Cover Letter

Dear Dr. Ramasamy

We, at Anti Biologics, would like to thank you for your interest. This letter is to introduce you to our cost effective, cutting edge product used to prevent patients from rejecting transplanted kidneys, a procedure over 35,000 people undergo each year. This product is monoclonal antibodies, whose generic name is Olizumab, that are developed from murine myeloma cells and immunized C5 B cells that fuse in the process to form hybridomas capable of producing thousands of immunoglobulins within seconds. This process is far more cost effective than rival processes due to its conservative use of machinery and cell cultures; where other companies would produce on massive scales, our use of small bioreactors and tangential flow perfusion systems ensures optimal growth and little error. With reactions that have a 96% conversion rate, the antibodies are produced quickly and skillfully.

Additionally, due to the small scale and advanced technology, costs are minimized; only 11 cells are needed per culture to produce 100 g of mAbs, a number that amounts to over 300 doses in a single day. The process uses easily available materials, such as murine myeloma cells and polyethylene glycol, materials that cost less than $1,000 per day. Once the shelf life of a year is factored in, the profit margin for these antibodies is dramatically increased.

Because our company, Anti Biologics, operates on such a fine-tuned basis, quality, upkeep, and competitive prices are at the forefront of our business strategy. We seek to help patients first, and with our technology, their lives can be improved, even lengthened, for years to come. Our biochemical processes ensure that patients will not reject the kidneys they have waited years and undergone extensive procedures for. We understand patients are often buried under bills, and we do not wish to contribute to this burden.

With such high demand, large profit margins, low prices, and newly developed technology, our company tackles an issue that no other medical procedure can. Thus, we hope to gain your support as an investor in our powerful monoclonal antibodies and as an investor in us.

Thank you again for your consideration,
Anti Biologics

Tasks by each member:

Cover Letter: Jordan Bragg
Introduction to the Project: Conner Tate
Product Information and Background: Jordan Bragg
Problem Definition with QFD: Liam Kozma
Project Planning: Jessica Shiffman
Process Description: Jordan Bragg
Design Basis Worksheet: Jordan Bragg
Process Flow Diagram: Liam Kozma
Detailed Description of Units of Operations: Conner Tate
Process Calculations for 3 major Units: Jessica Shiffman
Health Safety and Environmental Concerns: Liam Kozma
Cost Estimate for the Process: Conner Tate
Economic Analysis of the Project: Conner Tate
Marketing and Business Partnership Strategy: Jordan Bragg
Conclusions: Jordan Bragg

References: Jordan Bragg
Appendices: Team

Introduction to Project

Founded in 2014, Anti Biologics is a biotechnology company that specializes in monoclonal antibody production for kidney transplant rejection. We are based out of Athens, GA and we have a production volume of 36.5 kg of mAb product annually.

Monoclonal antibodies are used to treat a variety of diseases, including antibody-mediated rejection of transplanted kidneys, and our role is to supply these antibodies to companies who need them. Anti Biologics is passionate about the health industry and will do what is needed to deliver a quality product to the patients. To achieve this goal, our antibodies must be produced at large quantities.

Our facility will produce 100 grams of monoclonal antibodies per day using murine myeloma cells with C5 immunized B cells in a batch-fed/continuous process. Mice myeloma cells are cultured in small, stirred tank bioreactors, at standard culture conditions of pH, temperature, and oxygen saturation in a serum-free medium. Before this, the pre-cultures of the cells are grown in shake flasks. In the suspended cells, there is usually cell-free filtrate which must be separated. This is done by a tangential flow filtration perfusion system for cell retention and the cell-free filtrate is disposed of as waste. This filtrate is mixed with polyethylene glycol and a phosphate buffer solution and sent to an extraction column to separate the filtrate into a light phase and a heavy phase. The antibodies are dispersed throughout the light phase, and it must be separated further using filtration and chromatography purification. Once the antibodies are separated from the homogeneous filtrate, the liquid antibodies are solidified further, lyophilized, and sent for packaging.

From just the reactants, it costs $20 to produce a single gram of mAb's, which is $2,000 a day. There are various initial costs like our equipment which includes: glass spinner flasks, magnetic stirrers, 5L bioreactor, liquid addition bottles, biological safety cabinet, incubator, microcentrifuge, and a chromatography machine. The profit potential for this product is massive. 1 gram of antibodies sells for $800, so $80,000 is made per day, which is $78,000 of profit per day, and $28,470,000 profit per year. There is one other company, Soliris, that is our competition.

