drained gel
Dynamic binding capacity*:	~18 mg human IgG/ml drained gel
Bead structure:	4% highly cross-linked agarose
Bead size range:	45-165 µm
Mean bead size:	approx. 90 µm
Max linear flow rate: ( $\frac{\text{volumetric flow rate (cm}^3/\text{h)}}{\text{column cross-sectional area (cm}^2\text{)}}$ )	>1 300 cm/h at 0.05 MPa (0.5 bar) in a XK 16/20 column, bed height 5 cm
Max operating backpressure:	0.1 MPa (1 bar, 14 psi)
pH working range:	2-9**
pH stability	
Long term:	3-9***
Cleaning-in-place:	3-9***
Chemical stability:	The IgG binding capacity and recovery was maintained after storage for: (a) 7 days at 37°C in: 1 M acetic acid pH 2.0, 20 mM sodium phosphate, 1% SDS, pH 7.0, 6 M guanidine-HCl, 70% ethanol. (b) 2 hours at room temperature in: 0.1 M HCl, pH 1.0, 8 M Urea, pH 10.5, 0.1 M Glycine-NaOH, pH 11
Physical stability:	Negligible volume variation due to changes in pH or ionic strength.
Sanitization:	Sanitize the column with 70% ethanol.
* The dynamic capacity was calculated as the amount of human IgG adsorbed to the gel before the flow through exceeded 1% of the absorbance of the incoming solution. The capacity was determined at a linear flow rate of 30 cm/h and with a sample concentration of 0.92 mg/ml.	
** Protein G may hydrolyse at low pH.	
*** Estimates to the best of our knowledge and our experience.	

pH adjustment

Elution pH = 2.85

$$10^{-2.85} = [H^+] = 0.0014125 \text{ mol/L}$$

desired pH = 7.0       $10^{-7} \text{ mol/L} = [H^+]$ 

$$\text{~~0.00~~ } 10^{-2.85} - 10^{-7} = 0.0014115 \frac{\text{mol } [H^+]}{\text{L}}$$

 $[H^+] + [OH^-] \rightarrow H_2O$  neutralization

$$0.0014115 \frac{\text{mol } [OH^-]_{\text{needed}}}{\text{L}} \cdot \frac{\text{mol NaOH (1M)}}{\text{mol } [OH^-]}$$

Stream P-19 contains 232.437 L

$$232.437 \text{ L} \cdot \frac{0.0014115 \text{ L 1M NaOH}}{\text{L}} = \boxed{0.328 \text{ L 1M NaOH input}}$$

Final Concentration adjustments  
in MF Tank

Prof. Diamond's specs 2M Arginine

↳ 140 mM salt - already met

~ 100 mM sucrose

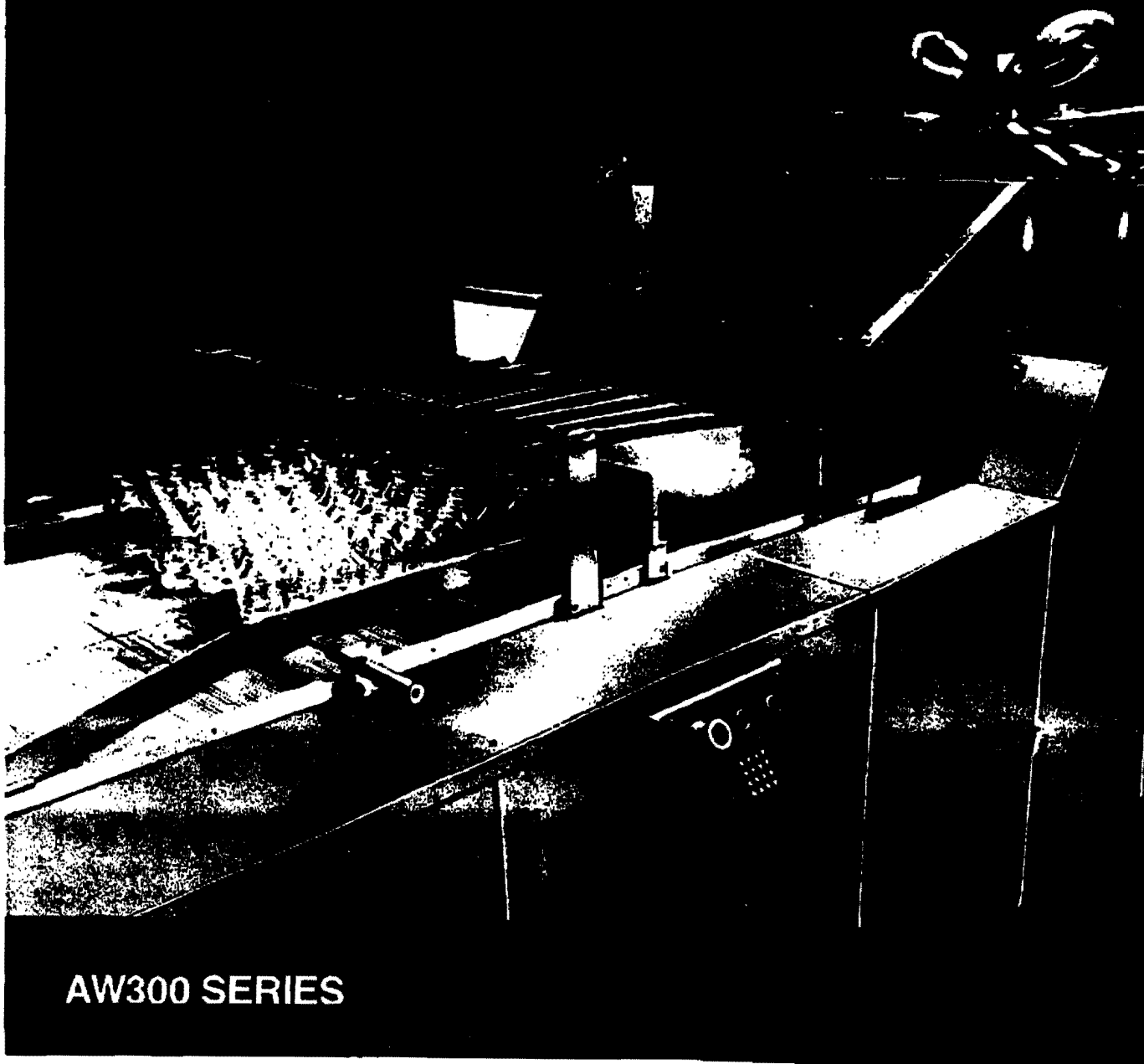
Superpro used to calculate inputs by trial &amp; error

sucrose &amp; Arg. needed to be added

volume increased to ~ 400 L for bottling purposes

# COZZOLI

## AUTOMATIC CLEANING/ WASHING SYSTEMS FOR PHARMACEUTICAL GLASSWARE



AW300 SERIES

**COZZOLI**  
MACHINE COMPANY

SENT BY:

VISCOSITY		FEED	FEED	FEED
Unscramble + Flush Fill + Cap		Unscramble + Flush Fill + Plug		
6	12	1	1	2
Up to 800 mPa		Up to 50 ml		
2		1		
Up to 4 1/2" (114mm)		1" (25mm)		
Conventional - Stopped		Screw Cap + Dropper Assembly		Plugs + Stoppers
28mm - 30mm		3/4" (8mm) OD to 1 1/4" (32mm)		
Vials - Bottles		Vials - Screw Neck Tubes	Vials - Tubes	Vials - Straight Wall Tubes
Up to 8" (229mm)		1 1/4" - 4 1/4" (35mm - 111mm)		
Up to 2 1/4" (67mm)	Up to 2" (51mm)	3/4" - 1 1/4" (8mm - 38mm)		
Up to 200	Up to 300	Up to 55		110
		Up to 55		
		Intermittent		
2 1/4" (67mm)	2" (51mm)	1 1/4" (38mm)		
		Unscrambler		
		Chain		
120" (3048mm)		N/A		
230V, 3PH, 60 Hz 1/2 HP	115V, Single PH, 60 Hz, 1/2 HP	1/2 HP	1/2 HP	1/2 HP
Start/Stop Push Button DC Drive		115V, Single PH; 50/60 Hz		
26 1/4" x 30" x 57 1/4" 26 1/4" x 30" x 57 1/4"	17 1/2" x 54 1/4" x 68" 443mm x 1396mm x 1727mm	60" x 48" x 60" 1524mm x 1219mm x 1524mm	72" x 48" x 48" 1829mm x 1219mm x 1219mm	
140" x 60" x 70" 3556mm x 1524mm x 1778mm	180" x 70" x 79" 4571mm x 1778mm x 2007mm	108" x 60" x 108" 2743mm x 1524mm x 2743mm	120" x 84" x 108" 3048mm x 2134mm x 2743mm	
3000 lbs. 1361 kg.	3600 lbs. 1631 kg.	900 lbs. 409 kg.	1600 lbs. 726 kg.	
4000 lbs. 1814 kg.	4500 lbs. 2045 kg.	1250 lbs. 568 kg.	2500 lbs. 1133 kg.	

# Fill heads  
Max FTL

Max ~~NO~~ NOZLE DIVE  
stopper/closure type

NOTES:

1. Expandable to double or triple fill.
1. Expandable to double fill only.
2. Depends on number of filling heads.
3. Also brush and cap, cologne dabbers - maximum 3 1/2" (89mm) long.
4. Optional parts required.
5. Depending on fill volume.
6. Other electricals available.
7. Approximate.
8. Machine dimensions include sample weighing system.
- Depends upon size of container opening.
- Larger sizes with optional change parts.
- Container set up selection PC controlled using servo/stepper motor drives

max speed

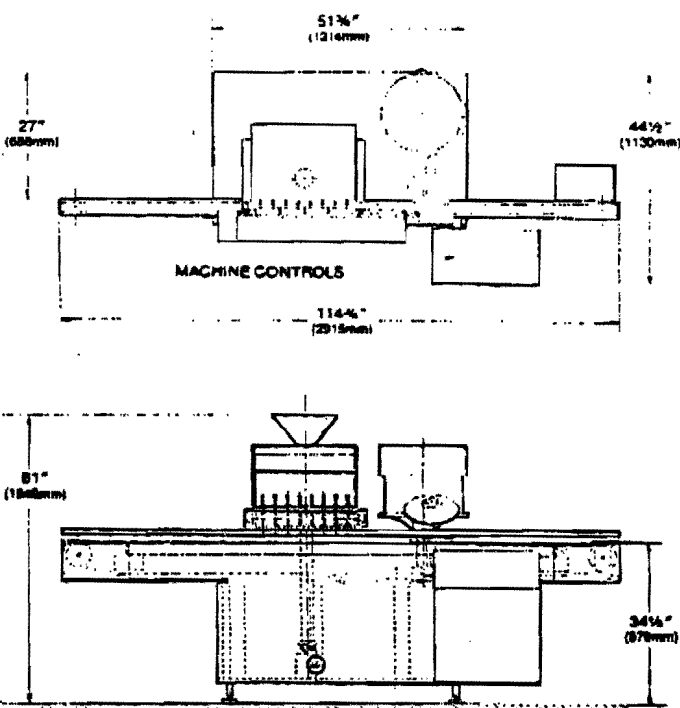
max diameter

Conveyor length

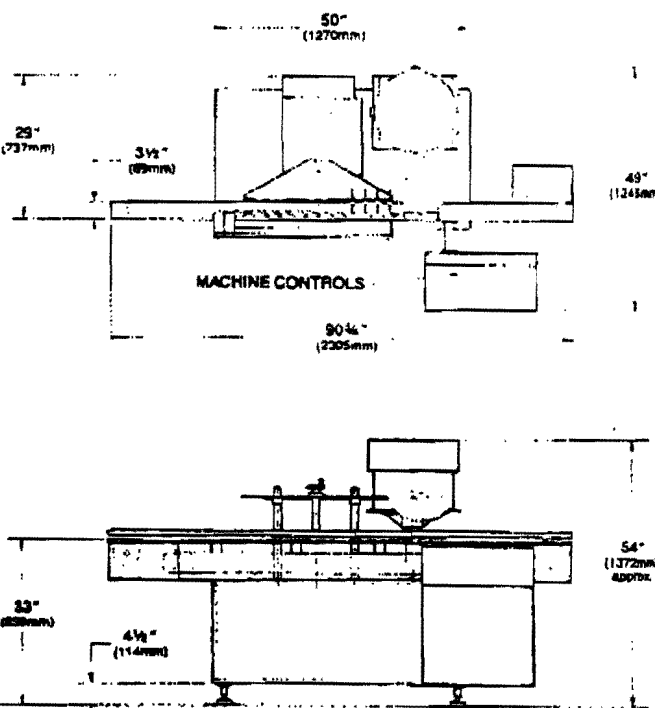
Cozzoli Machine Company maintains a continuing development program; equipment manufactured after publication may vary from specifications given here.

VR 840 S - 180,000

MODEL PF/S



MODEL VR2/S







# AW Series Washers

## Models AW160, AW240, AW300, AW400, AW500

MACHINE MODEL	AW160	AW240	AW300	AW400	AW500
VALS PER CARRIER	8	12	15	20	25
* MIN./MAX. CONTAINER DIAMETER	1/2" to 3/4" 13mm to 84.1mm	1/2" to 2 1/4" 13mm to 52.3mm	1/2" to 1 1/2" 13mm to 41.3mm	1/2" to 1 1/2" 13mm to 38.4mm	1/2" to 1 1/2" 13mm to 38.4mm
WASHING NEEDLE CENTERS	3 1/2" (88.9mm)	2 3/4" (55.5mm)	1 3/4" (44.4mm)	1 1/2" (31.75mm)	1 1/2" (31.75mm)
* MAX. CONTAINER HEIGHT	7" (177.8mm)				
MACHINE SPEED	6 to 20 cycles per minute				
MAX. HOURLY PRODUCTION	9600	14,400	18,000	24,000	30,000
WASH TIME PER STATION	Programmable				
PROCESSING LINE CONNECTIONS	1 1/2" O.D. Tri-Clover Clamps				
DRAIN CONNECTION	1 1/2" Tri-Clover Clamps and 2 1/2" O.D. Tube				
AIR VACUUM CLEAN - 2 STATIONS - EACH STATION					
MAX. AIR CONSUMPTION at 50 p.s.i. (3.5kg/cm <sup>2</sup> )	80 C.F.M. 2264 L.P.M.	120 C.F.M. 3376 L.P.M.	150 C.F.M. 4245 L.P.M.	200 C.F.M. 5640 L.P.M.	250 C.F.M. 7075 L.P.M.
AIR FLOW RATE at 50 p.s.i. (3.5kg/cm <sup>2</sup> )	5.6 C.F.M. 150 L.P.M.	8.4 C.F.M. 230 L.P.M.	10.5 C.F.M. 297 L.P.M.	14 C.F.M. 376 L.P.M.	17.5 C.F.M. 495 L.P.M.
VACUUM REQUIREMENTS at 3.5" Hg. (89mm Hg.)	100 C.F.M. 2832 L.P.M.				
INTERNAL WASH - 6 STATIONS (3 Water, 3 Air) - 20 P.S.I. (1.4kg/cm <sup>2</sup> ) - EACH STATION					
*** WATER CONSUMPTION	83 G.P.H. 296 L.P.H.	126 G.P.H. 489 L.P.H.	158 G.P.H. 515 L.P.H.	210 G.P.H. 682 L.P.H.	261 G.P.H. 850 L.P.H.
WATER FLOW RATE	2.1 G.P.H. 0 L.P.H.	3.2 G.P.H. 12 L.P.H.	4 G.P.H. 15 L.P.H.	5.3 G.P.H. 20 L.P.H.	6.6 G.P.H. 25 L.P.H.
AIR CONSUMPTION	80 C.F.M. 2264 L.P.M.	120 C.F.M. 3376 L.P.M.	150 C.F.M. 4245 L.P.M.	200 C.F.M. 5640 L.P.M.	250 C.F.M. 7075 L.P.M.
AIR FLOW RATE	5.6 C.F.M. 150 L.P.M.	8.4 C.F.M. 230 L.P.M.	10.5 C.F.M. 297 L.P.M.	14 C.F.M. 376 L.P.M.	17.5 C.F.M. 495 L.P.M.
EXTERNAL WASH - 2 STATIONS - 20 P.S.I. (1.4kg/cm <sup>2</sup> ) - EACH STATION					
*** WATER CONSUMPTION (= WATER FLOW RATE)	38 G.P.H. 144 L.P.H.				
EXTERNAL AIR BLOW - 1 STATION - 20 P.S.I. (1.4kg/cm <sup>2</sup> )					
AIR CONSUMPTION	129.4 C.F.M. 5104 L.P.H.	194.4 C.F.M. 7776 L.P.H.	243 C.F.M. 9720 L.P.H.	324 C.F.M. 12960 L.P.H.	405 C.F.M. 16200 L.P.H.
AIR FLOW RATE	8.64 C.F.M. 352 L.P.H.	12.96 C.F.M. 520 L.P.H.	16.2 C.F.M. 640 L.P.H.	21.6 C.F.M. 800 L.P.H.	27 C.F.M. 1100 L.P.H.
LIQUID CONTACT PARTS	Stainless Steel				
ELECTRICALS	1/2 and 1/4 h.p.; B.C. Drive PLC Controlled, Includes Message Center				
DIMENSIONS - W x D x H	See Layout Drawing				
DIMENSIONS - CRATED (2 CRATES) - W x D x H	Crate 1 96" x 67" x 67" Crate 2 140" x 70" x 75"				Crate 1 96" x 67" x 67" Crate 2 144" x 70" x 77"
NET WEIGHT	Crate 1 1200 lbs. (545kg.) Crate 2 3500 lbs. (1591kg.)				Crate 1 1200 lbs. (545kg.) Crate 2 3800 lbs. (1727kg.)
GROSS WEIGHT (2 CRATES)	Crate 1 1500 lbs. (682kg.) Crate 2 4000 lbs. (1818kg.)				Crate 1 1500 lbs. (682kg.) Crate 2 4200 lbs. (1905kg.)
* Larger containers may be handled with optional change parts - please consult.					
** Based on full speed of 20 cycles per minute. Actual spray times can be adjusted up or down.					
*** Based on continuous flow. Intermittent flow available.					
NOTE: Air, vacuum and other cleaning media supplied by user.					
ABBREVIATION KEY: G.P.H. = gallons per hour L.P.H. = liters per hour					
C.F.M. = cubic feet per hour G.P.M. = gallons per minute C.F.M. = cubic feet per minute					

sterilizer  
TUNNEL NOT SHOWN - 600,000.

Post-It® Fax Note	7671	Date	# of pages ▶
To <b>CHRIS</b>	From <b>Barry</b>		
Co./Dept.	Co. <b>COZZOLI</b>		
Phone #	Phone #		
Fax # <b>215 573 2093</b>	Fax #		

**COZZOLI**  
MACHINE COMPANY

*B. Campbell*  
**BIGCAMPKID@AOL.COM.**

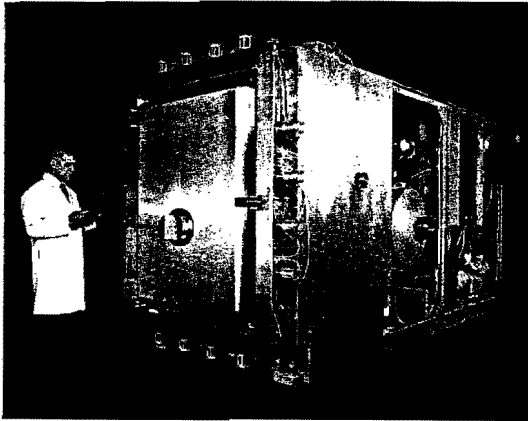
# PERFORMANCE-MATCHED LYOPHILIZERS FROM VIRTIS



**FLEXIBILITY  
&  
CONTROL**

**CONFIGURED TO  
CUSTOMER ORDER**

## WE'RE LISTENING TO YOU



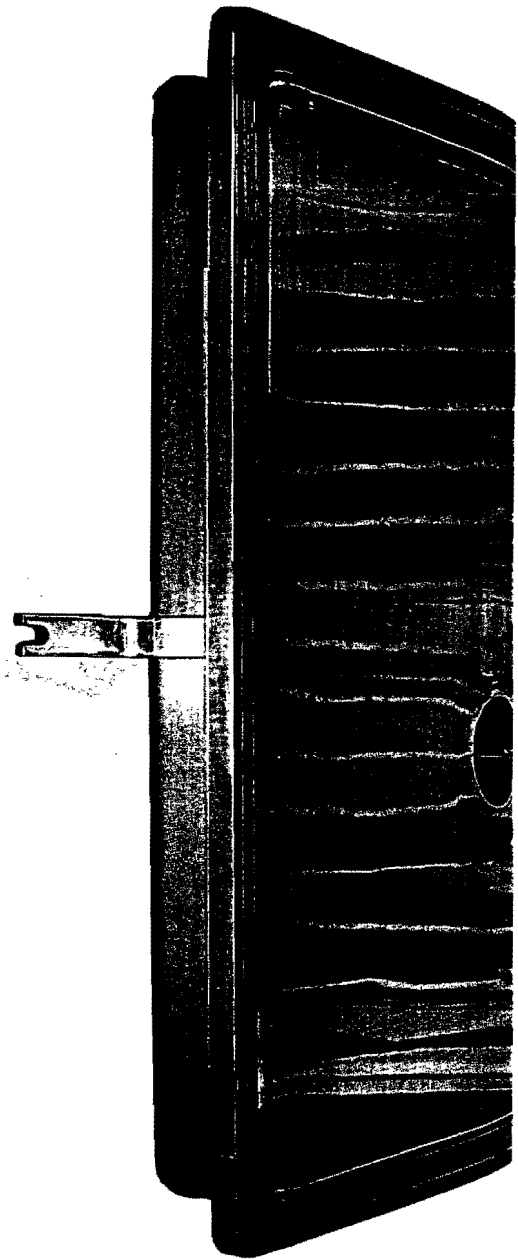
**Benchmark SQ Series lyophilizer configurations include Steam In Place (S.I.P.), Clean In Place (C.I.P.), Automated Locking Door, as well as a wide variety of optional refrigeration and condenser types to match any processing requirements.**

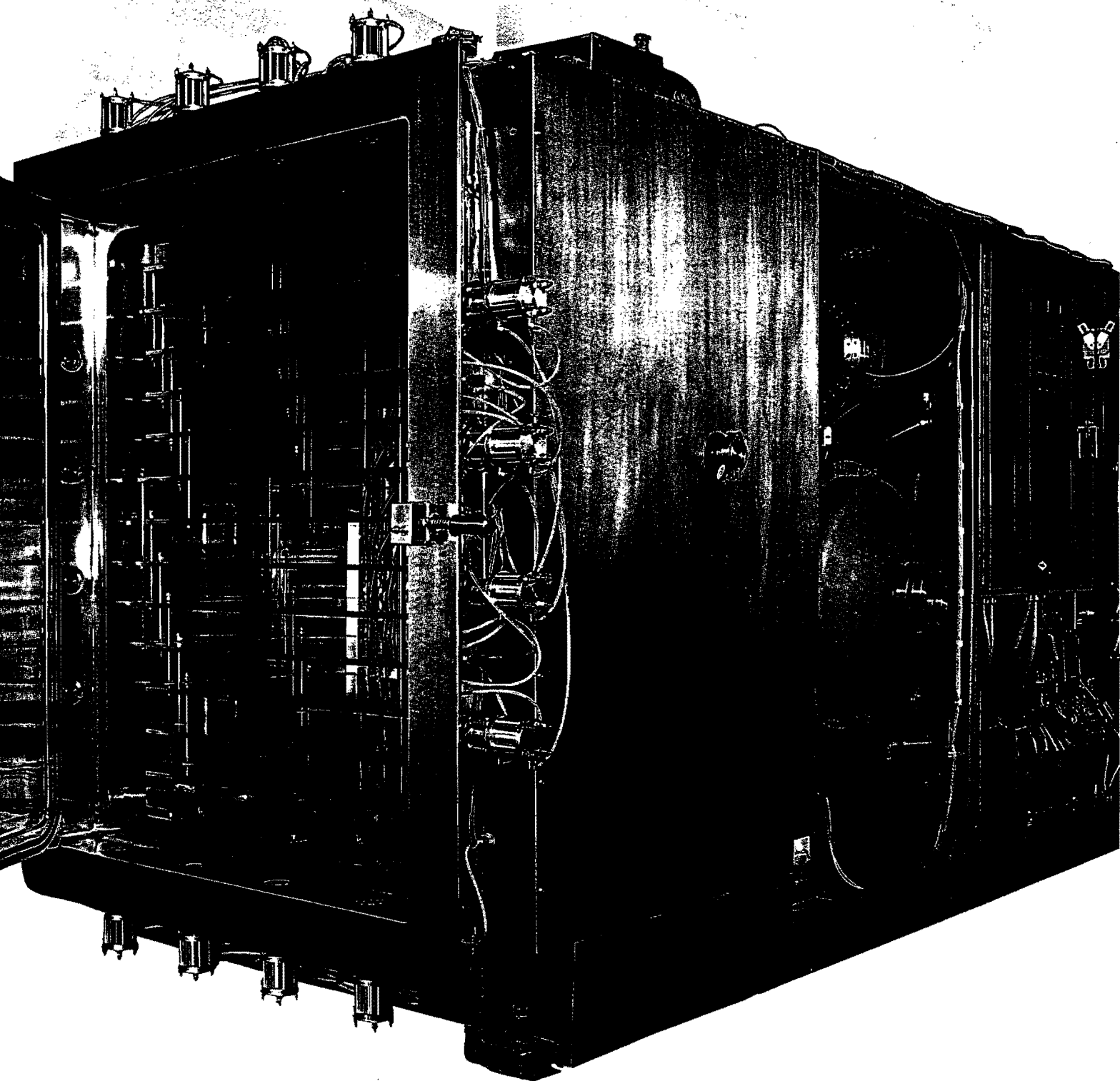
◆ For lyophilization equipment that meets your production expectations and volume requirements every time... for greater flexibility and more shelf size configurations... for lyophilizers that are designed to meet your own special needs, turn to VirTis.

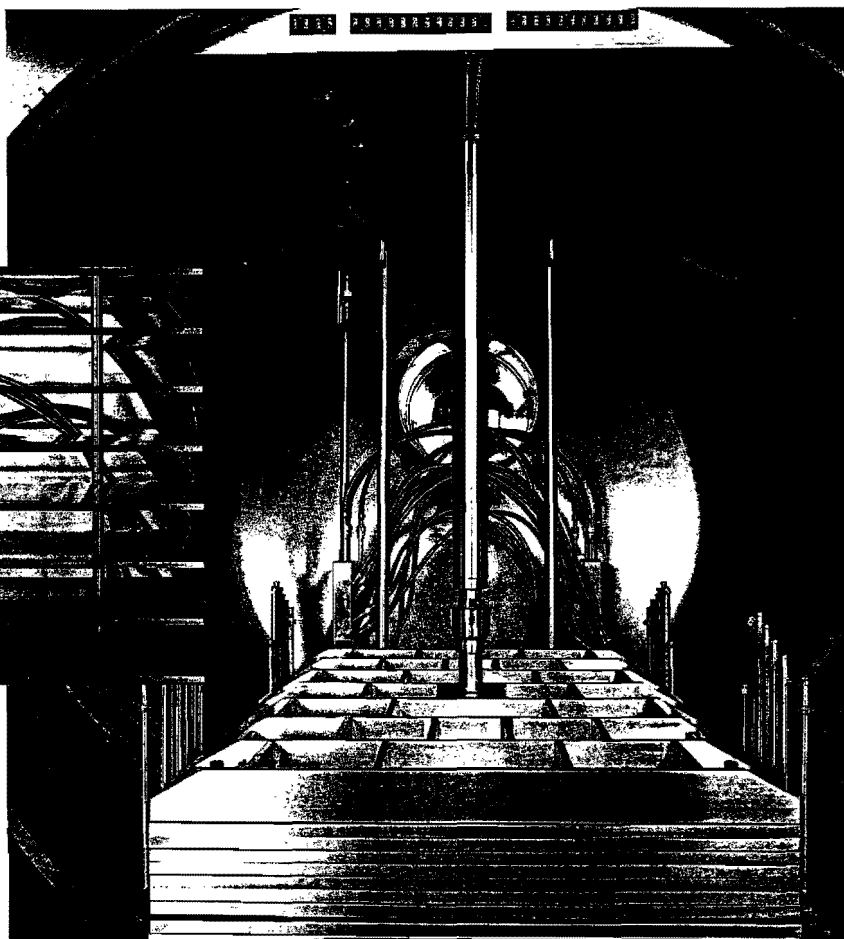
The VirTis Company understands your needs and your frustrations. We recognize that your requirements aren't like those of the guy down the street. And we have some answers.

Every VirTis Benchmark™ lyophilizer is configured to match your processing and production environment and requirements. For more than 42 years, VirTis has applied advanced lyophilization techniques to solve difficult product preservation problems. This gives us the knowledge and experience to meet your needs for equipment, technology and post-sale validation support... today, tomorrow, and into the next century.

We're proud to support VirTis freeze dryers with a worldwide sales and service network extending to seventy countries and covering the world's largest installed base. Whether you're producing antibiotics, blood fractions, diagnostic, veterinary or biopharmaceutical products, we make a lyophilization plant that can be configured, delivered and commissioned in less time and with fewer headaches than you ever thought possible.







**Benchmark Plus top-down hydraulic stoppering permits complete shelf stack closure for routine cleaning. Quick-change shelf spacing links make it easy to change the amount of useable shelf area and shelf interdistance to accommodate any size product container.**

## **CONFIGURED TO MATCH YOUR REQUIREMENTS—BOTH PRODUCTION AND PHYSICAL**

If your facility was designed and built for smaller batch production, we can help you maximize the space you have, by creating a unit with higher capacity that will fit right in. If extra large or small containers or variable production runs are part of your picture, our unique shelving system can be easily adjusted to fit. Only VirTis offers you a choice of economical round-chambered lyophilizers, as well as our unique square chambered (SQ) configurations for maximum capacity. Utilizing an SQ model can accommodate larger runs in less space, or do with one machine what might have required two. We'll even build a lyophilizer designed for your most demanding clean area size constraints while being able to handle your process requirements.

The VirTis industrial series extends from our Genesis™ SQ Series for pilot runs or small scale production at 6.94 ft<sup>2</sup> (0.65m<sup>2</sup>) to our largest standard Benchmark Plus lyophilization plant with 416 ft<sup>2</sup> (38.6m<sup>2</sup>) of usable shelf area, all built with the same level of quality and fine attention to detail.

## **WORLD-STANDARD CONTROLLERS MAKE VALIDATION SIMPLE**

You don't need to be a computer genius to take advantage of VirTis's advanced control systems. We deliver complete lyophilization control and documentation in a user-friendly environment.

Our VirTuoso™ and Maestro™ lyophilization control systems can handle every detail of your complex product cycle with complete accuracy and a full complement of safeguards. A Windows™-based operating system coupled with the powerful Intellution FIX DMACS™ supervisory package guarantees the system is extremely easy to use, simplifying the process of meeting validation requirements. An Allen Bradley PLC-based system provides you with proven performance hardware for unparalleled reliability. An IBM PC-compatible computer, SVGA color monitor and color printer furnish clear, vibrant display, graphical trending and solid documentation, backed by worldwide support.

VirTuoso and Maestro let you safely implement complex drying cycles with all the benefits of thermal treatment, pressure rise and validation testing. Sophisticated VirTuoso coupled to one of our Benchmark SQ or Plus Series lyophilizers, easily automates your lyophilization and

Windows™ is a trademark of Microsoft Corporation.

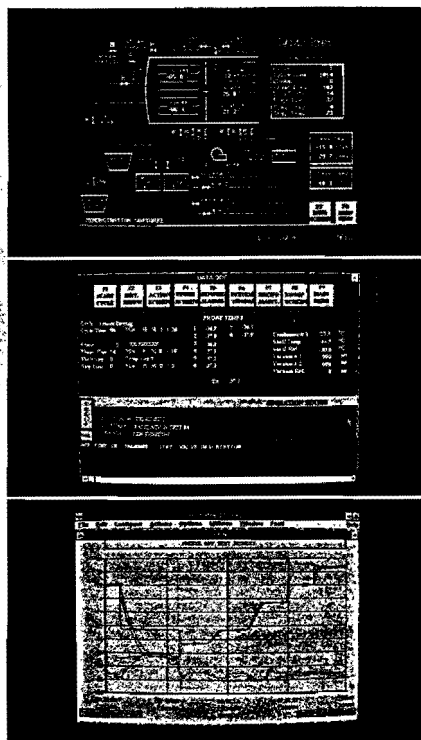
FIX DMACS™ is a trademark of Intellution.

sterilization cycles, vacuum control, barometric end point detection, automatic defrosting, eutectic control and monitoring and automatic validation testing of all systems and subsystems, as well as accommodating steam sterilization and control of product profile cycles.

Both systems include multi-level password protection to safeguard sensitive information and products, report generator for hard copy detailing all aspects of your lyophilization cycle, and an easy-to-use historical trending package with zoom control. An extensive defense system provides total product and equipment safety. And an inexpensive optional remote view package allows complete network or off-site monitoring and control with guarded password protection.

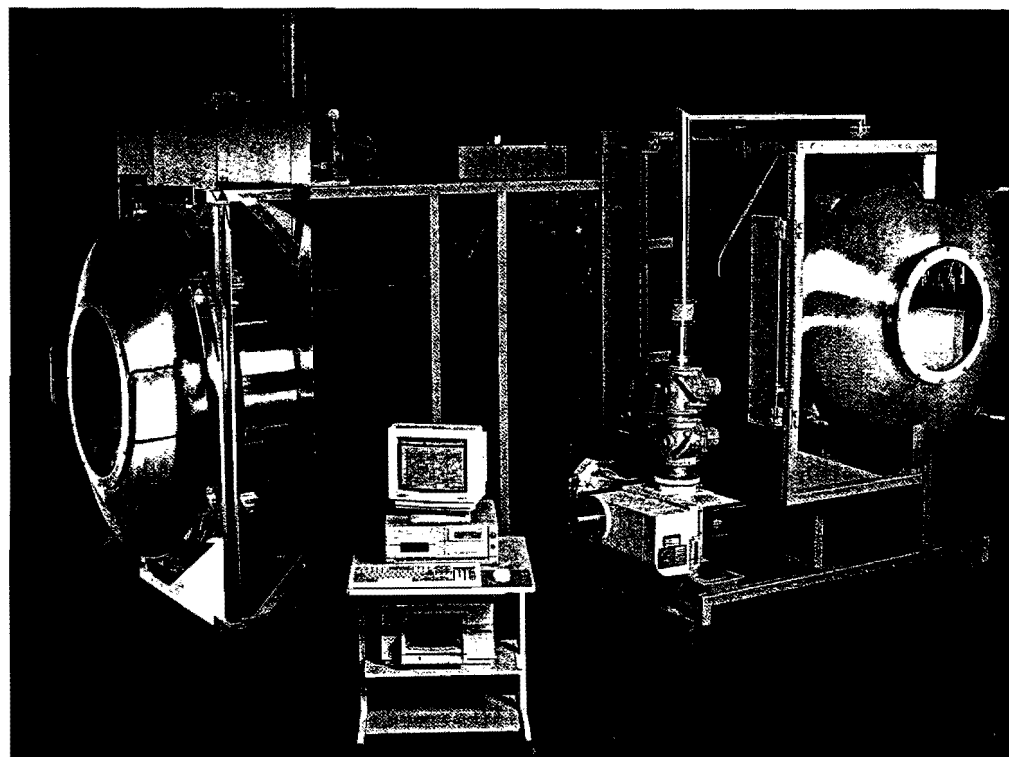
## PURITY AND INTEGRITY THAT GO BEYOND FDA STANDARDS

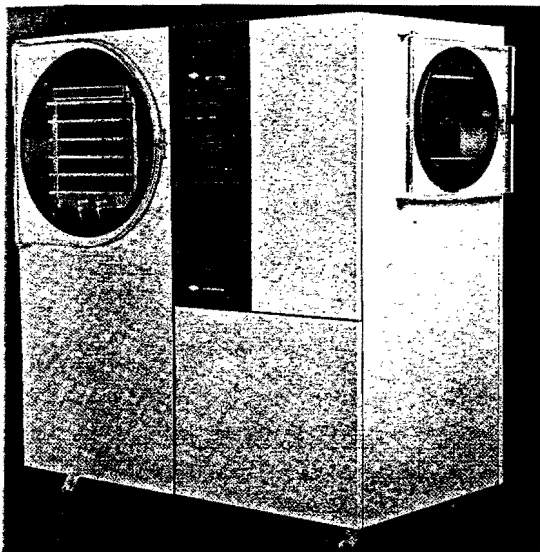
To ensure performance that will pass the scrutiny of any regulatory agency, we build each VirTis lyophilizer to meet stringent ISO-9001 certification and current good manufacturing practices [cGMP]. Every VirTis system is extensively factory-tested to verify critical design and performance parameters, and we not only invite but encourage you to visit during manufacturing and final qualification testing.