Product Information and Background

The product is freeze-dried monoclonal antibodies (mAbs) used to treat paroxysmal nocturnal haemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), and, the primary focus of this drug, antibody-mediated rejection of transplanted kidneys.[1] Monoclonal antibodies are proteins that are genetically identical to one another and function in the host immune system by allowing the host to recognize specific antigens. The drug, whose generic name is Olizumab, is named according to mAb nomenclature, and acts on the patient's immune system. The mAbs function by recognizing the C5 protein and preventing its cleavage, thus preventing the C5 cascade that facilitates the formation of the membrane attack complex which keeps the patient's immune system from attacking the donor cells.[2]

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[1] *Eculizumab Overview*. Eculizumab Overview - Creative Biolabs. (n.d.). Retrieved December 2, 2021.

[2] Bayly-Jones, C., Bubeck, D., & Dunstone, M. A. (2017, June 19). *The mystery behind Membrane Insertion: A review of the complement membrane attack complex*. Philosophical Transactions of the Royal Society B: Biological Sciences.

The mAbs are manufactured by using a cell line, in this case murine myeloma cells, and a specific B cell from mice immunized to the C5 protein. These cells are prepared using standard conditions, 1 atmosphere and room temperature, in shake flasks within serum, SFM4CHO, in the pre-culture process. These pre-culture procedures will be done in a batch process to ensure the freshest and purest cell lines, as well as ensuring no cells go to waste by leaving them unattended for an excess of time. Murine myeloma cell lines will be purchased and used in order to increase the speed at which the cells culture, and get rid of the process of extracting cells from mice. These cell lines also culture better within the medium than typical murine cells, increasing production as well. This is due to the fact that the myeloma cell lines are secreting cancer cells that have ramped production due to their uncontrolled growth. They are currently used in a variety of antibody therapies across the globe, and have been tested extensively to ensure they work well for human therapies. Additionally, murine cells are used since they are less expensive than other traditional methods, they require less genetic engineering than other methods, and they are easily available for purchase from multiple manufacturers.

Following this pre-culture process, the pre-cultures, containing both myeloma and B cells, will enter into a 5 L bioreactor where they will culture continuously for about 6 days. The cells will then pass on to the tangential flow perfusion system which keeps the cells in their growth phase for an extended period of time, and this system is beneficial to the process because the cells will continue to grow at an exponential rate for longer than they would if the tangential flow perfusion system were not used.[3] Traditionally, mAbs are manufactured using larger bioreactors, but use of smaller, 5 L bioreactors will ensure that quality is maintained at a higher standard. It also decreases the likelihood that excess cells will go to waste in case of a factory shutdown or in case of an error in processing.

Following the culturing process occurring in the tangential flow perfusion system, the cells will then be mixed with a solution of polyethylene glycol (PEG) and phosphate buffer solution. This mixture is used to form the final cell structure of a hybridoma, a fused form of the myeloma cells and the B cells, which only occurs in PEG because otherwise the cell membranes cannot fuse.[4] Once hybridomas, the cells are further mixed and the antibodies are extracted in an extraction column. This allows the light phase, which contains the mAbs, to be separated from the heavy phase, which contains excess cell features that weigh significantly more than the antibodies. The mAbs in solution can either be stored following this process, or they will travel to the microfiltration and ultrafiltration system where any remaining cell parts that are larger than 0.2 microns in size will be removed from the system as waste.

After the phases are separated, the light phase proceeds to an ion exchange chromatography exchanger where the negatively charged antibodies in solution are separated from the PEG and buffer solution due to their difference in charge. This ion exchange is followed up by a process involving hydrophobic interaction chromatography. This fine tunes the solution to ensure all excess buffer solution has been completely removed from the antibody solution. Last, these liquid antibodies are freeze dried, and they can either be stored for up to a year at about 2 degrees celsius, or they will be packaged and transported if purchased.

Customers should purchase from the company, Anti Biologics, because it uses the highly specific aforementioned process, and Anti Biologics is dedicated to the well-being of patients, use of quality materials, and a higher standard of production. Over 35,000 people in 2021 alone received kidney transplants, and each and every one required some sort of immune system

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[3] *Perfusion culture with ATF cell retention*. Cytiva. (n.d.). Retrieved December 2, 2021.
[4] *Monoclonal antibody production*. Molecular Devices. (n.d.). Retrieved December 2, 2021.

treatment in order to prevent rejection.[5] It is one of few medications on the market that is able to safely supply patients with antibodies that can recognize the donor's antibodies and prevent the patient from rejecting the transplanted organ. Thus, the product has high demand and necessity in the present day, and the need for these antibodies is unlikely to decline as there is no modern medicine that can currently replace the use of kidney transplants. Additionally, the mAbs allow for better donor matching and increase the likelihood that the surgery will be successful, as well as increasing the longevity of the organ itself. This product is administered noninvasively before and after the surgery via intravenous injections of mAbs in solution; it does not require demanding drug regimens nor prolonged use after the surgery. This is a direct result of the mAbs becoming incorporated into the body and preventing immune system activation by donor cells.

The process of producing the mAbs is incredibly conservative, as stated above; the process provides little valuable waste products, since the buffer and phosphate solutions do not leave as pure, and it lacks the potential for recycling the waste products back into the process stream since they contain components irrelevant to the process. These waste products are of little use since they would require further refining processes to be recycled, and these costs far outweigh the benefits. Thus, the byproducts are sent off as waste.

Problem Definition and QFD

Our goal of this project is to design a biochemical facility to produce 100 grams per day of monoclonal antibodies developed from murine myeloma cells and immunized C5 B cells from mice. This production must be designed upholding safe and ethical concerns, as well as following all regulatory laws. Our goal at Anti Biologics is to produce these antibodies continuously while beating our competitors' cost by at least 10% all within 18 months. The antibodies will also have a shelf life of at least 1 year.

Objectives:

- Beat competitor's cost by 10%
- Large-scale production process
- Produce 100g/day
- Continuous Process

Constraints:

- Follow all regulations
- Begin production in at least 18 months
- Shelf life for at least a year

[5] *Organ transplant trends: More transplants than ever*. UNOS. (2021, August 6). Retrieved December 2, 2021.

Project:	Olizumab
Date:	Dec. 9, 2021

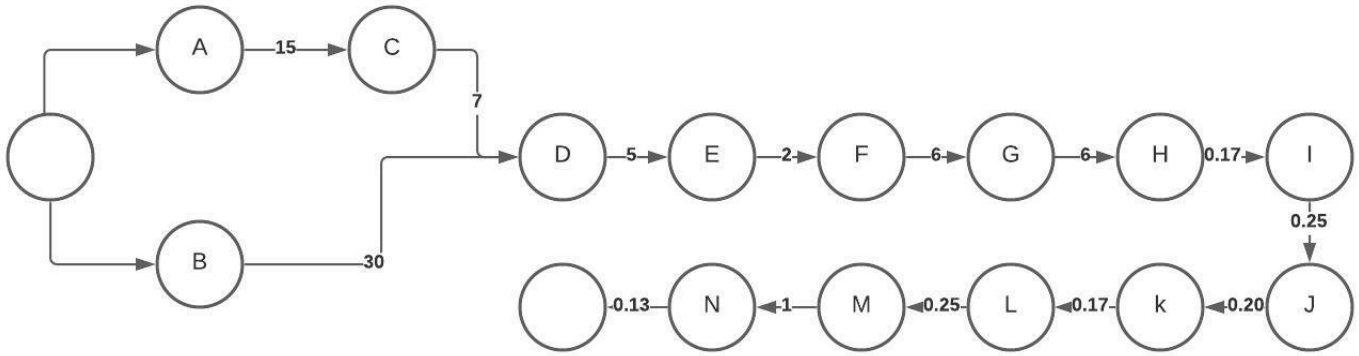
					+					Correlations	
				+	+	-					
			+	+	+	-	+			Positive	+
			Functional Requirement							Negative	-
		Direction of Improvement	▲	□	▲	▲	□	Comp.Ass		No Correlation	
Relative Weight	Customer Importance	Customer Requirements	Conservative Supply Chain	Media Prep	Sterile Filtration	Cold Product Storage	Safe Waste Disposal	Our Product	Soliris®	Direction of Improv.	
7%	5	Low Infection Rate	▽	▽	●	○	▽	9	9	Maximize	▲
13%	9	Continuous Production	●	○	○	●	○	9	NA		
10%	7	Careful Dissection (quality)	●	●	●	●	▽	1	NA	Target	□
13%	9	100g/day mAb	●	○	▽	▽	▽	9	NA	Minimize	▼
4%	3	Cost-effectiveness	●	○	○	○	○	7	NA	**Relationships**	**Weight**
11%	8	Increase-life expectancy	○	○	●	▽	▽	9	9	Strong	● 9
6%	4	Shelf Life	▽	▽	●	●	▽	7	NA	Medium	○ 5
10%	7	Chain-Quality Independence	●	●	●	▽	○	6	NA	Weak	▽ 1
13%	9	Cost ≥ 10% of Next Competitor	○	○	○	○	○	9	0		
13%	9	Start in 28 Months	○	○	○	○	○	9	NA		
		Importance Rating Sum (Importance x Relationship)	.5714	.5714	.7142	.1428	.4285				
		Relative Weight	25%	20%	24%	18%	12%				
		Our Product	7	4	9	9	4				
		Competitor 1	NA	NA	NA	9	NA				
		Technical Competitive Assessment									