**Left: VirTuoso and Maestro PLC-based control systems offer the ultimate in product and system protection. Special validation function testing routines provide documented performance of all major systems and subsystems. User-friendly Windows-based Intellution FIX DMACS makes the most complex and demanding pharmaceuticals processing tasks easy and worry free.**

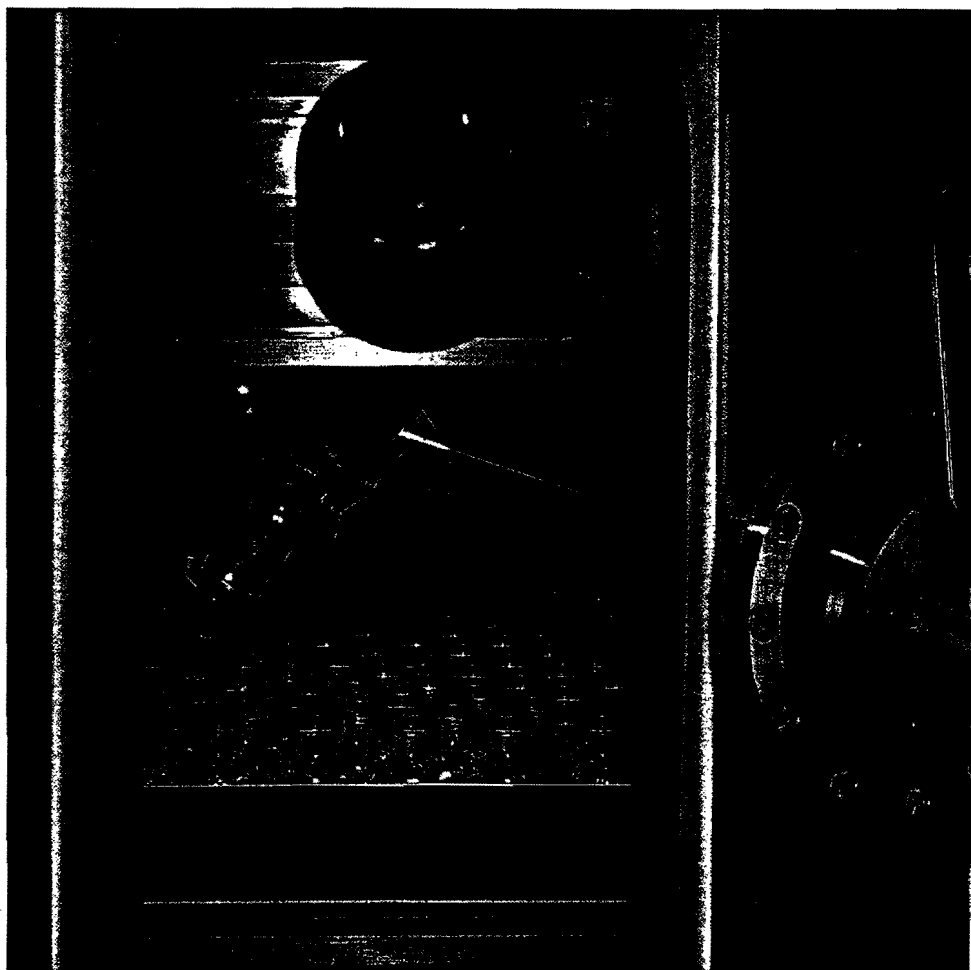
**Below: The Benchmark Plus Series in one of many possible configurations.**





**Above: Cabinetized mid-range Benchmark lyophilizers are the most flexible pilot plant and production lyophilizers ever produced, with the shortest delivery times in the industry. Bulk and vial processing models are available with usable shelf areas up to 48ft<sup>2</sup> (4.5m<sup>2</sup>), and shelf processing temperatures below -70°C. Over 100 available options suit all processing requirements and space constraints.**

**Right: Sample Thief simplifies the optimization process by allowing sample removal without breaking the cycle.**



## **WE WON'T ABANDON YOU AFTER DELIVERY**

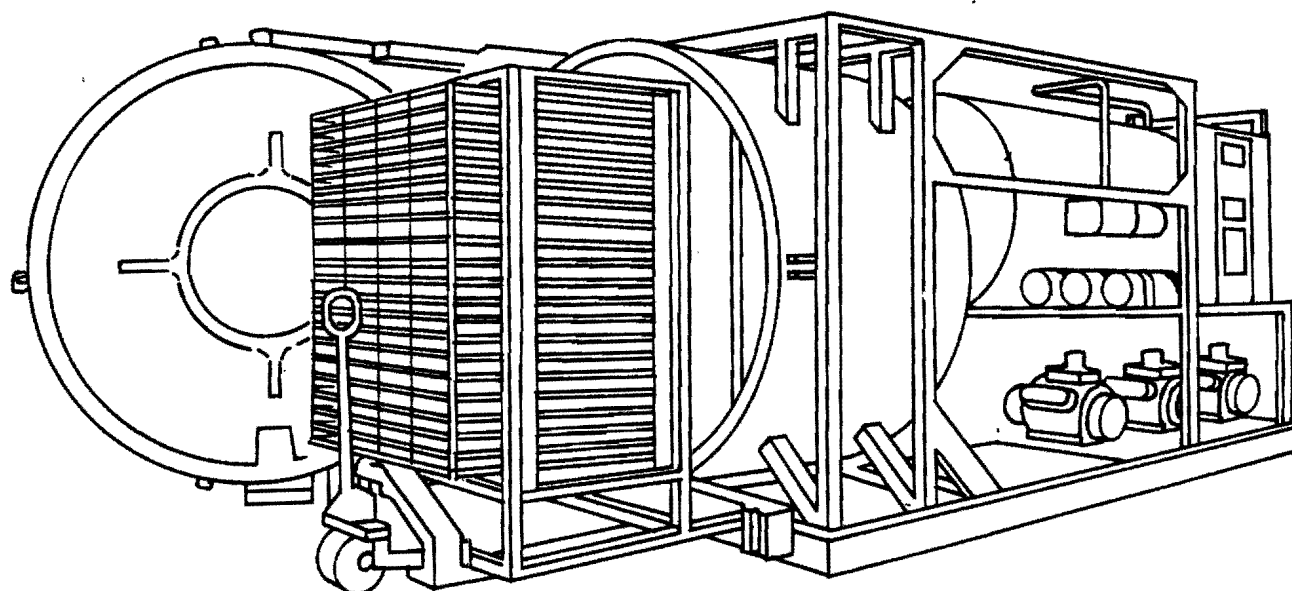
What is your most problematic stage of installing a new system? If it's getting your new equipment up and running, be assured that our Start Up Assistance Programs are designed to provide operator training after verification of your critical performance parameters. By working with your engineers, operators and maintenance staff, we can guarantee a timely and efficient start-up of your system, and support for the long life of your equipment.

Our trained field engineers assist your validation staff with the system testing and documentation required during the installation qualification and operational qualification stages. And, we'll train your staff to make in-house routine maintenance easier throughout the long life of your capital investment. *Validation Assistance Workbooks* consist of an extensive series of documented tests designed to audit system performance and clearly define product processing parameters.



# LYOPHILIZ

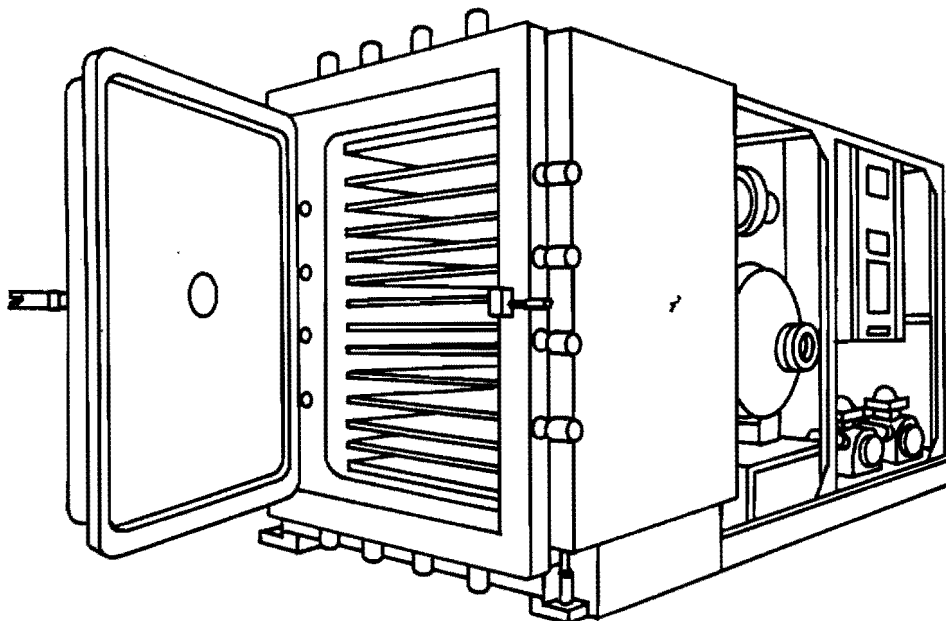
LYOPHILIZER DESIGNATION Configuration	BENCHMARK 1000		BENCHMARK 2000		BENCHMARK 3000		BENCHMARK PLUS 4	
	Bulk	Stoppering	Bulk	Stoppering	Bulk	Stoppering	Bulk	Stopper
Max. Number of Shelves	6	6	6	6	8	8	15	15
Max. Shelf Area: ft <sup>2</sup> (m <sup>2</sup> )	16.0(1.48)	16.0(1.48)	25.0(2.32)	25.0(2.32)	48.0(4.46)	48.0(4.46)	90.0(8.4)	78.0(7.2)
Shelf Interdistance at Max.: in (mm)	1.89(48)	1.89(48)	2.5(63)	2.5(63)	2.16(54)	2.16(54)	1.68(42)	2.40(61)
Min. Number of Shelves	3	2	3	2	3	3	6	4
Min. Shelf Area: ft <sup>2</sup> (m <sup>2</sup> )	8.0(0.74)	5.3(0.49)	12.5(1.16)	8.3(0.77)	18.0(1.67)	18.0(1.67)	36.0(3.34)	24.0(2.23)
Shelf Interdistance at Min.: in (mm)	4.28(108)	6.68(169)	5.5(139)	8.5(215)	6.59(167)	6.59(167)	4.67(118)	8.8(224)
Shelf Size: Width in (mm)	16(41)	16(41)	20(51)	20(51)	24(61)	24(61)	24(61)	24(61)
Length in (mm)	24(61)	24(61)	30(76)	30(76)	36(91)	36(91)	36(91)	36(91)
Refrigeration System Data	SL	SL	SL	SL	SL	SL	SL	SL
Standard System Shelf Temp °C	-55	-55	-55	-55	-55	-55	-55	-55
Condenser Temp °C	-70	-70	-70	-70	-70	-70	-70	-70
Standard Condenser Capacity in 24 hours: liters (type)	20.0(Ext.)	20.0(Ext.)	34.0(Ext.)	34.0(Ext.)	42.0(Ext.)	42.0(Ext.)	100.0(Ext.)	65.0(Ext.)
Optional Condenser Capacities in 24 hours: liters (type)	14.0(Ext.)	14.0(Ext.)	20.0(Int.)	20.0(Int.)	34.0(Int.)	34.0(Int.)	50.0(Int.)	50.0(Int.)
					64.0(Ext.)	64.0(Ext.)	65.0(Ext.)	100.0(Ext.)
							150.0(Ext.)	150.0(Ext.)



**THE BENCHMARK PLUS SERIES  
WITH LOADING AND UNLOADING CART**

## CTION GUIDE

BENCHMARK PLUS 5000		BENCHMARK PLUS 6000		BENCHMARK SQ 4000		BENCHMARK SQ 5000		BENCHMARK SQ 6000	
Bulk	Stoppering	Bulk	Stoppering	Bulk	Stoppering	Bulk	Stoppering	Bulk	Stoppering
15	14	26	20	15	13	21	14	26	20
0[22.3]	168.0[15.7]	416.0[38.6]	240.0[22.42]	90.0[8.4]	78.0[7.3]	336.0[31.2]	168.0[15.7]	416.0[38.6]	240.0[22.42]
30[46]	2.5[63]	1.80[46]	2.5[63]	1.68[42]	2.40[60]	1.80[46]	2.5[63]	1.80[46]	2.5[63]
7	4	12	6	6	4	9	4	12	6
2[10.4]	48.0[4.48]	192.0[17.8]	72.0[6.72]	36.0[3.34]	24.0[2.23]	144[13.4]	48.0[4.48]	192.0[17.8]	72.0[6.72]
0[109]	10.6[270]	4.35[110]	10.0[255]	4.67[118]	8.8[225]	4.60[117]	10.6[270]	4.35[110]	10.0[255]
24[610]	36[914]	2x,24[610]	36[914]	24[610]	24[610]	2x,24[610]	36[914]	2x,24[610]	36[914]
8[1220]	48[1220]	2x,48[1220]	48[1220]	36[915]	36[915]	2x,48[1220]	48[1220]	2x,48[1220]	48[1220]
LL	LL	LL	LL	SL	SL	LL	LL	LL	LL
-55	-55	-55	-55	-55	-55	-55	-55	-55	-55
-70	-70	-70	-70	-70	-70	-70	-70	-70	-70
0[Ext.]	120.0[Ext.]	300.0[Ext.]	220.0[Ext.]	100.0[Ext.]	65.0[Ext.]	220.0[Ext.]	120.0[Ext.]	300.0[Ext.]	220.0[Ext.]
0[Int.]	100.0[Int.]	220.0[Int.]	200.0[Int.]	50.0[Int.]	50.0[Int.]	100.0[Int.]	100.0[Int.]	220.0[Int.]	200.0[Int.]
0[Ext.]	220.0[Ext.]	300.0[Ext.]	300.0[Ext.]	65.0[Ext.]	100.0[Ext.]	120.0[Ext.]	220.0[Ext.]	300.0[Ext.]	300.0[Ext.]
0[Ext.]	300.0[Ext.]	450.0[Ext.]	450.0[Ext.]	150.0[Ext.]	150.0[Ext.]	300.0[Ext.]	300.0[Ext.]	450.0[Ext.]	450.0[Ext.]
		600.0[Ext.]	600.0[Ext.]					600.0[Ext.]	600.0[Ext.]

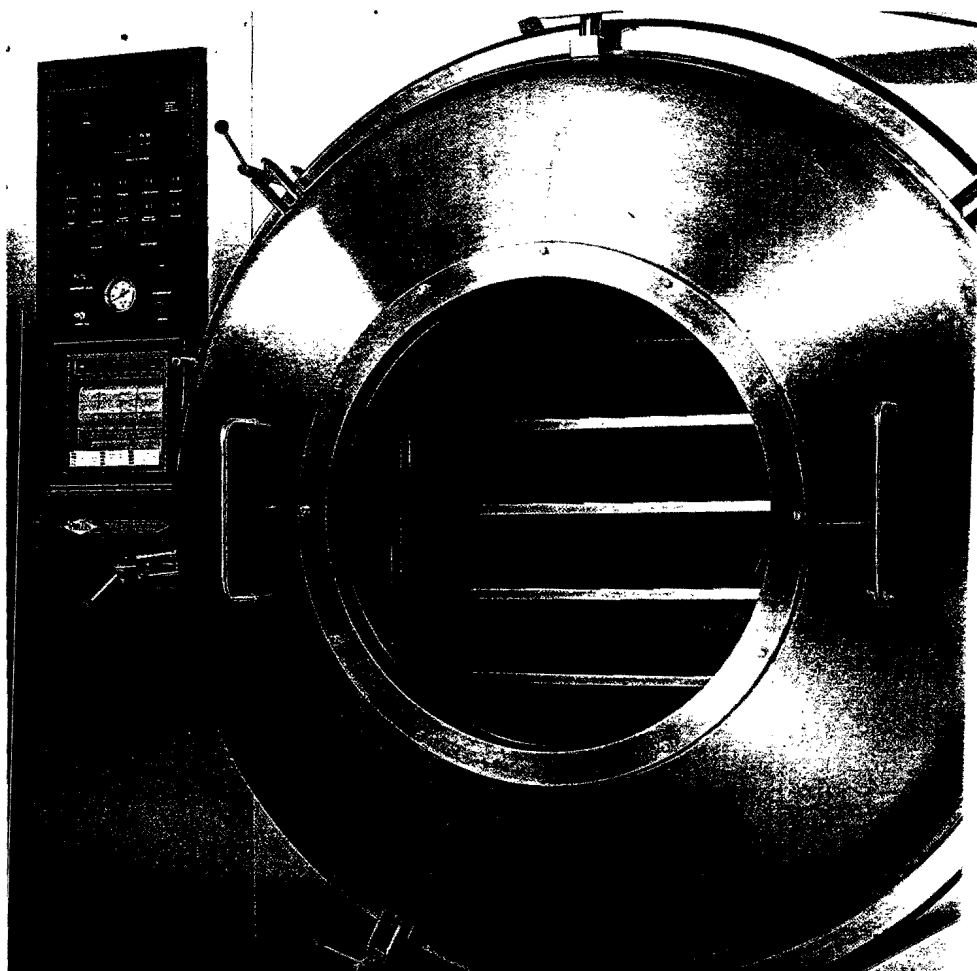


Virtis Benchmark Series Lyophilizers can be constructed on virtually any single or multiple frame configuration required by the customer, and all are available in either square chamber or round chamber models.

**THE BENCHMARK SQ SERIES  
CONSTRUCTED ON A MONOBLOCK FRAME**



Once a unit has been validated and brought up to a full production schedule, VirTis continues to back up its customers. A worldwide network of sales and service support provide a full range of post-sale assistance. Lyophilization and service support seminars, maintenance and performance audit contract programs, and a 24-hour hot-line are designed to keep production on schedule.



#### *Finishes*

All Benchmark lyophilizer product chambers, condensers and product shelves are engineered of pharmaceutical grade #4, (RA Value less than 25 micro inches), 220 grit finish, with welds ground smooth and flush. Mirror finish or electropolishing is optional.

#### *Heat Transfer Systems*

Benchmark incorporates an extremely efficient silicone heat transfer system that can provide uniform temperatures from  $-70^{\circ}\text{C}$  to  $+65^{\circ}\text{C}$ . With either the standard manual control system or our optional VirTuoso or Maestro systems, you can easily achieve shelf temperature control of  $\pm 1^{\circ}\text{C}$ .

### *Automated Controls*

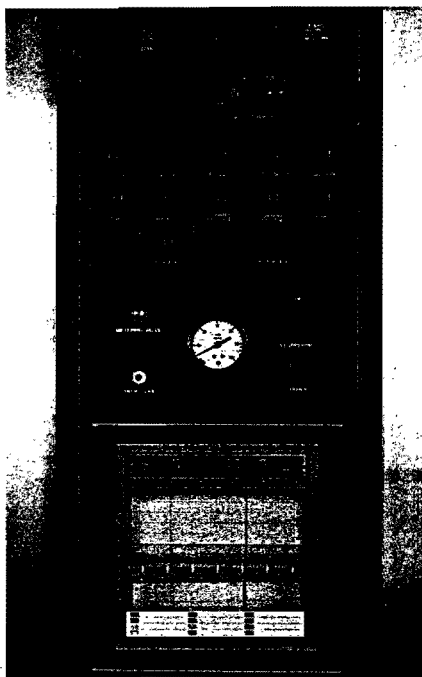
For the "hands-off" operation that can save you time and money, Benchmark Plus and Benchmark SQ Series lyophilizers can be equipped with our optional VirTuoso or Maestro PLC-based control system. Use them to control every detail of your lyophilization cycle, including thermal treatment, precise vacuum control, primary and secondary drying, pressure rise barometric end point detection and validation testing of systems and subsystems.

### *Durable Vacuum Components*

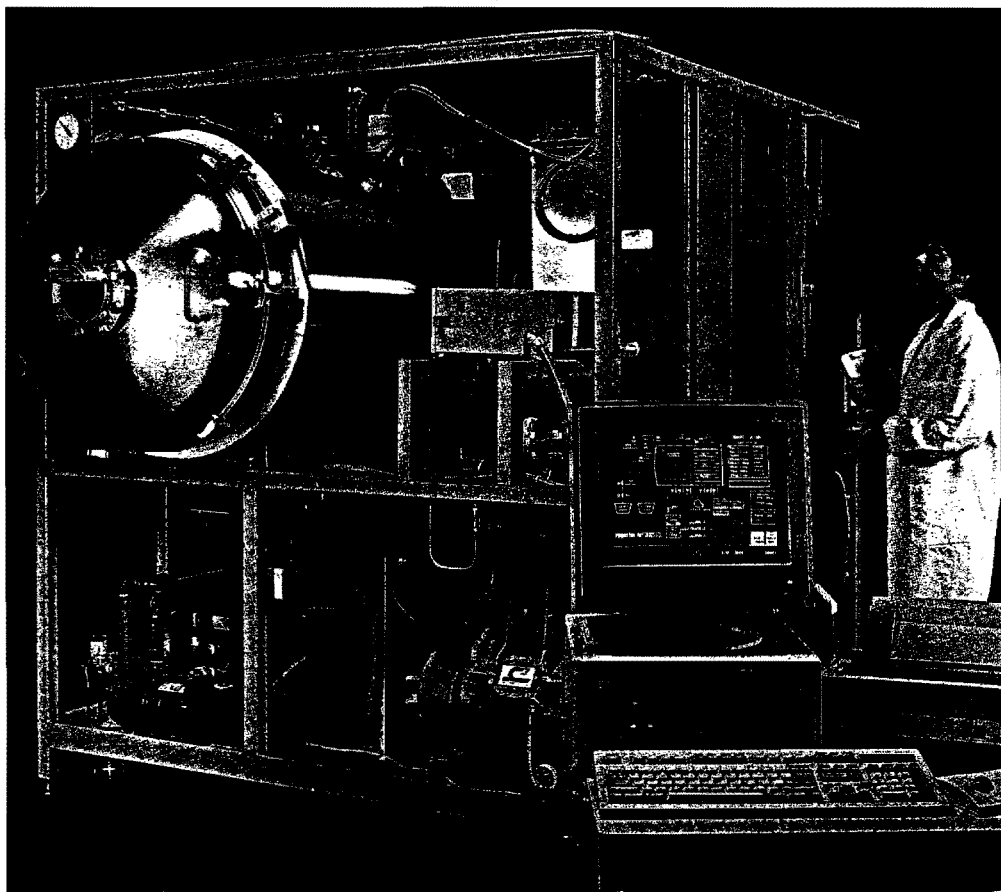
All vacuum components are selected for long life and leak-free operation, with vacuum pump-down times that meet the most pressing processing or product schedule. All connections and piping are resistant to metal azide formation. The optional Maestro or VirTuoso automated control system tests leak rates automatically. We can also equip Benchmark systems with optional standby vacuum pumps, chemical resistant pumps, oil filtration systems and roots blower packages.

### *Sterilization Systems*

Benchmark's optional steam sterilization system incorporates an efficient multi-evacuation heat-up cycle and sterilization system rated and documented to meet ASME vessel code at 25 PSIG. Higher coded pressure vessel ratings are available upon request. A rapid cool-down option improves turn-around time. A variety of optional gas sterilization systems are available.



**Redundant manual backup controls or sophisticated PLC-based automated controls deliver consistent product quality during lyophilization, providing automation of required support cycles. Special password-protected remote monitoring systems update operators on system status constantly, at their convenience.**



## BENCHMARK SQ AND PLUS SERIES BULK DRYING LYOPHILIZERS

Series/Bulk	4000 SQ, Plus	5000 Plus	5000 SQ	6000 SQ, Plus
Shelf Size in (mm)	24x36/[610x915]	Shelf Stack: 2 - 24x48 (610 x 1220)		
6 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	36 (3.3)/4.67 [118]	—	—	—
7 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	42 (3.9)/3.96 [100]	112 (10.4)/4.30 [109]	—	—
8 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	48 (4.5)/3.43 (87)	128 (11.9)/3.70 [94]	—	—
9 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	54 (5.0)/3.01 (76)	144 (13.4)/3.25 [83]	144 (13.4)/4.60 [117]	—
10 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	60 (5.6)/2.68 (68)	160 (14.9)/2.90 [73]	160 (14.9)/4.11 [104]	—
11 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	66 (6.1)/2.41 (61)	176 (16.4)/2.60 [66]	176 (16.4)/3.71 [94]	—
12 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	72 (6.7)/2.18 (55)	192 (17.8)/2.35 (60)	192 (17.8)/3.38 (85)	192 (17.8)/4.35 (110)
13 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	78 (7.3)/1.99 (50)	208 (19.3)/2.15 (54)	208 (19.3)/3.09 (78)	208 (193)/4.00 [101]
14 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	84 (7.8)/1.82 (46)	224 (20.8)/1.95 (50)	224 (20.8)/2.85 (72)	224 (20.8)/3.65 (93)
15 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	90 (8.4)/1.68 (42)	240 (22.3)/1.80 (46)	240 (22.3)/2.64 (67)	240 (22.3)/3.40 (86)
16 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	—	—	256 (23.8)/2.45 (62)	256 (23.8)/3.15 (80)
17 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	—	—	272 (25.3)/2.29 (58)	272 (25.3)/2.95 (75)
18 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	—	—	288 (26.8)/2.15 (54)	288 (26.8)/2.80 (70)
19 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	—	—	304 (28.2)/2.02 (51)	304 (28.2)/2.60 (66)
20 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	—	—	320 (29.7)/1.90 (48)	320 (29.7)/2.45 (62)
21 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	—	—	336 (31.2)/1.80 (46)	336 (31.2)/2.30 (59)
22 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	—	—	—	352 (32.7)/2.20 (56)
23 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	—	—	—	368 (34.2)/2.10 (53)
24 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	—	—	—	384 (35.7)/2.0 (50)
25 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	—	—	—	400 (37.2)/1.90 (48)
26 Shelf: ft <sup>2</sup> (m <sup>2</sup> ) / Clearance: in (mm)	—	—	—	416 (38.6)/1.80 (46)

## BENCHMARK SQ, BENCHMARK PLUS BULK MODELS PRODUCT LOADING PER SHELF

Model Shelf Size in (mm)	10mm Bulk Depth (Liters)	12mm Bulk Depth (Liters)	15mm Bulk Depth (Liters)	20mm Bulk Depth (Liters)
4000 SQ, 4000 Plus 24 x 36 [610 x 915]	5.34	6.36	7.98	10.62
5000 SQ, 5000 Plus Two 24 x 48 [610 x 1220]	14.24	16.96	21.28	28.32
6000 SQ, 6000 Plus Two 24 x 48 [610 x 1220]	14.24	16.96	21.28	28.32

## BENCHMARK SQ AND BENCHMARK PLUS CONDENSER SYSTEMS

Condenser Selections By Model	Benchmark 4000 SQ And Benchmark 4000 Plus Models	Benchmark 5000 SQ And Benchmark 5000 Plus Models	Benchmark 6000 SQ And Benchmark 6000 Plus Models
Condenser Configurations	Liters in 24 Hours (Compressor Sizing) [Condenser Area]	Liters in 24 Hours (Compressor Sizing) [Condenser Area]	Liters in 24 Hours (Compressor Sizing) [Condenser Area]
Standard Internal	50 Liters (One 20 Hp) [21 Square Feet]	100 Liters (One 30 Hp) [50 Square Feet]	200 Liters (Two 20 Hp) [100 Square Feet]
Standard Stoppering External	65 Liter (One 20 Hp) [35 Square Feet]	120 Liters (One 30 Hp) [60 Square Feet]	220 Liters (Two 20 Hp) [120 Square Feet]
Standard Bulk External	100 Liters (One 20 Hp) [60 Square Feet]	220 Liters (Two 20 Hp) [120 Square Feet]	300 Liters (Two 30 Hp) [160 Square Feet]
Heavy Duty External	150 Liters (One 30 Hp) [80 Square Feet]	300 Liters (Two 30 Hp) [160 Square Feet]	450 Liters (Two 30 Hp) [250 Square Feet]
Extra Heavy Duty External	Consult Factory	Consult Factory	600 Liters (Three 30 Hp) [320 Square Feet]

## VIRTIS BENCHMARK SQ AND PLUS SERIES STOPPERING LYOPHILIZERS

Series/Stoppering	4000	5000	6000
Shelf Size in (mm)	24x36 (610x915)	36x48 (915x1220)	36x48 (915x1220)
4 Shelf: ft <sup>2</sup> (m <sup>2</sup> )	24.0 (2.23)	48.0 (4.48)	—
Clearance: in (mm)	8.8 (225)	10.6 (270)	—
5 Shelf: ft <sup>2</sup> (m <sup>2</sup> )	30.0 (2.79)	60.0 (5.61)	—
Clearance: in (mm)	7.0 (175)	8.4 (210)	—
6 Shelf: ft <sup>2</sup> (m <sup>2</sup> )	36.0 (3.34)	72.0 (6.72)	72.0 (6.72)
Clearance: in (mm)	5.7 (145)	6.8 (170)	10.0 (255)
7 Shelf: ft <sup>2</sup> (m <sup>2</sup> )	42.0 (3.90)	84.0 (7.85)	84.0 (7.85)
Clearance: in (mm)	4.8 (120)	5.8 (145)	8.5 (215)
8 Shelf: ft <sup>2</sup> (m <sup>2</sup> )	48.0 (4.46)	96.0 (8.97)	96.0 (8.97)
Clearance: in (mm)	4.2 (105)	4.9 (125)	7.3 (185)
9 Shelf: ft <sup>2</sup> (m <sup>2</sup> )	54.0 (5.05)	108.0 (10.09)	108.0 (10.09)
Clearance: in (mm)	3.6 (92)	4.1 (110)	6.4 (163)
10 Shelf: ft <sup>2</sup> (m <sup>2</sup> )	60.0 (5.61)	120.0 (11.21)	120.0 (11.21)
Clearance: in (mm)	3.3 (82)	3.8 (95)	5.7 (145)
11 Shelf: ft <sup>2</sup> (m <sup>2</sup> )	66.0 (6.17)	132.0 (12.34)	132.0 (12.34)
Clearance: in (mm)	2.9 (72)	3.4 (85)	5.1 (130)
12 Shelf: ft <sup>2</sup> (m <sup>2</sup> )	72.0 (6.72)	144.0 (13.45)	144.0 (13.45)
Clearance: in (mm)	2.6 (65)	3.0 (75)	4.6 (115)
13 Shelf: ft <sup>2</sup> (m <sup>2</sup> )	78.0 (7.28)	156 (14.58)	156.0 (14.58)
Clearance: in (mm)	2.4 (60)	2.8 (70)	4.2 (105)
14 Shelf: ft <sup>2</sup> (m <sup>2</sup> )	—	168 (15.70)	168.0 (15.70)
Clearance: in (mm)	—	2.5 (63)	3.9 (100)
15 Shelf: ft <sup>2</sup> (m <sup>2</sup> )	—	—	180.0 (16.82)
Clearance: in (mm)	—	—	3.6 (90)
16 Shelf: ft <sup>2</sup> (m <sup>2</sup> )	—	—	192 (17.94)
Clearance: in (mm)	—	—	3.3 (85)
17 Shelf: ft <sup>2</sup> (m <sup>2</sup> )	—	—	204 (19.06)
Clearance: in (mm)	—	—	3.0 (75)
18 Shelf: ft <sup>2</sup> (m <sup>2</sup> )	—	—	216 (20.18)
Clearance: in (mm)	—	—	2.8 (72)
19 Shelf: ft <sup>2</sup> (m <sup>2</sup> )	—	—	228.0 (21.3)
Clearance: in (mm)	—	—	2.6 (65)
20 Shelf: ft <sup>2</sup> (m <sup>2</sup> )	—	—	240.0 (22.42)
Clearance: in (mm)	—	—	2.5 (63)

### BENCHMARK SQ, BENCHMARK PLUS MODELS: VIAL LOADING PER SHELF +/-3% (STOPPERING MODELS ONLY)

Stoppering Model Shelf Size in (mm)	15 mm Dia	23 mm Dia	25 mm Dia	32 mm Dia	43 mm Dia	52 mm Dia
4000 SQ, 4000 Plus 24 x 36 (610 x 915)	2598	1050	858	540	294	198
5000 SQ, 5000 Plus 36 x 48 (914 x 1220)	5196	2100	1716	1080	588	396
6000 SQ, 6000 Plus 36 x 48 (914 x 1220)	5196	2100	1716	1080	588	396

### COMMON VIAL AND SERUM BOTTLE SIZES

Size	Diameter x Height	Required Clearance Without Stopper	Required Clearance With Stopper
2 ml	15 mm x 40 mm	44 mm	51 mm
5 ml	23 mm x 47 mm	51 mm	60 mm
10 ml	25 mm x 54 mm	58 mm	67 mm
20 ml	32 mm x 58 mm	62 mm	71 mm
50 ml	43 mm x 73 mm	77 mm	86 mm
100 ml	52 mm x 95 mm	99 mm	108 mm

## Equipment Design for pumps in Separation Section

## UF1 Pump

Pressure required at inlet of UF1 = 40 psi

$$F := 1825.47 \frac{\text{lb}}{\text{hr}} \quad \text{Flow through Pump}$$

$$\rho := 63.72 \frac{\text{lb}}{\text{ft}^3} \quad \text{Density of Water}$$

$$\eta := 0.80 \quad \text{Pump efficiency}$$

$$\Delta P := 40 \cdot \text{psi} - 14.7 \cdot \text{psi}$$

$$\Delta P = 25.3 \cdot \text{psi}$$

## Sizing the Pump:

$$\text{Size} := F \cdot \frac{\Delta P}{\rho}$$

$$\text{Size} = 0.053 \cdot \text{hp}$$

$$\text{Hours per year: } t := 7920 \cdot \text{hr}$$

$$\text{Power} := \frac{\text{Size}}{\eta}$$

$$\text{Power} = 49.135 \cdot \text{W}$$

$$\text{Electricity} := \text{Power} \cdot t$$

$$\text{Head} := \frac{\Delta P}{\rho \cdot g}$$

$$\text{Head} = 57.175 \cdot \text{ft}$$

$$\text{Electricity} = 389.149 \cdot \text{kW} \cdot \text{hr}$$

## UF2 Pump

Pressure required at inlet of UF2 = 40 psi

$$F := 1325 \cdot \frac{\text{kg}}{\text{hr}} \quad \text{Flow through Pump}$$

Pressure Drop over filter

$$\rho := 63.72 \cdot \frac{\text{lb}}{\text{ft}^3} \quad \text{Density of Water}$$

$$\Delta P := 40 \cdot \text{psi} - 14.7 \cdot \text{psi}$$

$$\Delta P = 25.3 \cdot \text{psi}$$

$$\eta := 0.80 \quad \text{Pump efficiency}$$

## Sizing the Pump:

$$\text{Size} := F \cdot \frac{\Delta P}{\rho}$$

$$\text{Size} = 0.084 \cdot \text{hp}$$

$$\text{Hours per batch: } t := 7920 \cdot \text{hr}$$

$$\text{Power} := \frac{\text{Size}}{\eta}$$

$$\text{Power} = 78.626 \cdot \text{W}$$

$$\text{Electricity} := \text{Power} \cdot t$$

$$\text{Head} := \frac{\Delta P}{\rho \cdot g}$$

$$\text{Head} = 57.175 \cdot \text{ft}$$

$$\text{Electricity} = 622.718 \cdot \text{kW} \cdot \text{hr}$$



## MF Pump

Assume an average pressure drop over the microfilter of 20 psi

$$F := 1135.6 \frac{\text{kg}}{\text{hr}} \quad \text{Max flow through filter}$$

$$\rho := 63.72 \frac{\text{lb}}{\text{ft}^3} \quad \text{Density of Water}$$

$$\eta := 0.80 \quad \text{Pump efficiency}$$

Pressure Drop over filter

$$\Delta P := 20 \cdot \text{psi}$$

Sizing the Pump:

$$\text{Size} := F \cdot \frac{\Delta P}{\rho} \quad \text{Size} = 0.057 \cdot \text{hp}$$

$$\text{Power} := \frac{\text{Size}}{\eta} \quad \text{Power} = 53.27 \text{ W}$$

$$\text{Head} := \frac{\Delta P}{\rho \cdot g} \quad \text{Head} = 45.198 \cdot \text{ft}$$

$$\text{Hours per batch: } t := 7920 \cdot \text{hr}$$

$$\text{Electricity} := \text{Power} \cdot t$$

$$\text{Electricity} = 421.901 \cdot \text{kW} \cdot \text{hr}$$

## Impeller Power Calculations for Separation section:

Assuming all impellers run for 7920 hrs/yr

7920 is about 400 hrs more than the most used tank (FD Tank)

$$t := 7920 \cdot \text{hr}$$

Power requirements for large tanks (4000-5000 L): 10 hp

$$P_{\text{large}} := 10 \cdot \text{hp}$$

$$\text{Holduptank} := P_{\text{large}} \cdot t$$

$$\text{UF1Tank} := P_{\text{large}} \cdot t$$

$$\text{Holduptank} = 5.906 \cdot 10^4 \cdot \text{kW} \cdot \text{hr}$$

$$\text{UF1Tank} = 5.906 \cdot 10^4 \cdot \text{kW} \cdot \text{hr}$$

Power requirements for smaller tanks (300-500 L): 5 hp

$$P_{\text{small}} := 5 \cdot \text{hp}$$

$$\text{UF2Tank} := P_{\text{small}} \cdot t$$

$$\text{UF2Tank} = 2.953 \cdot 10^4 \cdot \text{kW} \cdot \text{hr}$$

Process includes two of each of these tanks:

$$\text{AffinityTanks} := 2 \cdot P_{\text{small}} \cdot t$$

$$\text{FDTanks} := 2 \cdot P_{\text{small}} \cdot t$$

$$\text{AffinityTanks} = 5.906 \cdot 10^4 \cdot \text{kW} \cdot \text{hr}$$

$$\text{FDTanks} = 5.906 \cdot 10^4 \cdot \text{kW} \cdot \text{hr}$$

$$\text{ERTanks} := 2 \cdot P_{\text{small}} \cdot t$$

$$\text{MFTanks} := 2 \cdot P_{\text{small}} \cdot t$$

$$\text{ERTanks} = 5.906 \cdot 10^4 \cdot \text{kW} \cdot \text{hr}$$

$$\text{MFTanks} = 5.906 \cdot 10^4 \cdot \text{kW} \cdot \text{hr}$$

$$\text{Total} := \text{Holduptank} + \text{UF1Tank} + \text{UF2Tank} + \text{AffinityTanks} + \text{FDTanks} + \text{ERTanks} + \text{MFTanks}$$

$$\text{Total} = 3.839 \cdot 10^5 \cdot \text{kW} \cdot \text{hr}$$

383900 kW\*hr/yr required for Separation Impellers

Electrical Costs: Separation section

Elec. for refrigeration: 4,000 ft<sup>2</sup> for 2 sep. trains  
 "rules of thumb" from Rich Metallo:  $\frac{1.25 \text{ tons refrigeration}}{300 \text{ ft}^2}$

for low temp cooling:  $\frac{1.0 \text{ kW}}{\text{ton ref.}}$

Load factor = 0.6  $\rightarrow$  cooler in winter time  
 often holding at 4°C only

Condition air 24 hrs/day, 365 days/yr  
 = 8760 hrs/yr

$$4000 \text{ ft}^2 \times \frac{1.25 \text{ tons ref.}}{300 \text{ ft}^2} \times \frac{1.0 \text{ kW}}{\text{ton ref.}} \times 0.6 \times \frac{8760 \text{ hr}}{\text{yr}} = \boxed{\frac{87,600 \text{ kW} \cdot \text{hr}}{\text{yr}}}$$

for refrigeration

Electricity for pumping requirements:

Calculations on Mathcad printout

- assuming pumping occurs 7920 hr/yr  $\rightarrow$  gross overestimation

done on  
 Dr. Rivnick's advice

Pump	$\frac{\text{kW} \cdot \text{hr}}{\text{yr}}$	
UF1 Pump	389.15	
UF2 Pump	622.72	
MF Pump	421.9	
Aff. Col. Pump	25.52	} pressure drops rounded from 2 psi to 10 psi
ER Col. Pump	2.08	

$$\boxed{\frac{1461.37 \text{ kW} \cdot \text{hr}}{\text{yr}}} \text{ for pumping}$$

Impeller Power requirements (see Mathcad sheets: page

$$383,900 \text{ kW}\cdot\text{hr}/\text{yr}$$

Centrifuge Power requirements

max kW = 7.5 kW (see specs page)

assume 24 hrs/batch  $\rightarrow$  more than double the running time of 10 hrs (calc's page)

$$7.5 \text{ kW} \times \frac{24 \text{ hrs}}{\text{batch}} \times \frac{50 \text{ batches}}{\text{yr}} = \frac{9000 \text{ kW}\cdot\text{hr}}{\text{yr}}$$

Bottler/Tray loader Power req

(see specs page:)

$$\left\{ \begin{array}{l} \text{Bottler} = 0.5 \text{ hp} \\ \text{Loader} = 0.5 \text{ hp} \rightarrow \text{voltage \& freq. same as Bottle} \\ \text{Washer} = 0.5 \text{ hp} \end{array} \right.$$

$$1.5 \text{ hp} = 1.12 \text{ kW}$$

assume all three run for 146 hrs/batch

actually about 30 hrs/batch

$$1.12 \text{ kW} \times \frac{25 \text{ batches}}{\text{mach}\cdot\text{yr}} \times \frac{146 \text{ hrs}}{\text{batch}} \times 2 \text{ machines} = \frac{8176 \text{ kW}\cdot\text{hr}}{\text{yr}}$$

Freeze Dryer Power req.