Project Planning

Construction Tasks	Activity Notation	Duration (days)	Preceded by
Order parts of machinery	A	15	
Order mouse myeloma cells	B	30	
Construct set up	C	7	A
Test system	D	5	B, C
Adjust System	E	2	D
Upstream Tasks[6]		Duration (days)	
Preculture	F	6	E
Culture in bioreactor	G	6	F
Downstream Tasks		Duration (hours)	
Tangential flow filtration	H	4	G
Polyethylene glycol and phosphate buffer mix with cell-free filtrate	I	6	H
Light and Heavy phase separate in extractor column	J	5	I
Micro and ultrafiltration process with centrifugation	K	4	J
Chromatography purification with the light phase	L	6	K
Lyophilization	M	24	L

[6] *Flow chart: Mab Upstream Process - biomanufacturing*. Northeast Biomanufacturing Center. (n.d.). Retrieved December 6, 2021.

Packaging	N	3	M



Production of Monocolonal Antiobodies

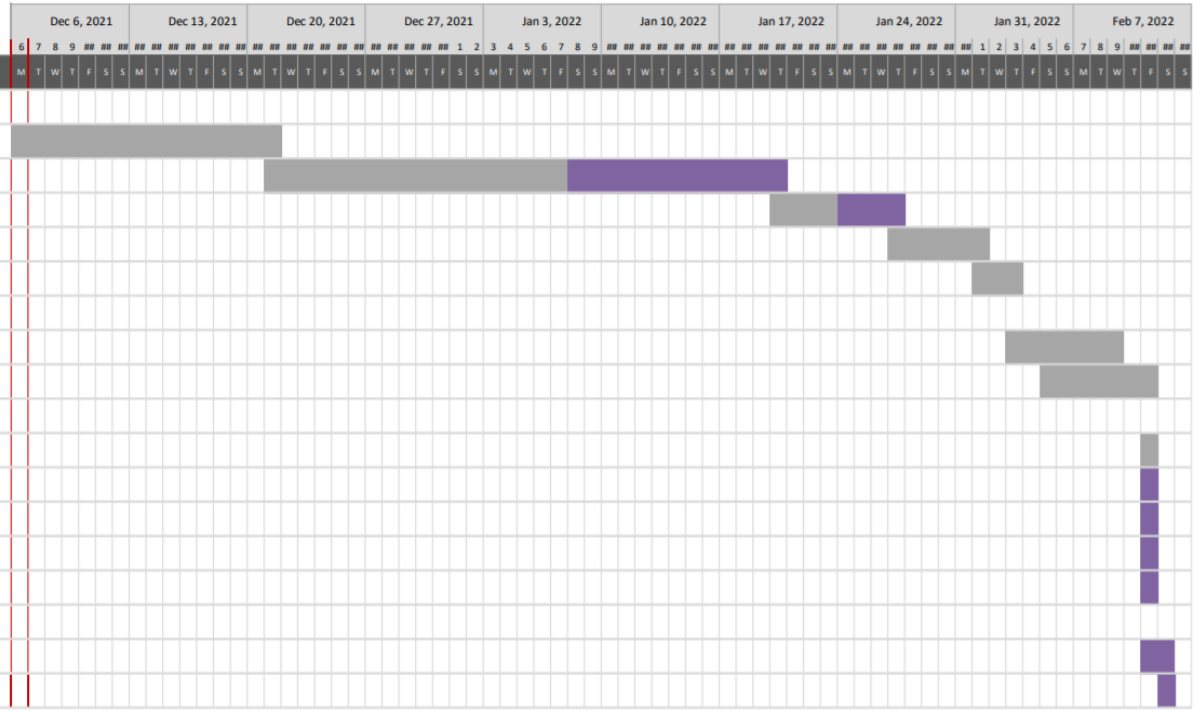
SIMPLE GANTT CHART template by Vertex42.com
https://www.vertex42.com/ExcelTemplates/simple-gantt-chart.html

Company Name Antibiologics

Project Start: Mon, 12/6/2021

Display Week: 1

TASK	ASSIGNED TO	PROGRESS	START	END
Constuction and Planning				
Task 1	Ordering fermentors and other parts	100%	12/6/21	12/21/21
Task 2	Order mouse myeloma cells	60%	12/21/21	1/20/22
Task 3	Construct set up	50%	1/20/22	1/27/22
Task 4	Test system without cells	100%	1/27/22	2/1/22
Task 5	Udjust system	100%	2/1/22	2/3/22
Upstream				
Task 1	Preculture	100%	2/3/22	2/9/22
Task 2	Culture in bioreactor	100%	2/5/22	2/11/22
Downstream				
Task 1	Tangential flow filtration	100%	2/11/22	2/11/22
Task 2	C2H6O2 and buffer solution mixture	100%	2/11/22	2/11/22
Task 3	Extractor column	100%	2/11/22	2/11/22
Task 4	Light phase ultrafiltration	100%	2/11/22	2/11/22
Task 5	Chromatographypurification	100%	2/11/22	2/11/22
Packaging				
Task 1	Luphilization	100%	2/11/22	2/12/22
Task 2	Package	100%	2/12/22	2/12/22



Process Description

The process of creating mAbs has only one reaction, and the remainder of the processes are physical reactions such as mixing or separating. Thus the basis of the reaction was determined using stoichiometry and the assumption that production will be based on the minimum amount of reactants needed. Production capacity could be increased significantly with ease, but due to the desire to have only 100 g of finalized product per day, the following calculations utilize the minimum quantities of reactants to achieve this. The reactants of the process are murine myeloma cells and immunized B cells which fuse to create hybridomas. These hybridomas are then purified to eventually reach the final product of mAbs. Thus the total reaction is $Cells_{murine}$+ $Cells_B$ ⇒ mAbs with a stoichiometric ratio of 1:1:1. Additionally, the PEG and phosphate buffer solutions are added to the process, yet subsequently removed in the same chemical formation as they began; no reaction occurs with the PEG and phosphate buffer solution.

The basis of the reaction is 11 cells total. The following statistics are necessary to perform the calculations:

- 1 monoclonal antibody weighs 150kDa (1.66E-21g)[7]
- 1 cell weighs 27E-12 g[8]
- The culturing process takes 6 days
- 1 cell can create 10,000 mAbs in 1 second[9]
- 1 murine myeloma cell can undergo 40 rounds of division[10]
- 1 antigen weighs about 8 kDa (1.328E-20g)[11]

Thus the following calculations were performed:

$100\ g\ mAbs\ \times \frac{1\ mAbs}{1.660*10^{-21}\ g\ mAbs} = 6.024\ *\ 10^{22}$ mAbs needed

$6\ days\ \times \frac{24\ hours}{1\ day} \times \frac{3600\ sec}{1\ hour} \times \frac{10{,}000\ mAbs}{1\ sec} = 5.184\ *\ 10^{9}$ mAbs produced by 1 cell in 6 days

$6.024\ *\ 10^{22}\ mAbs \times \frac{1\ cell}{5.184*10^{9}\ mAbs} = 1.160\ *\ 10^{13}$ cells needed at the end of 6 days

$(2\ cells)^{40\ rounds} = 1.099\ *\ 10^{12}$ cells after 40 rounds

$1.160\ *\ 10^{13}\ cells\ after\ 6\ days\ \times \frac{1\ cell\ before\ division}{1.099*10^{12}\ cells\ after\ division} = 10.57$ cells needed

In order to simplify the aforementioned process 10.57 was rounded to 11 cells needed in the pre-culture shake flasks with some expected excess. It is also important to note that the assumed reaction that occurs prior to pre-culture for these calculations is 1 cell + 1 antigen ⇒ 10,000 mAbs.

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[7] *Antibody Basics*. Sigma Aldrich. (n.d.). Retrieved December 7, 2021.

[8] American Physical Society. (2012, September 13). How much does a cell weigh? Physics. Retrieved December 7, 2021.

[9] Ghose, T. (2020, July 17). What are antibodies? LiveScience. Retrieved December 7, 2021.

[10] Khan Academy. (n.d.). *Cancer and the cell cycle | biology (article)*. Khan Academy. Retrieved December 7, 2021.

[11] *Antigens*. Abcam. (2021, November 24). Retrieved December 7, 2021.

This reaction was used to address the question of limiting reactants instead of the overall system reaction of $Cells_{murine}$+ $Cells_B \Rightarrow$ mAbs. This is because the B cells without the antigen are useless, thus calculating the yield, extent of the reaction, and other characteristics of the system using that reaction would ultimately be redundant despite immunizing the B cells not being a part of the biochemical process. A basis of 1 g Ag per 1 mL of solution was decided upon as well, as the antigen has very little mass and is easily obtained from manufacturers in this amount. Thus the following characteristics could be concluded using a basis of 11 antigens to correspond to the 11 cells found above:

$mass\ of\ Ag\ = 8\ kDa$

$8\ kDa \times \frac{1.660*10^{-24}\ g}{1\ kDa} = 1.328\ *\ 10^{-20} g\ Ag$

$11\ Ag\ \times 1.328\ *\ 10^{-20}\ g\ Ag\ = 1.461\ *\ 10^{-19} g\ Ag$ needed for the reaction

Thus, it can be concluded that the antigen is in excess and the cells are the limiting reactant since the cells are synthesizing the antibodies, and the amount of antibodies clearly depends on the cell's machinery. The yield based on feed of the reaction was found to be 31.9% by dividing 100 g of mAbs, the desired mass of product, by (1.16E13 cells)*(27E-12 g/cell) which is the mass of the limiting reactant fed. The ξ^{max}, or the extent of the reaction, was found to be 6.67E-4 by dividing 6.67E-4, which is the moles of mAbs reacted, by 1, which is the stoichiometric coefficient of the mAbs. The selectivity was found to be 9.344E20 which was found by dividing ξ^{max} by 7.138E-25 which is the moles of the undesired product produced; in this case the undesired product is excess antigen. Last, the conversion was found to be 96% which was found by dividing the actual mass of the limiting reactant that is reacted, (10.57 cells)*(27E12 g/cell), by the mass of the limiting reactant fed, (11 cells)*(27E12 g/cell). This percent conversion was purposeful since the process should be conservative in order to avoid excess costs, and the process is strictly maintained to ensure the conversion is high.

Unfortunately, because the process of synthesizing monoclonal antibodies is so strict, recycle streams are far more costly to include than simply disposing of the products as waste. This is primarily due to the fact that the useless cell components, the polyethylene glycol, the phosphate buffer solution, any materials removed from the chromatography machines, or other removed components would require filtration and purification prior to being recycled since they could have contaminants from the machines, or chemical moieties that can damage the mAbs. Thus, the aforementioned components are removed from the process in purge streams and are disposed of as hazardous waste materials.

The project comprises various unit operations that utilize both major and minor units. The pre-culture operation is the beginning with cells in shake flasks. This is followed by the bioreactor, where the cells culture; immediately following this process, the cells enter the tangential flow perfusion system, where they remain in their exponential growth phase until they enter the mixer. Once in the mixer, a mixture of PEG and phosphate buffer solution will weaken the cell membranes, allowing the myeloma cells and the B cells to fuse. Then, the cells will pass on to an extraction column where they will be separated. From here, the antibodies will be filtered in both an ultrafiltration and a microfiltration system to ensure any and all impurities are removed. Succeeding this process, the antibodies will pass onto the chromatography machines. The process is concluded by a freeze drying the antibodies in their liquid either for storage or for transportation..

The conditions for these processes typically mimic physiological conditions with a pH ranging from 7.3 to 7.4, a temperature of approximately 37℃, a pressure of 1 atm, all with the

presence of about 5% carbon dioxide.[12] This is all strictly maintained to ensure the environment does not deviate from biological conditions or else the cells' growth will be impaired.

Raw materials and feedstock will be acquired from chemical manufacturers, such as Sigma-Aldrich, who source their cells and equipment from trusted labs and producers. The materials must be handled in accordance with their material safety procedures which include, but are not limited to, proper storage, ventilation, and training. The product must be handled with care, and it must be transported immediately or stored after freeze drying, as the antibodies will denature if not at proper conditions for extended periods of time. Neither the antibodies, nor the materials used to synthesize them are particularly dangerous, however gloves, goggles, and face masks are enforced for all who handle these items directly in order to prevent skin contact. This is because burns, allergic reactions, dermatitis, or more can occur from contact with said chemicals.

Presently, there are few competitive processes for the synthesis of monoclonal antibodies, even less that target the C5 protein of the membrane attack complex. Competitors may use other variations of mammalian cells, such as rabbit cells, but murine cells have proven the most similar to humans, thus they provide the best machinery for the product. Additionally, murine cells tend to be less costly than other mammalian cells because they are so readily available. Otherwise, there are few competitive processes for this highly specific technology.

[12] Morgan, S., Campbell, L., Allison, V., Murray, A., & Spears, N. (2015, March 17). *Culture and co-culture of mouse ovaries and ovarian follicles*. Journal of visualized experiments : JoVE. Retrieved December 7, 2021.