Max 46.1 kW (Quote from Tray at Vintros 3/29/00)

146 hrs/batch

$$46.1 \text{ kW} \times 2 \text{ FD} \times \frac{25 \text{ batches}}{\text{FD}\cdot\text{yr}} \times \frac{146 \text{ hrs}}{\text{batch}} = \frac{336,530 \text{ kW}\cdot\text{hr}}{\text{yr}}$$

Total Power Requirements for Separation:

Refrigeration: 87,600 kW.hr/yr

Centrifuge: 9,000

Pumps: 1,461.37

Impellers: 383,900

Bottlers: 8,176

Freeze Dryers: 336,530

Total = 826,667 kW.hr  
yr

## Equipment Design for water purification Section

## Power for Pump #1 P-1 (10 Micron Filter Pump)

Pressure Drop over the first ultrafilter: 10 psi

Water flow through pump = 9.17 gal/min = 73.55 ft<sup>3</sup>/hr, We can calculate F by multiply flow rate in ft<sup>3</sup>/hr with water density in lb/ft<sup>3</sup>

$$\begin{aligned}
 F &:= 4688 \cdot \frac{\text{lb}}{\text{hr}} && \text{Flow through Pump} && \text{Pressure Drop over filter} \\
 \rho &:= 63.72 \cdot \frac{\text{lb}}{\text{ft}^3} && \text{Density of Water} && P_{\text{in}} := 14.7 \cdot \text{psi} \\
 \eta &:= 0.80 && \text{Pump efficiency} && P_{\text{out}} := (10 + 14.7) \cdot \text{psi} \\
 &&& && \Delta P := P_{\text{out}} - P_{\text{in}} \quad \Delta P = 10 \cdot \text{psi}
 \end{aligned}$$

Sizing the Pump:

$$\begin{aligned}
 \text{Size} &:= F \cdot \frac{\Delta P}{\rho} && \text{Size} = 0.054 \cdot \text{hp} \\
 \text{Power} &:= \frac{\text{Size}}{\eta} && \text{Power} = 49.875 \text{ kg} \cdot \text{m}^2 \cdot \text{sec}^{-3} \quad \text{Power} = 49.875 \text{ W}
 \end{aligned}$$

Hours per batch:  $t := 8000 \cdot \text{hr}$  # of batches:

$$\text{Electricity} := \text{Power} \cdot t \quad \text{Electricity} = 399 \cdot \text{kW} \cdot \text{hr}$$

## Power for Pump #2 P-2 (Carbon Filter Pump)

Pressure Drop over the carbon filter: 12 psi

Water flow through pump = 5 gal/min = 40.1 ft<sup>3</sup>/hr, We can calculate F by multiply flow rate in ft<sup>3</sup>/hr with water density in lb/ft<sup>3</sup>

$$\begin{aligned}
 F &:= 2555 \cdot \frac{\text{lb}}{\text{hr}} && \text{Flow through Pump} && \text{Pressure Drop over filter} \\
 \rho &:= 63.72 \cdot \frac{\text{lb}}{\text{ft}^3} && \text{Density of Water} && P_1 := 14.7 \cdot \text{psi} \\
 \eta &:= 0.80 && \text{Pump efficiency} && P_2 := 14.7 \cdot \text{psi} + 12 \cdot \text{psi} \\
 &&& && \Delta P := P_2 - P_1 \\
 &&& && \Delta P = 12 \cdot \text{psi}
 \end{aligned}$$

## Sizing the Pump:

$$\text{Size} := F \cdot \frac{\Delta P}{\rho}$$

$$\text{Size} = 0.035 \text{ hp}$$

$$\text{Hours per batch: } t := 8000 \text{ hr}$$

$$\text{Power} := \frac{\text{Size}}{\eta}$$

$$\text{Power} = 32.619 \text{ kg} \cdot \text{m}^2 \cdot \text{sec}^{-3}$$

$$\text{Electricity} := \text{Power} \cdot t$$

$$\text{Head} := \frac{\Delta P}{\rho \cdot g}$$

$$\text{Head} = 27.119 \text{ ft}$$

$$\text{Electricity} = 260.95 \text{ kW} \cdot \text{hr}$$

## Power Pump #3 P-3 (Water Softener Pump)

Pressure Drop over water softener: 10 psi

Water flow through pump = 20 gal/min = 160.42 ft<sup>3</sup>/hr, We can calculate F by multiply flow rate in ft<sup>3</sup>/hr with water density in lb/ft<sup>3</sup>

$$F := 10222 \cdot \frac{\text{lb}}{\text{hr}}$$

Flow through Pump

Pressure Drop over filter

$$P_1 := 14.7 \text{ psi}$$

$$\rho := 63.72 \cdot \frac{\text{lb}}{\text{ft}^3}$$

Density of Water

$$P_2 := 14.7 \text{ psi} + 10 \text{ psi}$$

$$\eta := 0.80 \quad \text{Pump efficiency}$$

$$\Delta P := P_2 - P_1$$

$$\Delta P = 10 \text{ psi}$$

## Sizing the Pump:

$$\text{Size} := F \cdot \frac{\Delta P}{\rho}$$

$$\text{Size} = 0.117 \text{ hp}$$

$$\text{Hours per batch: } t := 8000 \text{ hr}$$

$$\text{Power} := \frac{\text{Size}}{\eta}$$

$$\text{Power} = 108.751 \text{ kg} \cdot \text{m}^2 \cdot \text{sec}^{-3}$$

$$\text{Electricity} := \text{Power} \cdot t$$

$$\text{Head} := \frac{\Delta P}{\rho \cdot g}$$

$$\text{Head} = 22.599 \text{ ft}$$

$$\text{Electricity} = 870.004 \text{ kW} \cdot \text{hr}$$

## Reverse Osmosis System

The manufacturer give the power requirement for the Reverse Osmosis Pump

$$\text{Power} := 1.5 \cdot \text{hp} \quad \text{Power} = 1.119 \cdot 10^3 \text{ kg} \cdot \text{m}^2 \cdot \text{sec}^{-3}$$

$$\text{Hours per batch:} \quad t := 8000 \cdot \text{hr}$$

$$\text{Electricity} := \text{Power} \cdot t$$

$$\text{Electricity} = 8.948 \cdot 10^3 \text{ kW} \cdot \text{hr}$$

## Power for the UV Light System

From the Manufacturer, the power required for the UV lamb

$$\text{Power} := 0.021 \quad \text{kW}$$

$$\text{if running the light} \quad t := 8000 \quad \text{hr}$$

$$\text{Electricity} := \text{Power} \cdot t$$

$$\text{Electricity} = 168 \quad \text{kW} \cdot \text{hr}$$



## Power for pump #4 P-4 (UV system pump)

Pressure drop across the UV system : 5 psi

Water flow through pump = 6 gal/min = 48.125 ft<sup>3</sup>/hr, We can calculate F by multiply flow rate in ft<sup>3</sup>/hr with water density in lb/ft<sup>3</sup>

$$\begin{aligned}
 F &:= 3066.5 \cdot \frac{\text{lb}}{\text{hr}} && \text{Flow through Pump} && \text{Pressure Drop over filter} \\
 \rho &:= 63.72 \cdot \frac{\text{lb}}{\text{ft}^3} && \text{Density of Water} && P_{\text{in}} := 14.7 \cdot \text{psi} \\
 \eta &:= 0.80 && \text{Pump efficiency} && P_{\text{out}} := (5 + 14.7) \cdot \text{psi} \\
 &&& && \Delta P := P_{\text{out}} - P_{\text{in}} \quad \Delta P = 5 \cdot \text{psi}
 \end{aligned}$$

Sizing the Pump:

$$\begin{aligned}
 \text{Size} &:= F \cdot \frac{\Delta P}{\rho} && \text{Size} = 0.017 \cdot \text{hp} \\
 \text{Power} &:= \frac{\text{Size}}{\eta} && \text{Power} = 16.312 \text{ kg} \cdot \text{m}^2 \cdot \text{sec}^{-3} \quad \text{Power} = 16.312 \text{ W}
 \end{aligned}$$

Hours per year:  $t := 8000 \cdot \text{hr}$ 

$$\text{Electricity} := \text{Power} \cdot t \quad \text{Electricity} = 130.496 \cdot \text{kW} \cdot \text{hr}$$

## Power for pump #5 P-5 (0.2 micron filter pump)

Pressure drop across the filter : 10 psi

Water flow through pump = 8 gal/min = 64.17 ft<sup>3</sup>/hr, We can calculate F by multiply flow rate in ft<sup>3</sup>/hr with water density in lb/ft<sup>3</sup>

$$\begin{aligned}
 F &:= 4088.7 \cdot \frac{\text{lb}}{\text{hr}} && \text{Flow through Pump} && \text{Pressure Drop over filter} \\
 \rho &:= 63.72 \cdot \frac{\text{lb}}{\text{ft}^3} && \text{Density of Water} && P_{\text{in}} := 14.7 \cdot \text{psi} \\
 \eta &:= 0.80 && \text{Pump efficiency} && P_{\text{out}} := (10 + 14.7) \cdot \text{psi} \\
 &&& && \Delta P := P_{\text{out}} - P_{\text{in}}
 \end{aligned}$$

Sizing the Pump:

$$\text{Size} := F \cdot \frac{\Delta P}{\rho} \quad \text{Size} = 0.047 \text{ hp}$$

$$\text{Power} := \frac{\text{Size}}{\eta} \quad \text{Power} = 43.499 \text{ kg} \cdot \text{m}^2 \cdot \text{sec}^{-3} \quad \text{Power} = 43.499 \text{ W}$$

$$\text{time running} \quad t := 8000 \cdot \text{hr} \quad \text{per year}$$

$$\text{Electricity} := \text{Power} \cdot t$$

$$\text{Electricity} = 347.993 \text{ kW} \cdot \text{hr}$$

Power for pump #6 P-6

Pressure drop : 20 psi

Water flow through pump = 5 gal/min = 40.1 ft<sup>3</sup>/hr, We can calculate F by multiply flow rate in ft<sup>3</sup>/hr with water density in lb/ft<sup>3</sup>

$$F := 2555.4 \cdot \frac{\text{lb}}{\text{hr}} \quad \text{Flow through Pump}$$

$$\rho := 63.72 \cdot \frac{\text{lb}}{\text{ft}^3} \quad \text{Density of Water}$$

$$\eta := 0.80 \quad \text{Pump efficiency}$$

Pressure Drop over filter

$$P_{\text{in}} := 14.7 \cdot \text{psi}$$

$$P_{\text{out}} := (20 + 14.7) \cdot \text{psi}$$

$$\Delta P := P_{\text{out}} - P_{\text{in}}$$

Sizing the Pump:

$$\text{Size} := F \cdot \frac{\Delta P}{\rho} \quad \text{Size} = 0.058 \text{ hp}$$

$$\text{Power} := \frac{\text{Size}}{\eta} \quad \text{Power} = 54.373 \text{ kg} \cdot \text{m}^2 \cdot \text{sec}^{-3} \quad \text{Power} = 54.373 \text{ W}$$

$$\text{time running} \quad t := 8000 \cdot \text{hr} \quad \text{per year}$$

$$\text{Electricity} := \text{Power} \cdot t$$

$$\text{Electricity} = 434.985 \text{ kW} \cdot \text{hr}$$

pump → water from outlet

$$P = WI$$

14 7.5 A  
330 V

## COMPLETE WATER TREATMENT SYSTEM

A 25' water line → 15' water

AmeriWater's complete water treatment system is designed to be simple to install. All components are equipped with hosing and quick connects so that the system can be easily joined together.

Cost of resin included?

110 LPM

**WATER SOFTENER** (what type of resin?). port (20-30 GPM) \$4,537.00

Model WTMD16 meter initiated system. 132,000 grain capacity.

~~Model WTMD16~~  
**CARBON SYSTEM**

Model WTTS16C backwashing carbon system. 40 gal/min.

Model WTTS24C

**REVERSE OSMOSIS SYSTEM\***

Model IRO2. 220 volt system is capable of producing up to 4,600 gallons per day. Includes two model 4 x 40 high output membranes and particulate filter.

(16,000 L)

**HOLDING TANK**

4000 polyethylene, horizontal holding tank, 95" dia. x 140" high. Includes inlet/outlet mercury float switch with plug in cord and cam locks for easy connection.

**REPRESSURIZATION SYSTEM**

Model LB-5 stainless steel pump, 115 volt. Provides flow up to 30 GPM.

Deionizer or Filter? **INVESTMENT**

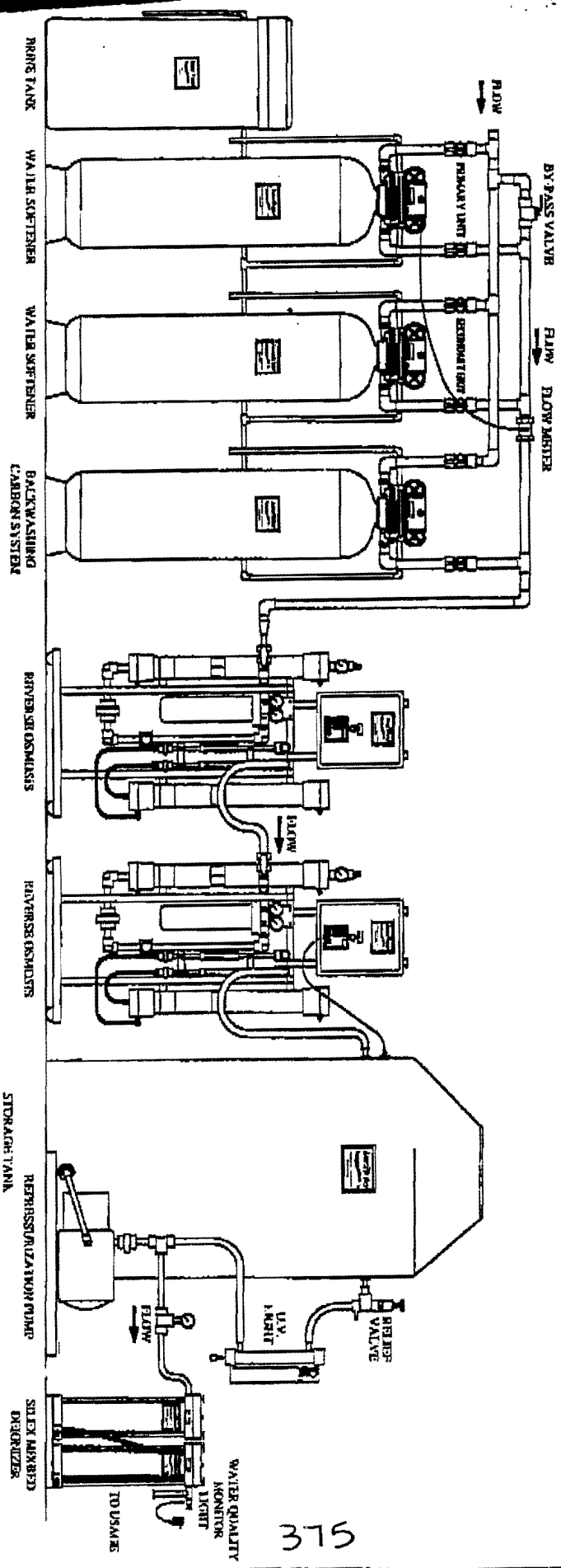
\*Product flow rate varies with temperature. All models are rated at 77 degrees, with feed water of 1500 mg/l NaCl @ 200 PSI and pH of 7.5

\*\*Holding tank will be drop shipped from the manufacturer. Our price does not include freight.

### RECIRCULATION OPTION

To prevent bacteria growth in the holding tank, we recommend that you keep the water recirculating through an ultra violet light system. The price for this option is as follows:

UV System - 7 GPM	\$850.00
Relief Valve	\$ 82.00
Interconnecting Piping	\$225.00
Spray Ball Assembly	\$260.00
<b>TOTAL</b>	<b>\$1417.00</b>



(Filter 97)

375

**AmeriWater®**

1217 Soudby Avenue, Dayton, Ohio 45404  
Phone: 937/261-6311, Fax: 937/261-5585  
Fax: 937/261-1888

**Model 24**  
R/O SYSTEM, UP TO 10 G.P.M.

System Size: 0 to 10 G.P.M. 0 to 100 G.P.M. 100 to 1000 G.P.M.  
Flow Rate: 0 to 10 G.P.M. 0 to 100 G.P.M. 100 to 1000 G.P.M.  
Flow Rate: 0 to 10 G.P.M. 0 to 100 G.P.M. 100 to 1000 G.P.M.

**Subject: Technical Question - Your E-mail, 28 Feb 00**

**Date:** Wed, 1 Mar 2000 09:01:35 -0600

**From:** "Lewis, James" <JLewis@osmonics.com>

**To:** "'kasidit@seas.upenn.edu'" <kasidit@seas.upenn.edu>

Dear Mr. Nootong,

Thank you for inquiry regarding Osmonics' Pleated Filter Cartridges. For any of our cartridges, we recommend a maximum flow rate of 5 gpm/Ten Inch Equivalent (TIE) length. Thus, a 20" long cartridge would have a maximum flow rate of 10 gpm. The maximum can be reduced by other factors such as the micron rating of the cartridge, the solution viscosity and available pressure drop.

When we size a housing for a given filtration, typically we would include enough TIEs to keep the initial differential pressure at 2 psid or less. For the Flotrex-AP021 cartridge this rate would be 5 gpm. For the Memtrex-FE921, this rate would be about 3.5 gpm. In each case, a fluid with a viscosity of water was assumed. The data necessary for these calculations is available on our website, osmonics.com.

Please let us know if you have further questions. Good luck on your project.

Best regards,

Jim Lewis  
Application Engineer



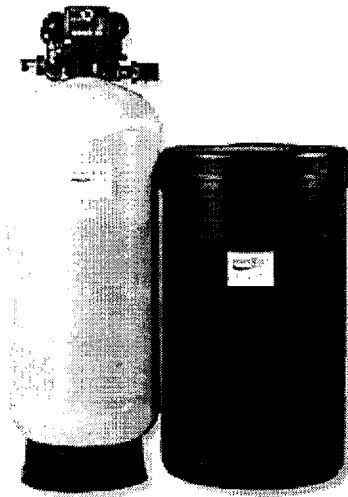
Industrial

Services

Accessories

## Water Treaters Series

- Softeners
- Dealkalizers
- Filters



The price is right

Quick installation  
that's hassle-freeEasy maintenance  
cuts downtime

## Softening

### Water softening cation sodium form resin

Ultra high exchange capacity, better than 8% cross linked gelular, strongly acidic, cation exchange resin. Very good kinetics, excellent stability at elevated temperatures and good chemical resistance over a wide pH range.

Model	Mode	# Media Tanks	Flow Rate (GPM)	Capacity Grains
WT TS 16	Timer	1	40	140,000
WT MS 16	Meter	1	40	140,000
WT TD 16	Meter/Alternating	2	40	280,000
WT TS 24	Timer	1	80	280,000
WT MS 24	Meter	1	80	280,000
WT TD 24	Meter/Alternating	2	80	560,000

## Dealkalization

### Water Dealkalization Anion Chloride Form Resin

Very effective for the reduction of bicarbonate alkalinity. Type II gelular, strongly basic resin in the chloride form gives high capacity and good leakage characteristics.

Model	Mode	# Media Tanks	Flow Rate (GPM)	Capacity Grains
WT TS 16D	Timer	1	20	48,000
WT MS 16D	Meter	1	20	48,000
WT MD 16D	Meter	2	40	96,000
WT TS 24D	Timer	1	50	96,000
WT MS 24D	Meter	1	50	96,000
WT MD 24D	Meter	2	100	192,000

## Filtration

### Activated Carbon

Made from select grades of bituminous coal to produce a high density, durable granular product capable of withstanding hydraulic loading, back washing, and mechanical handling. Abrasion #80, Iodine #950, Backwash 8-10 GPM/sq.ft.

### Sediment filter Ag

Ag granuals have irregular surface characteristics for maximum removal with less pressure loss. Requires less backwash water. Backwash 8-10 GPM/sq.ft. Removal down to 15 microns.

### Manganese Greensand

Capable of removing iron, manganese and hydrogen sulfide from water through oxidation and filtration. Feed of chlorine or potassium permanganate will continue the oxidation process. Backwash 10-12 GPM/sq.ft. Iron limit is 15 PPM.

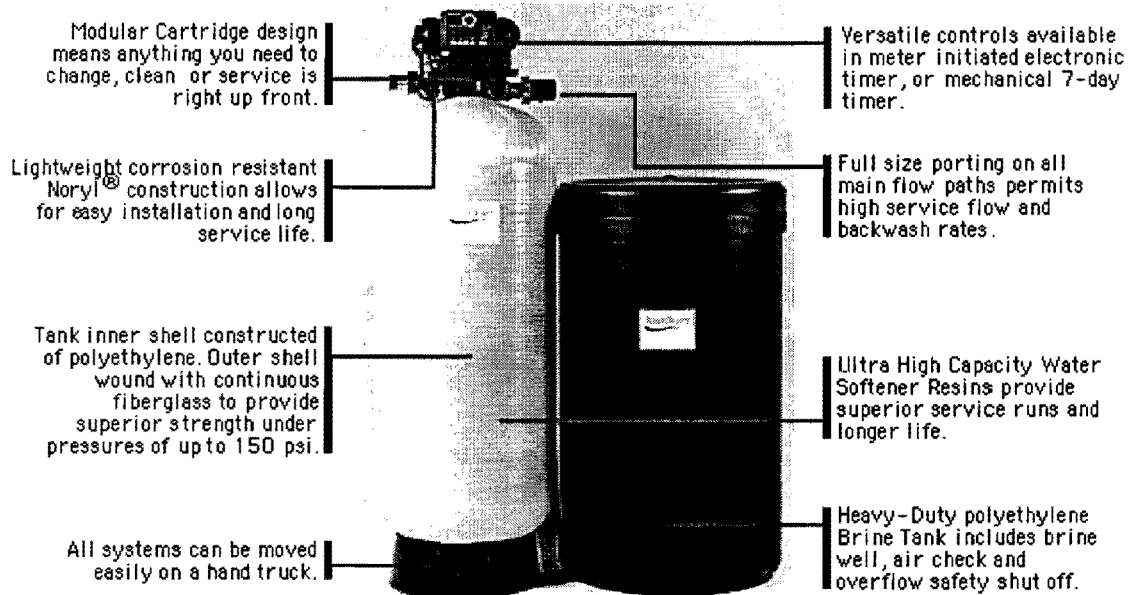
### Multi-Media

High quality quartz sand and gravel in layers provides for excellent particulate filtration. Backwash 10-12 GPM/sq.ft. Removal down to 20 microns.

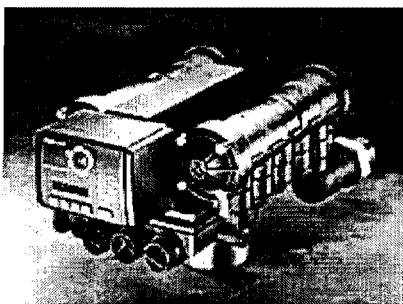
Model	Mode	# Media Tanks	Flow Rate (GPM)	Capacity Grains
WTTS 16 A	Timer	1	18	Sediment
WTTS 24 A	Timer	1	40	Sediment
WTTS 16 C	Timer	1	18	Carbon
WTTS 24 C	Timer	1	40	Carbon
WTTS 16 M	Timer	1	18	Multi Media
WTTS 24 M	Timer	1	40	Multi Media
WTTS 16 G	Timer	1	12	Green Sand
WTTS 24 G	Timer	1	26	Green Sand

## One versatile system for softening, dealkalizing, and filtering applications

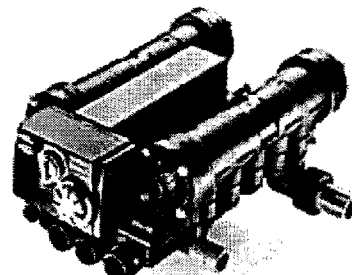
The AmeriWater Water Treaters Series of equipment is designed specifically for the water treatment professional. We offer a variety of sizes and media to meet the needs of your industry. Whether you need a softener, dealkalizer, or filter, you'll find our pricing to be very competitive, without sacrificing quality and durability.



Options include bypass or no bypass water during restoration and PVC or threaded brass installation adapters



2" Control with meter initiated electronic timer



1 1/2" Control with mechanical 7-day timer

## Water treaters series configurations



Water Softener or Dealkalizer Single Tank System



# SYBRON CHEMICALS INC.

SYBRON CHEMICALS INC.  
BIRMINGHAM ROAD, P.O. BOX 66, BIRMINGHAM, NEW JERSEY 08011 (609) 893-1100

## IONAC® C-249 Strong Acid Sodium (Brine) Cycle CATION EXCHANGE RESIN

IONAC C-249 is a premium quality cation exchange resin capable of meeting the most exacting requirements of both household and commercial water softeners and suitable for treating even potable water. Extremely low fines content, high operating capacity and excellent physical quality make IONAC C-249 - A PREMIUM PRODUCT AT STANDARD COST.

IONAC C-249 is a bead-form, standard crosslinked, polystyrene sulfonate cation exchange resin, possessing high cation exchange capacity, combined with excellent stability and operating characteristics. It contains a minimal amount of "fines" (-50 mesh) thus showing low pressure loss effects.

U. S. Standard Mesh	Size mm	Typical Distribution
+16	1.19	4.0 %
-16+20	1.19-0.84	29.0 %
-20+30	0.84-0.59	49.0 %
-30+40	0.59-0.42	16.0 %
-40+50	0.42-0.30	1.5 %
-50	0.30	0.5 %

IONAC C-249 is specially manufactured to eliminate color throw and to assure compliance with F.D.A. Regulations, Para. 173.25. It has also been approved by the Meat Inspection Division of the U.S. Department of Agriculture for use in the treatment of water for meat packaging plants. IONAC C-249 also meets the stringent specifications for extractable limits set by the French Ministry of Health for cation exchange resins used in potable water supplies and for food industry uses.

IONAC C-249 is supplied in the fully swollen moist sodium form, and its primary application (detailed in this bulletin) is in the softening of water, for household, municipal, and industrial use.

NOTE: Similar cation exchange resins with different crosslinkage, ionic form, particle size distribution, or degrees of purity are available.

### TYPICAL CHARACTERISTICS

#### Sodium Cycle Operation

Polymer Structure	Crosslinked Styrene/divinylbenzene
Functional Structure	.R-SO <sub>3</sub> -Na <sup>+</sup>
Form (Physical)	Spherical Beads
Form (Ionic), as shipped	Na <sup>+</sup>
Screen Size, U.S. Std. (Wet)	16-40
Particle Size	.04-1.2 mm
Total Capacity	Volumetric: 1.9 meq/ml Weight: 4.6 meq/gm
Swelling	H → Na = 5%
Water Retention	46-47 %
Moisture Content as Shipped	45-48 %
pH Range (stability)	0.14
Solubility	Insoluble in all Common Solvents
Approximate Shipping Weight	52 lb./cu. ft. (832 gm/l)
Standard Packaging (a)	7 cu. ft. in polyethylene lined fiber drums or 1 cu. ft. burlap bags.
(b)	156 liter polyethylene lined fiber drums or 25 liter burlap bags.

### SUGGESTED OPERATING CONDITIONS

	U. S. Units	Metric Units
Maximum Operating Temperature	280° F.	140° C.
Minimum Bed Depth*	24"	60 cm
Standard Operating Flow Rate	3 gpm/cu. ft.	24 l/hr/l
Design Rising Space	100%	100%
Back Wash Expansion**	50-75%	50-75%
Regenerant	NaCl	NaCl
Regenerant Strength	5-15%	5-15%
Regenerant Levels (100% NaCl)	5-15 lb/cu. ft.	80-240 gm/l
Regenerant Flow rate	0.5 gpm/cu. ft.	4 l/hr/l
Regenerant Injection Time	20-40 minutes	20-40 minutes
Slow Rinse Volume	20 gal/cu. ft.	2.7 l/l
Slow Rinse Rate	6 regen. flow rate	6 regen. flow rate
Fast Rinse Rate	2.0 gpm/cu. ft.	16 l/hr/l
Fast Rinse Volume	30 gal/cu. ft.	4 l/l
<b>INFLUENT LIMITATIONS</b>		
Maximum Free Chlorine		1.0 ppm
Maximum Turbidity		5 A.P.H.A. Units

\*For high total solids water or where effluent quality is critical, 30" (76 cm) minimum bed depth recommended.

\*\*See Figure 8

The data included herein are based on test information obtained by Sybron Chemicals Inc. These data are believed to be reliable but do not imply any warranty of performance guarantee. We recommend that the user determine performance by testing on his own processing equipment. We assume no liability or responsibility for patent infringement resulting from the use of this product.

## KEY FACTORS AFFECTING CAPACITY OF IONAC C-249

- Regenerant quantity, contact time with resin, and brine concentration
- Water flow rate during service run.
- Bed depth

## CAPACITY

Figures 1, 2, 5 and 6 illustrate the effect of total dissolved solids (T.D.S.) and various hardness levels on operating (softening) capacities at different salt (NaCl) dosages.

Figures 3 and 4 illustrate the effect of Sodium vs. Total Hardness on expected leakage.

For clarity the Total Dissolved Solids (Sodium plus Total Hardness as ppm  $\text{CaCO}_3$ ) is given as a parameter, vs. Influent Hardness. To obtain TOTAL DISSOLVED SOLIDS (T.D.S.):

... Add Sodium to Total Hardness (all expressed as  $\text{CaCO}_3$ )

Capacity ratings and related data are based upon capacity break-through, i.e. end-point of 10% rise from the average residual hardness.

### FOR METRIC CONVERSIONS:

Multiply gpm sq. ft. x 2.44	= Meters/hr.
Multiply gpm cu. ft. x 8	= l/hr./l
Multiply PSI/ft. bed x .22	= Atmosphere/m/bed depth
Multiply Bed depth, ft. x 30.48	= cm
Multiply lb./cu. ft. x 16	= Grams/l
Multiply Kgr./cu. ft. x 2.29	= Grams $\text{CaCO}_3$ /l

### CAPACITY CALCULATION:

U.S. gal. / regen. x Total Hardness (in ppm)  
Resin volume (cu. ft.) x 17.1 x 1000 = kilograins/cu. ft.

Metric conversion 1 Kgr/cu. ft. = 2.29 gm ( $\text{CaCO}_3$ ) per liter of resin  
(1 cu. ft. = 28.3 liters)

Figure 1

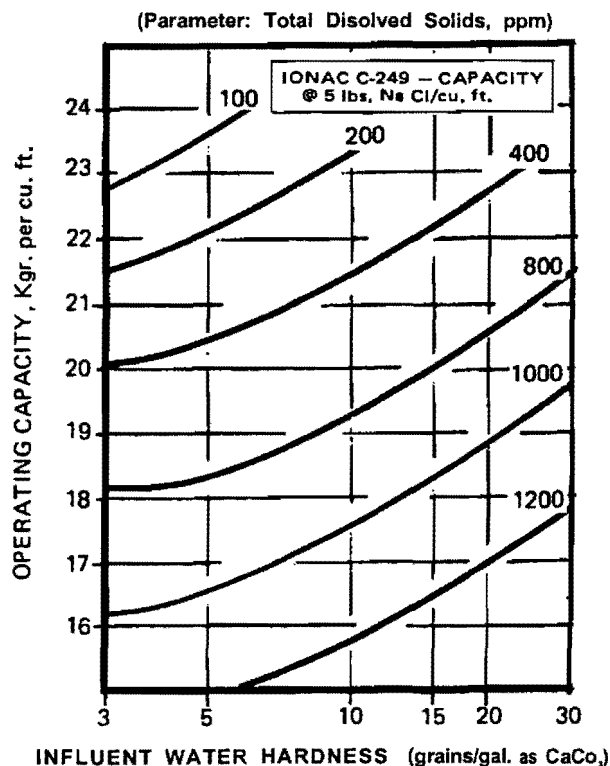


Figure 2

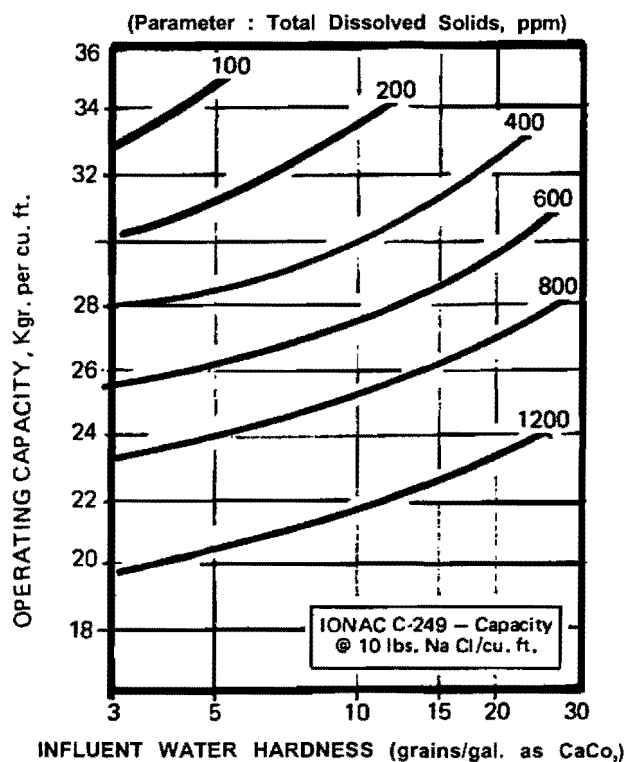


Figure 3

(Parameter, Total Dissolved Solids, ppm)

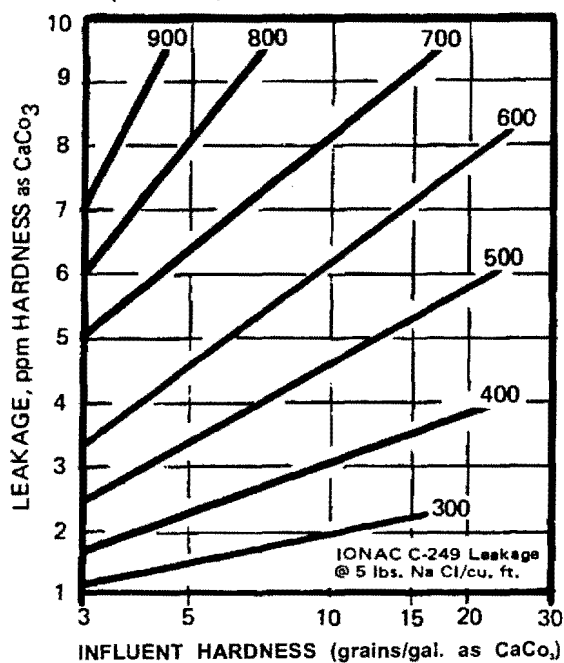


Figure 5

(Parameter : Total Dissolved Solids, ppm)

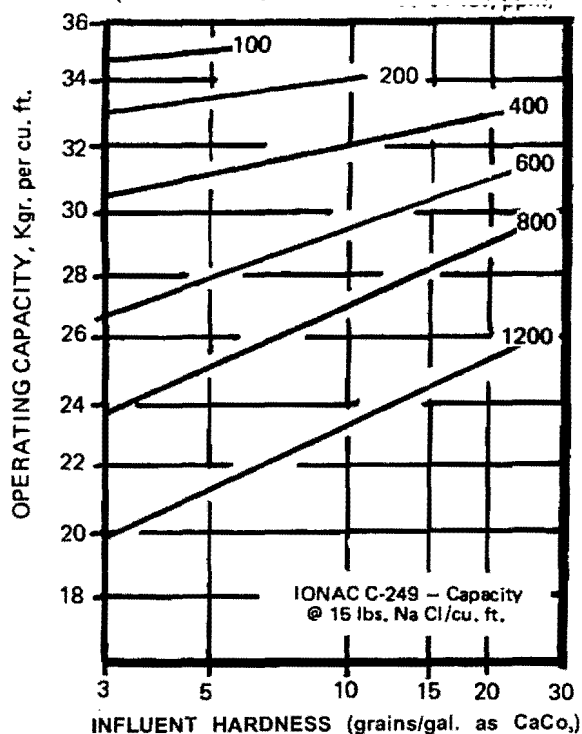


Figure 4

(Parameter: Total Dissolved Solids, ppm)