Design Basis Worksheet

Anti Biologics
Address

DESIGN BASIS

Form 22315-33-21

Project Name: Olizumab Synthesis
Project Number
Sheet: 1

REV	DATE	BY	APVD	REV	DATE	BY	APVD

1 General Information

Owners Name	Jordan Bragg	
Process Unit Name	Anti Biologics	
Plant Location	Athens, GA	
Correspondance Contacts Address Telephone / Fax E-mail	Jordan Bragg 1355 Willow Street 770-321-0331 bragg.antibiologics@anti.com	

2 Measurement System

(●) English ( ) Metric

3 Equipment Numbering System

Equipment will be identified by alphabetic prefix as defined here, followed by three-digit serial number unless otherwise indicated

First digit - process section
Second & third digits - equipment count

AC	Air cooler	G	Grinder, mill	PRV	Pressure relief valve
B	Boiler	H	Heater (fired or electric)	R	Reactor
C	Compressor, blower, fan	J	Ejector, jet, turboexpander	SP	Sample point
CT	Cooling tower	M	Motor	T	Storage tank
D	Dryer	ME	Miscellaneous equipment	V	Vessel (including columns)
E	Exchanger	MX	Mixer		
F	Filter, classifier	P	Pump		

4 Primary Products

Product Name Product Grade	monoclonal antibodies 100%					
MSDS Form Number	1000-19900					
Production Rate Tons per year Tons per day Other units	100 g/day 0.04 1.10*10^-4					
Product Purity (wt%)	99.90%					
Product shipment mode	Freight					
Additional Specifications	Temperature-controlled (< 2°C), no light exposure, handle with care					

5 Primary Raw Materials

(Attach additional sheets if needed)

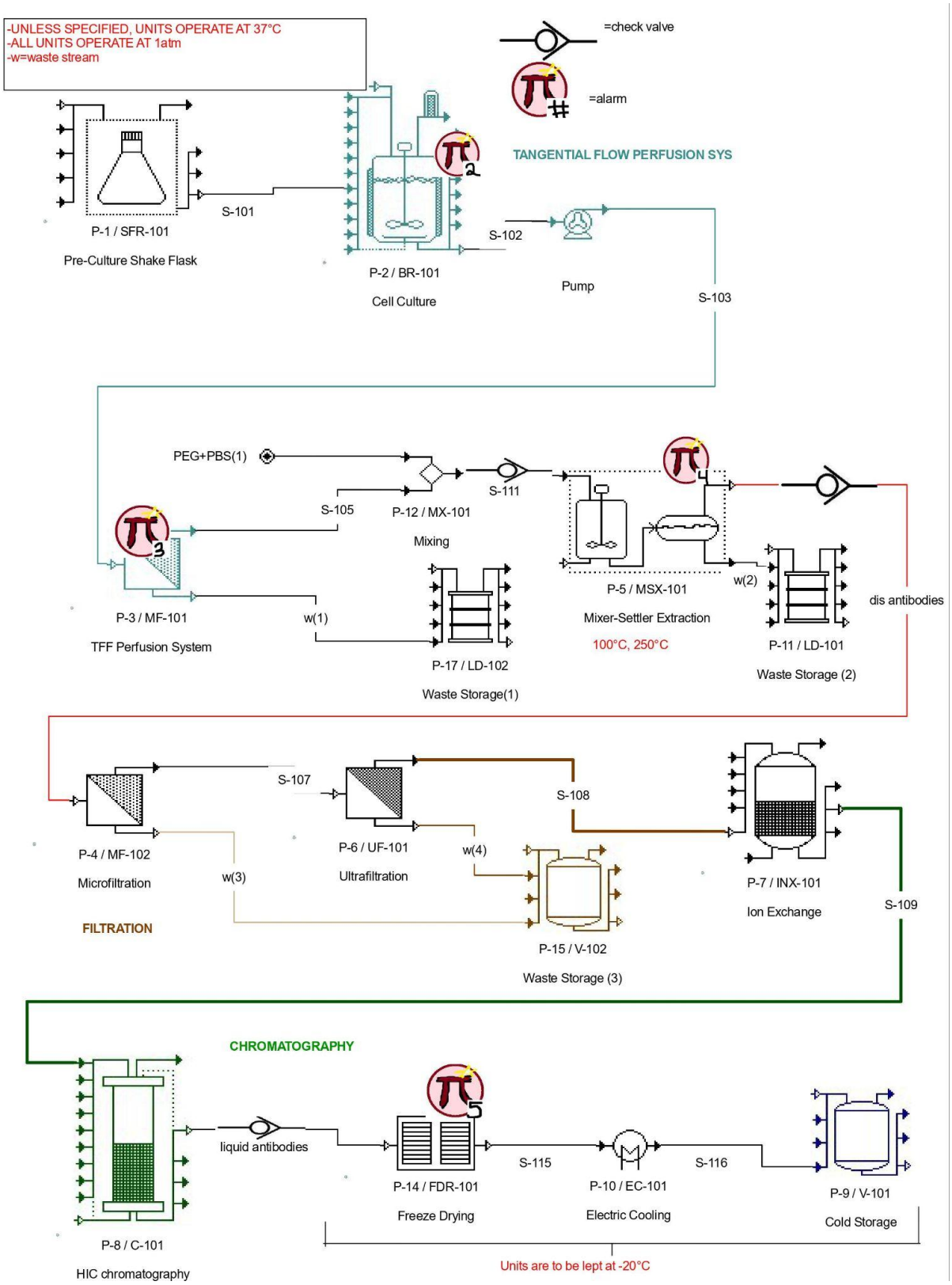
Feedstock name Feedstock grade	Murine Myeloma Cells		Buffer Solution					
MSDS form number								
Feedstock availability Tons per year Tons per day Other units	2.97E-10 g/day **1.19E-13** 3.26E-16		1 L/ day 1.29E-01 3.53E-04		2.97E-10 g/day 1.19E-13 3.26E-16			
Feedstock price ($/lb) (Default: open market price)	$150/ fl oz		$20/ L		$165/ fl oz			
Known feedstock impurities	Name	ppmw	Name	ppmw	Name	ppmw	Name	ppmw
	none		none		none			
Additional specifications								