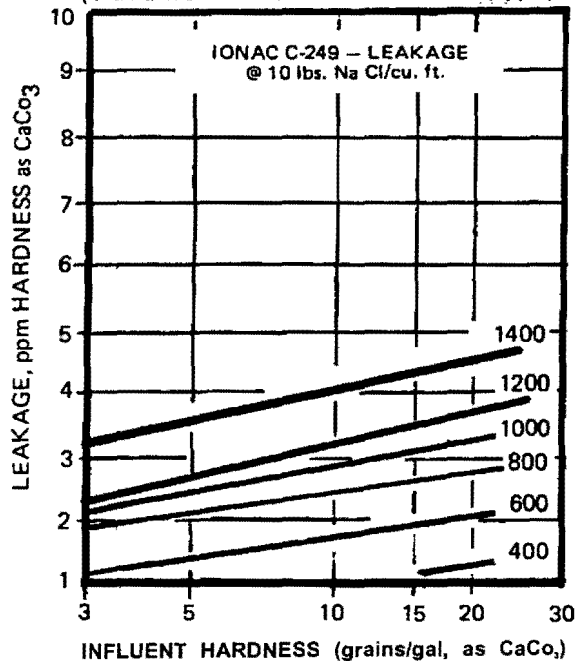
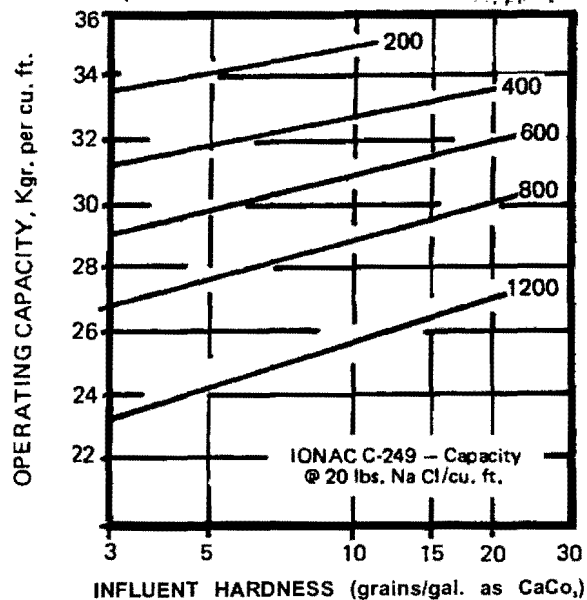


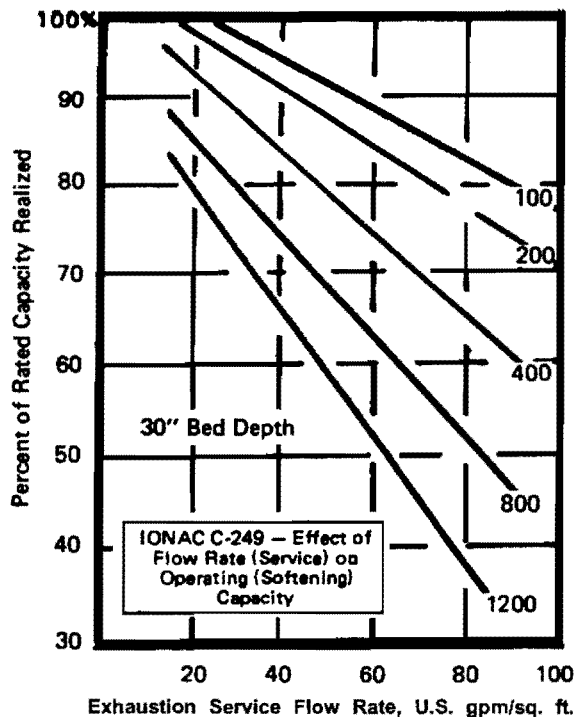
Figure 6

(Parameter : Total Dissolved Solids, ppm)

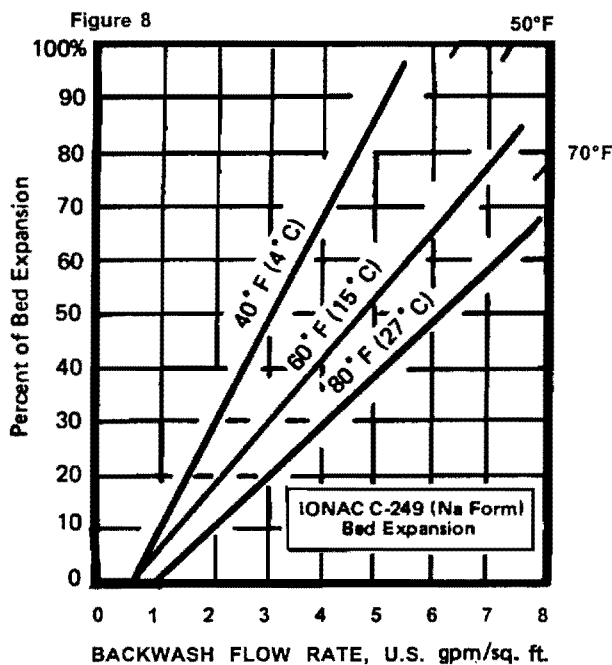


For 15 and 20 lb. Salt (NaCl) regeneration, per cu. ft., no leakage curves are presented. The reason for omitting these is that at the Total Solids and Total Hardness levels shown the leakage at 15 lb. of Salt is below 1.5 ppm; and at 20 lb. of Salt the leakage is below 0.5 ppm.

Figure 7 (Parameter: Total Dissolved Solids ppm)

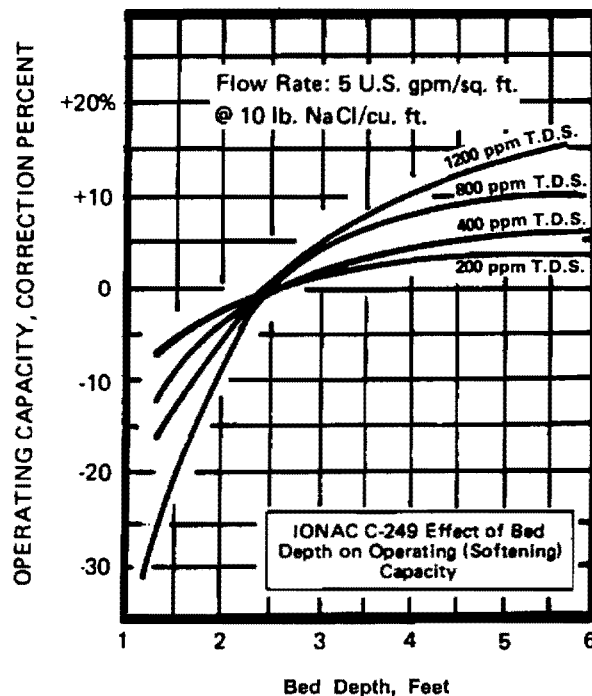


The backwash step removes particulate matter filtered out by the IONAC C-249 resin bed during exhaustion as well as regrading the bed and eliminating any channels which may have formed. Normally, a backwash rate that expands the bed 50 to 75 percent in a clean system for 5 to 10 minutes is recommended. The backwash flow rate should always be achieved gradually to prevent loss of resin through a surge carryover. Figure 8 shows the relationship between the temperature, backwash (upward) flow rate and the percent of bed expansion which can be achieved.

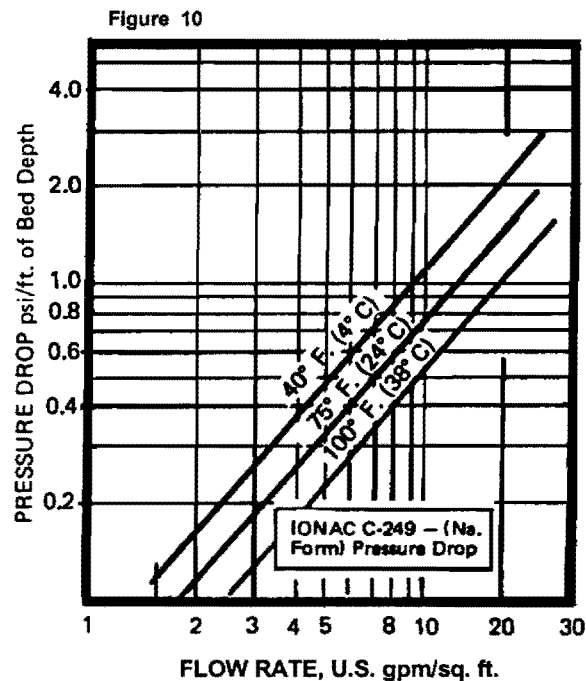


When Ionac C-249 ion exchange resin is to be used on the sodium cycle for softening potable water or for treating water for food processing, it should be contracted with a minimum of 60 gallons of potable water per cu. ft. of resin over a period of at least 15 minutes before being placed in service. This total

Figure 9



The pressure loss (or head loss) experienced in passing a solution through a bed of IONAC C-249 is directly related to the rate of flow and the temperature of the solution. Figure 10 illustrates these relationships and what amount of pressure loss can be expected in unit design. These data are based upon clean water and new resin. Any particulate matter in the water filtered out by the ion exchange bed can cause additional pressure loss.



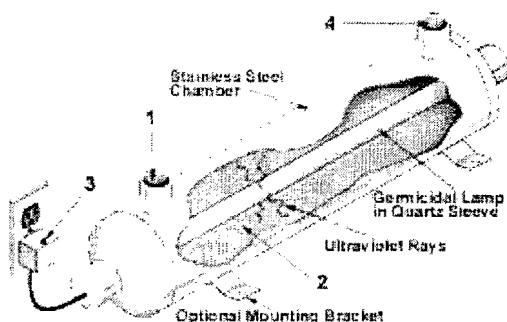
volume of water can be used in either the backwashing, brining or rinse portions of a normal regeneration cycle. When so contacted, Ionac C-249 will meet the extractive requirements of the Food Additives Regulations, Para. 173.25.

**SYBRON CHEMICALS INC.**

# MINIPURE

ULTRAVIOLET WATER PURIFIERS

## Principles of Operation



1. Water enters the purifier and flows into the annular space between the quartz sleeve and the outside chamber wall.

2. Within the chamber, water is exposed to intense germicidal ultraviolet radiation.

3. Transformer with LED indicator light provides visual indication of germicidal lamp operation.

4. Water leaving the purifier is instantly ready for use.

Minipure™ Water Purifiers are manufactured under one or more patents owned by Atlantic Ultraviolet Corporation.

Minipure™, Ster-L-Ray™ and Easy-Off™ Retainer Cap are trademarks of the Atlantic Ultraviolet Corporation.

UV - 2190T

# MINIPURE

ULTRAVIOLET WATER PURIFIERS

## Specifications for Standard Models

Model	GPM	GPH	Inlet/Outlet Size	Unit Dimensions (Inches)			Shipping Data		
				Length	Width	Height	Gross Wt.	Net Wt.	Cu. Ft.
MIN-1	1	60	1/4" FPT	12-3/8"	2-1/2"	3"	7 lbs.	5 lbs.	.30
MIN-1.5	1.5	90	1/4" FPT	15-3/8"	2-1/2"	3"	8 lbs.	6 lbs.	.35
MIN-3	3	180	3/4" MPT	16-1/4"	4-1/4"	5-9/16"	9 lbs.	7 lbs.	.90
MIN-6	6	360	3/4" MPT	22-1/4"	4-1/4"	5-9/16"	14 lbs.	10 lbs.	1.20
MIN-9	9	540	3/4" MPT	29-3/8"	4-1/4"	5-9/16"	18 lbs.	14 lbs.	1.51

- Maximum recommended operating pressure for all purifiers is 100 PSI.
- Pressure drop at maximum recommended flow rate is 5 PSI or less.
- All data shown above is for 118 volt 60 Hz. Units are also available for 220 volt 50 Hz and 12 volt DC operation.

Minipure™ Water Purifiers are manufactured under one or more patents owned by Atlantic Ultraviolet Corporation.

Minipure™, Ster-L-Ray™ and Easy-Off™ Retainer Cap are trademarks of the Atlantic Ultraviolet Corporation.

# MINIPURE

## ULTRAVIOLET WATER PURIFIERS

### Ster-L-Ray Lamp Data for Minipure™ Water Purifiers

05-1119	MIN-1	212	8-11/32	10 watts	2.3	10,000 Hours
05-1366	MIN-1.5	287	11-19/64	14 watts	3.7	10,000 Hours
05-1370	MIN-3	287	11-19/64	14 watts	3.7	10,000 Hours
05-1370	MIN-6	436	17-5/32	21 watts	6.4	10,000 Hours
05-0097A	MIN-9	620	24-13/32	29 watts	9.7	10,000 Hours

\*Wattage is lamp watts only and does not include ballast loss.

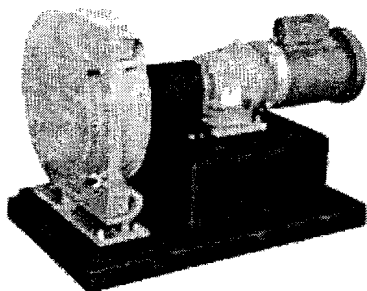
\*\*Maximum rated output at 254 nanometers.

All Minipure™ Water Purifiers employ genuine Ster-L-Ray™ germicidal lamps that afford the maximum efficiency and longevity in producing the required germicidal rays. In addition to the obvious advantages of high efficiency and low power requirements, there is no possibility of the purifier overheating, as is the case with some other types. Consequently the need for additional equipment to combat overheating is eliminated.

Minipure™ Water Purifiers are manufactured under one or more patents owned by Atlantic Ultraviolet Corporation.

Minipure™, Ster-L-Ray™ and Easy-Off™ Retainer Cap are trademarks of the Atlantic Ultraviolet Corporation.

# Large Volume Models Technical Data



Cat. No. 880-110, 880-120  
Cat. No. 880-110 Shown

## Standard Model - Fixed Speed

The Series 880 is offered with choice of two fixed speed motors. Motors offered include a 100 rpm and 190 rpm 3/4 hp - 115/230 volt - 60 hz - single phase gearhead open type. Units are mounted on a durable cast metal base. Available in explosion proof model also. Refer to chart below for flow rates,

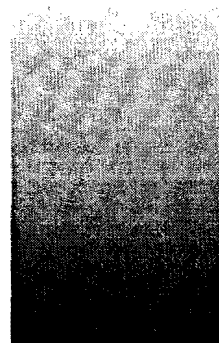
specifications and shipping weight.

Dimensions: L 29" x W 17" x H 21" Cat No. 880-300

## Variflow Model - DC Control

The Series 880-300 (not shown) is offered with a 220 volt single phase control to a geared 190 rpm DC motor. The Vari-Flow control permits accurate adjustment with forward and reverse capabilities.

Dimensions: L 29" x W 17" x H 21"



## Technical Data - Series 880 - Models

Cat.#.	Mode	Pump Speed rpm.	Motor hp.	Output gpm		Ship. wt.
				Flow Rate 3/4" ID Tubing	1" ID Tubing	
880-PHO	Pumphead only					115 lb.
880-110	Std. model - fixed speed	100	3/4	6	12	240 lb.
880-120	Std. model - fixed speed	190	3/4	11	21	240 lb.
880-300	Vari-flow speed control	190	1	1/2-11	1-21	250 lb.
880-400	Air-motor	225	.7	3-14	5-23.5	210 lb.

# RANDOLPH AUSTIN COMPANY

Pump - 880-330



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## Back to Intermediate Volume Page

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### Series 750 - Models

Cat.#.	Model	Pump Speed rpm.	Motor hp.	Output gph Flow Rate		Ship. wt.
				5/8" ID Tubing	3/4" ID Tubing	
750-PHO	Pumphead only					20 lb.
750-000	Pumphead and pulley					20 lb.
750-100	Std. model- single phase motor	430	1/3	390	570	65 lb.
750-101	Zero-max speed control model	0-400	1/2	360	470	120 lb.
750-200	Explosion proof model	430	1/3	390	570	120 lb.
750-201	Expl. proof model/speed control	0-400	1/2	360	470	140 lb.
750-332	Vari-flow speed control model	3-125	1/4	8.2-113	12-166	66 lb.
750-342	Vari-flow speed control model	4-165	1/4	10-150	14.6-219	66lb.
750-352	Vari-flow speed control model	6-250	1/4	14-227	20-332	66 lb.
750-362	Vari-flow speed control model	12-500	1/4	24-454	36-662	66 lb.
750-400	Air drive model - ARO		10-90 psi	102-390	126-570	42 lb.
750-450	Air drive model - Gast		10-90 psi	92-288	111-544	38 lb.

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## Back to Intermediate Volume Page

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Pump 750-362

# FLOTREX™-AP FILTERS

POLYPROPYLENE MICROFIBER  
(0.65, 1, 2, 3, 5, 10, 20, AND 40μm ABSOLUTE RATINGS)



Constructed of thermally-bonded polypropylene fiber media, absolute-rated Flotrex-AP (FAP) filters combine exceptional solids holding capacities with precise micron retention ratings. The FAP filters are constructed of high-purity polypropylene and are made with all FDA-acceptable materials.

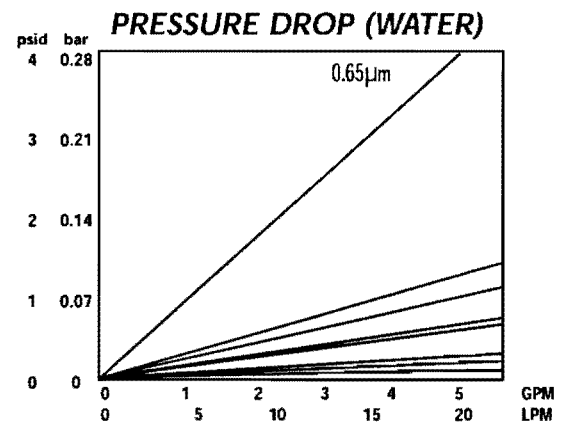
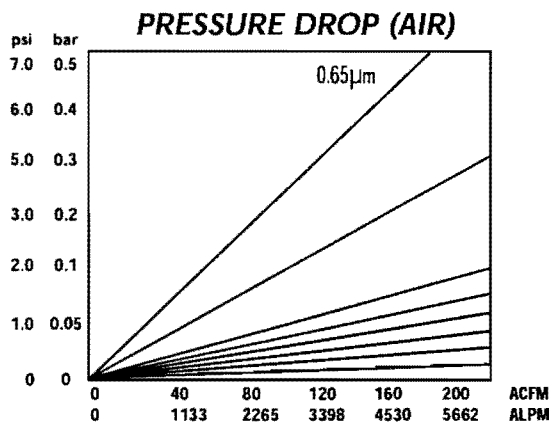
FAP filters are absolute-rated for air, gas and liquid filtration with low pressure drop across the wide range of 0.65 to 40 micron. The graded sheets of melt-blown media are layered to provide absolute particle retention, high solids loading and long service life.

The FAP filter is just one example of our strong commitment to liquid, gas and air treatment. Our complete portfolio includes filters for every stage of processing, and we offer custom solutions for your unique applications. Osmonics is your complete source for filters, housings and other filtration equipment.

Whether you require an integrated solution or a single component for a specific application, look to Osmonics first. From one end of the filtration spectrum to the other, Osmonics has a total commitment to fluid purity.

## FAP ADVANTAGES

- Broad chemical compatibility
- Dependable protection for final filters
- Efficient removal of *Cryptosporidium* and *Giardia* cysts
- High throughput
- High efficiency
- Long service life
- Absolute rating (99.9%)
- Fast rinse up to 18 megohm
- Thermally-bonded polypropylene fiber media
- All FDA-acceptable materials



ACFM = Actual Cubic Feet per Min.

ACFM = SCFM at 70°F and 14.7 psia

Note: For pressures and temperatures other than 14.7 psia (0 psig) and 70°F, ACFM can be approximated with the following formula:  $ACFM = SCFM \left[ \frac{14.7 \text{ psia}}{(psig + 14.7)} \right] \left[ \frac{°F + 460}{530} \right]$

## SPECIFICATIONS

### Effective Filtration Area for 10-inch Equivalent

0.65µm	- 5.0 ft <sup>2</sup> (0.45 m <sup>2</sup> )
1µm	- 5.0 ft <sup>2</sup> (0.45 m <sup>2</sup> )
2µm	- 5.2 ft <sup>2</sup> (0.48 m <sup>2</sup> )
3µm	- 5.2 ft <sup>2</sup> (0.48 m <sup>2</sup> )
5µm	- 6.1 ft <sup>2</sup> (0.57 m <sup>2</sup> )
10µm	- 6.7 ft <sup>2</sup> (0.62 m <sup>2</sup> )
20µm	- 6.9 ft <sup>2</sup> (0.64 m <sup>2</sup> )
40µm	- 7.2 ft <sup>2</sup> (0.67 m <sup>2</sup> )

### Operational Data

Maximum Rated Differential Pressure -

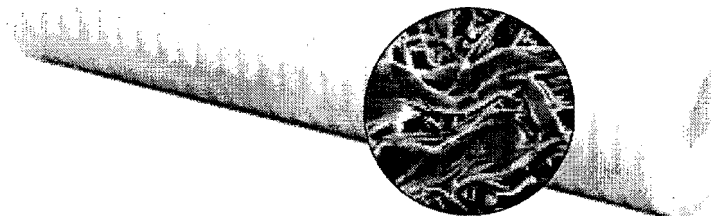
- Forward flow - 60 psi (4.14 bar)
- Reverse flow - 30 psi (2.07 bar)

Maximum Rated Operating Temperature -

- 180°F (82°C) at 10 psid
- (0.69 bar) in water

### Cartridge Dimensions (Nominal)

- Outside diameter -  
2.75 inches (70 mm)
- Inside diameter -  
1.25 inch (31 mm)



Polypropylene Microfibers

## APPLICATIONS

Flotrex-AP filters are specifically designed for pharmaceutical prefiltration. Typical applications include:

### Prefiltration and Final Chemical Filtration

- Broad chemical compatibility

### Bottled Water Final Filtration

- Efficient removal of *Giardia* and *Cryptosporidium* cysts

### Prefiltration of Pharmaceuticals and Biological Fluids

- Dependable protection for final filters

### High Throughput for Beer Filtration

Integrity test and particle retention data available on request.

## ORDERING INFORMATION

Type	Absolute Micron Rating	Cartridge Length	End #1 Adapter	End #2 Adapter	Elastomer Material
FAP	96 = 0.65µm 01 = 1.0µm 02 = 2.0µm 03 = 3.0µm 05 = 5.0µm 10 = 10.0µm 20 = 20.0µm 40 = 40.0µm	1 = 10 Inch 2 = 20 Inch 3 = 30 Inch 4 = 40 Inch	A = Open End Gasket B = 120 O-Ring C = 213 O-Ring E = 222 O-Ring F = 226 O-Ring J = 020 O-Ring Q = 222 O-Ring Stainless Steel Support Ring Z = 226 O-Ring Stainless Steel Support Ring	A = Open End Gasket B = 120 O-Ring C = 213 O-Ring G = Closed End Cap H = Fin Adapter	B = Buna-N E = EPDM S = Silicone T = Teflon* Encapsulated (Only in 222 and 226 Sizes) V = Viton*

\* Viton and Teflon are registered trademarks of E.I. DuPont de Nemours and Company, Inc.

For more information call toll free in the USA (800) 848-1750

Manufactured in the USA



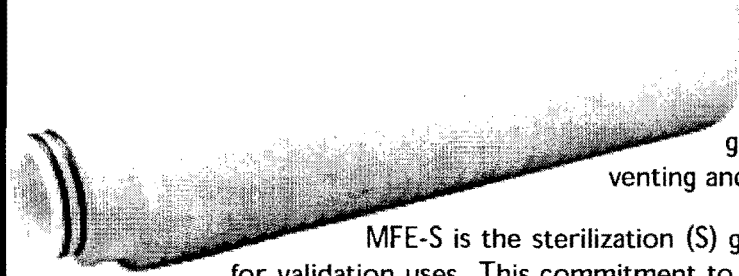
**OSMONICS**

5951 Clearwater Drive, Minnetonka, MN 55343-8995 USA  
Phone (612) 933-2277, Fax (612) 933-0141  
<http://www.osmonics.com>

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# MEMTREX™-FE-S FILTERS

PTFE MEMBRANE  
(0.2 µm ABSOLUTE RATING)



Memtrex-FE-S (MFE-S) validatable sterilizing grade filter cartridges are designed for sterile venting and final filtration of pharmaceutical fluids.

MFE-S is the sterilization (S) grade MFE for validation uses. This commitment to validation by Osmonics is based upon the FDA Guidelines that we "establish documented evidence of assurance that a specific process will consistently produce a product meeting its predetermined specification and quality attributes" (FDA 1987). MFE-S is designed for final sterile filtration of pharmaceutical products.

MFE-S is designed for vent and process fluid filtration in validated systems where critical fluids need absolute, aseptic and sterile hydrophobic or chemically resistant protection.

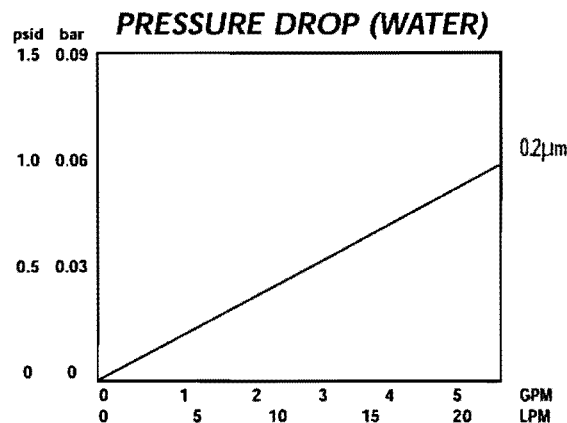
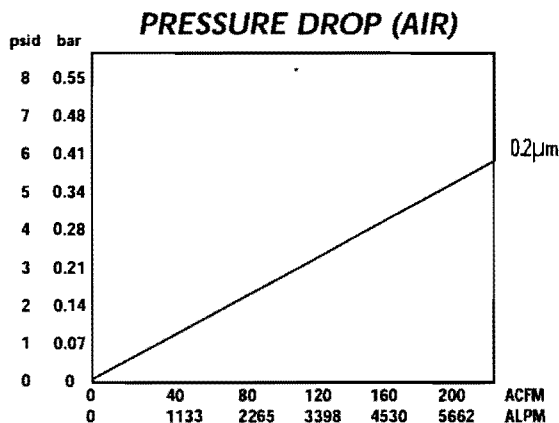
A detailed Validation Guide is available from Osmonics to document our rigorous testing for your records and reviews.

The MFE-S filter is just one example of our strong commitment to the pharmaceutical industry. Our complete portfolio includes filters for every stage of processing, and we offer custom solutions for your unique applications. Osmonics is your complete source for filters, housings and other filtration equipment.

\* Gore is a registered trademark of W.L. Gore & Associates, Inc.

## MFE-S ADVANTAGES

- Sterilization (S) grade MFE for validation uses
- High-purity Gore\* membrane
- High throughput
- Meets HIMA guidelines
- 100% integrity tested
- Steam sterilizable
- Meets USP 23 Class VI Plastic Test
- Passes MEM Elution Cytotoxicity tested



ACFM = Actual Cubic Feet per Min.

ACFM = SCFM at 70°F and 14.7 psia

Note: For pressures and temperatures other than 14.7 psia (0 psig) and 70°F, ACFM can be approximated with the following formula:  $ACFM = SCFM \left[ \frac{14.7 \text{ psia}}{(psig + 14.7)} \right] \left[ \frac{°F + 460}{530} \right]$

## SPECIFICATIONS

### Effective Filtration Area for 10-inch Equivalent

0.2µm - 9.0 ft<sup>2</sup> (0.9 m<sup>2</sup>)

### Operational Data

Maximum Rated Differential Pressure -

Forward flow - 60 psi (4.14 bar)

Reverse flow - 30 psi (2.07 bar)

Maximum Rated Operating Temperature -

180°F (82°C) at 10 pisd

(0.69 bar) in water

### Integrity Test

#### Diffusional Flow 60% IPA per 10-inch Element

0.2µm - 10 cc/min at 13 psig (0.90 bar)

### Sterilization

Autoclave at 250°F (121°C) -

Maximum 10 hours

Steam-in-Place at 257°F (125°C) -

Maximum 10 hours

### Biosafety

Passes USP 23 Class VI 250°F (121°C)

Plastics test passes MEM Elution

Cytotoxicity Test

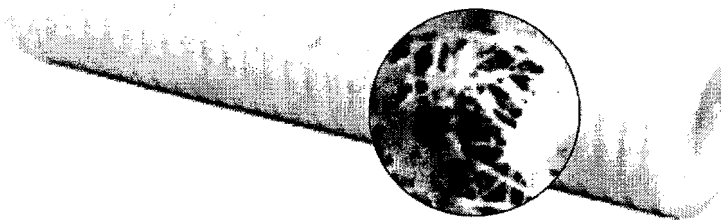
### Gravimetric Extractables

7.7 mg per 10 inch cartridge

### Bacterial Endotoxin

Leachate is below 0.25 EU/mL which meets

the USP 23 limit for Water For Injection (WFI)



PTFE Membrane

## APPLICATIONS

Memtrex-FE-S filters are specifically designed for chemical, air, gas and vent filtration. Typical applications include:

### Pharmaceutical Vailidation

- Ultra high purity
- Broad chemical compatibility

### Vent Filtration in Pharmaceutical Vailidated Process

### Acid, Base, and Oxidant Filtration

## ORDERING INFORMATION

Type	Absolute Micron Rating	Cartridge Length	End #1 Adapter	End #2 Adapter	Elastomer Material	Grade
MFE	92 = 0.2µm	1 = 10 Inch 2 = 20 Inch 3 = 30 Inch 4 = 40 Inch	Q = 222 O-Ring Stainless Steel Support Ring Z = 226 O-Ring Stainless Steel Support Ring	A = Open End Gasket B = 120 O-Ring C = 213 O-Ring G = Closed End Cap H = Fin Adapter	S = Silicone	S = Sterilizing Pharmaceutical

For more information call toll free in the USA (800) 848-1750

Manufactured in the USA



OSMONICS

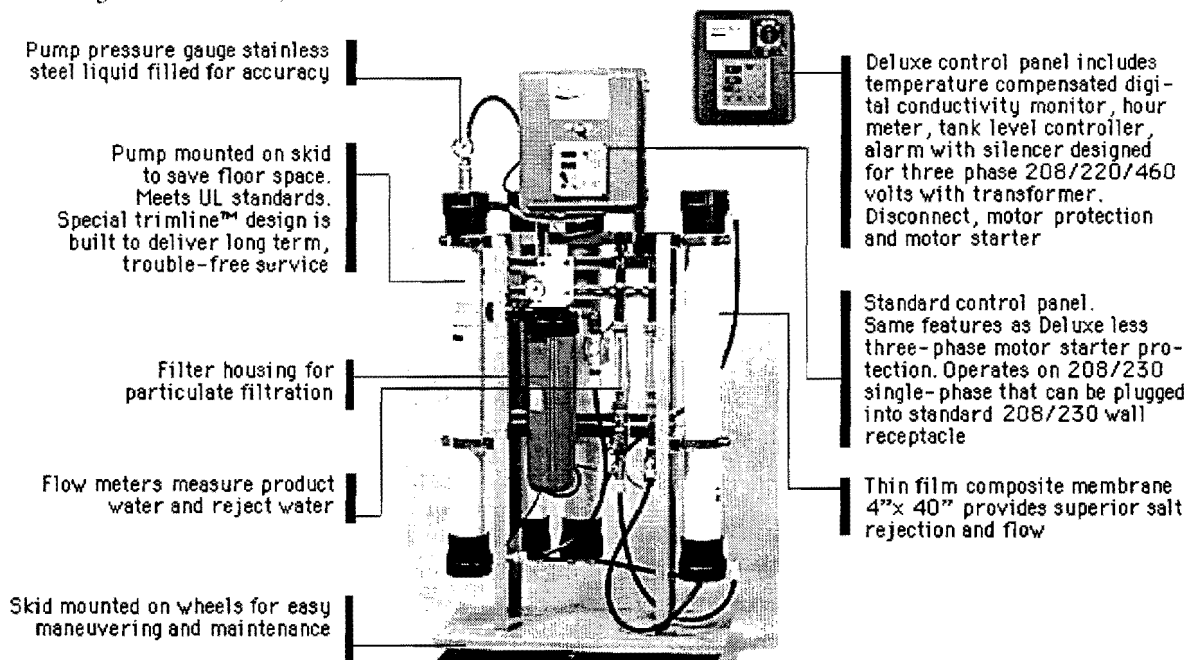
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## RO Systems from AmeriWater deliver versatility and value

### IRO open skid style RO system

Sizes range from 2,000-15,000 GPD



### IRO Model Specifications

Model:	IRO1 S/D	IRO2 S/D	IRO3 S/D	IRO4 S/D	IRO5 S/D	IRO6 S/D
<b>Performance:</b>						
Projected Rejection	>96%	>96%	>96%	>96%	>96%	>96%
Initial Production (GPD)*	2300	4600	6900	9200	11500	13800
Product Flow (GPM)*	1.5	3	4.5	6	7.5	9
Recovery Rate	50-75 %	50-75 %	50-75 %	50-75 %	50-75 %	50-75 %
<b>Electrical - NOTE: The only difference between models S and D is the control panel and Electrical specs</b>						
<b>All "S" models</b>						
Single Phase**	220V 13.5 Maximum Amps					
Pump Horse Power	1.5					
<b>All "D" models</b>						
Three Phase**	220V 7.5 Maximum Amps					
Pump Horse Power	1.5					
<b>Membranes:</b>						
Size (inches)	4 x40	4 x 40	4 x40	4 x40	4 x40	4 x 40
Quantity	1	2	3	4	5	6
Type	Thin Film Composite	Thin Film Composite	Thin Film Composite	Thin Film Composite	Thin Film Composite	Thin Film Composite
Feed Water	IRO1 S/D	IRO2 S/D	IRO3 S/D	IRO4 S/D	IRO5 S/D	IRO6 S/D

RO

Requirements	IKO1 S/D	IKO2 S/D	IKO3 S/D	IKO4 S/D	IKO5 S/D	IKO6 S/D
Max Free Chlorine	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Temperature Range	33-113 degrees F (1-45 degrees C)					
Operating pH Range	4-11	4-11	4-11	4-11	4-11	4-11
Cleaning pH Range	2.0 - 11.5	2.0 - 11.5	2.0 - 11.5	2.0 - 11.5	2.0 - 11.5	2.0 - 11.5
Prefiltration	5 micron	5 micron	5 micron	5 micron	5 micron	5 micron
Pressure Feed Range (PSIG)	20-80	20-80	20-80	20-80	20-80	20-80
Max Fouling Index (SDI)	<3	<3	<3	<3	<3	<3
Langelier Saturation Index	<0	<0	<0	<0	<0	<0
<b>Dimensions</b>						
Connections	Inlet/Outlet/Drain 1" FPT					
H x W x D (inches)	72 x 24 x 35					
Approx Shipping Weight (lbs)	492	512	542	567	592	622

\* Product flow rate varies with temperature. All model capacities are rated at 77 degrees F (25 C) with feed water of 1500 mg/L NaCl @ 200 PSI and pH of 7.5

\*\* 208V and 440V are also available

### Here are other AmeriWater RO systems:

#### **CRO cabinet style RO system**

for applications requiring from 300-2,000 GPD

#### **Custom designed systems**

for applications larger than 15,000 GPD

**AMERIWATER REVERSE OSMOSIS SYSTEMS ARE EASY TO MAINTAIN**



225 Fellowship Road  
P.O. Box 751  
Eagle, PA 19120  
(610) 458-1000  
(610) 458-9333  
(610) 471-1260 Fax

April 11, 2000

Kasadit Nootanq  
3900 Chestnut Street  
Apt. 409  
Philadelphia, PA 19104

RE: Research Wastewater

Dear Kasadit:

As per your preliminary specifications, EarthCare is pleased to submit this preliminary quotation for the transportation and disposal of the wastewater at University of Pennsylvania. Material will be transported to Gloucester County WWTP for treatment and disposal.

<b>Transportation and Disposal</b>	<b>Budget</b>	<b>\$0.16 to \$0.21/gallon</b>
		<b>6000 gal. minimum</b>
PH 5.5 to 9    TSS less than 1%	BOD less than 10,000 ppm	

One hour loading and 1/2 hour unloading is free; \$23.15 per quarter hour demurrage will be charged thereafter. It will be the responsibility of the Customer to pay for any tank clean-outs (if necessary). If a tank clean-out is required, then the cost plus demurrage will be charged.

**All pricing based on facility acceptance.** If this preliminary proposal is acceptable, we would require a sample and analysis of the waste (if not already provided). Upon review of the sample, a final proposal will be issued provided the material is acceptable. Should the material arrive at the disposal facility not meeting the above specifications, the waste will face possible rejection or surcharges.

This preliminary pricing is in effect for thirty (30) days from the date of this correspondence. Rates do not include any state, federal, or local taxes or fees, which may be imposed.

We appreciate this opportunity to assist in your environmental concerns. Upon agreeing to these terms and conditions, we ask for your acknowledgement in the below area provided. Should you have any questions regarding this transportation and disposal package, please feel free to call me at (610) 458-9333. I look forward to servicing your needs in the near future.

Very truly yours,

*Kathleen B. Roegner*  
Kathleen B. Roegner

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