6 Site Information

Low ambient temperature (F)	95
High ambient temperature (F)	99
Hight ambient relative humidity (%)	N/A
Site elevation (ft)	N/A
Maximum wind loading (mph)	N/A

Other site design requirements

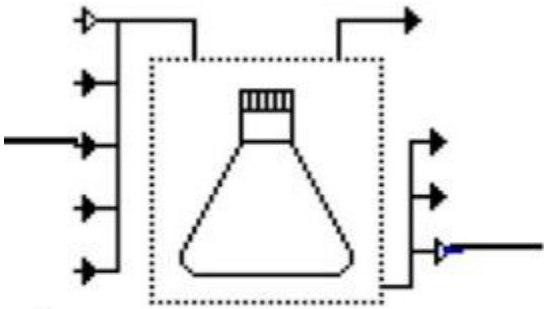
Process Flow Diagram


-UNLESS SPECIFIED, UNITS OPERATE AT 37°C
-ALL UNITS OPERATE AT 1atm
-w=waste stream
=check valve
=alarm
TANGENTIAL FLOW PERFUSION SYS
P-1 / SFR-101
Pre-Culture Shake Flask
S-101
P-2 / BR-101
Cell Culture
S-102
Pump
S-103
PEG+PBS(1)
S-105
P-12 / MX-101
Mixing
S-111
P-3 / MF-101
TFF Perfusion System
w(1)
P-17 / LD-102
Waste Storage(1)
P-5 / MSX-101
Mixer-Settler Extraction
100°C, 250°C
w(2)
P-11 / LD-101
Waste Storage (2)
dis antibodies
S-107
P-4 / MF-102
Microfiltration
w(3)
P-6 / UF-101
Ultrafiltration
w(4)
S-108
P-7 / INX-101
Ion Exchange
S-109
FILTRATION
P-15 / V-102
Waste Storage (3)
CHROMATOGRAPHY
liquid antibodies
P-14 / FDR-101
Freeze Drying
S-115
P-10 / EC-101
Electric Cooling
S-116
P-9 / V-101
Cold Storage
P-8 / C-101
HIC chromatography
Units are to be lept at -20°C

Detailed Description of Units Operation

Pre-culture:[13]

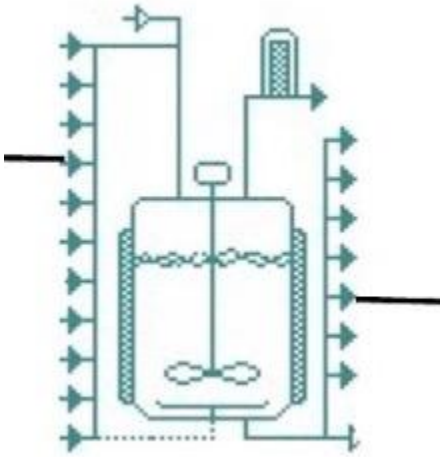
Prior to their culture in the bioreactor, cells go through a pre-culture in shake flasks. This helps to increase the rate of formation of the final product, which increases the yield of the final product. The pre-culture step will be done as a batch process, and fed into the bioreactor.. In the shake flask, the cells are suspended in a culture broth that prepares them for the bioreactor.



[13] Keil, T., Landenberger, M., Dittrich, B., Selzer, S., & Büchs, J. (2019, August 5). *Precultures grown under fed‐batch conditions increase the reliability and reproducibility of high‐throughput screening results*. Wiley Online Library. Retrieved December 8, 2021

5L Bioreactor:[14]

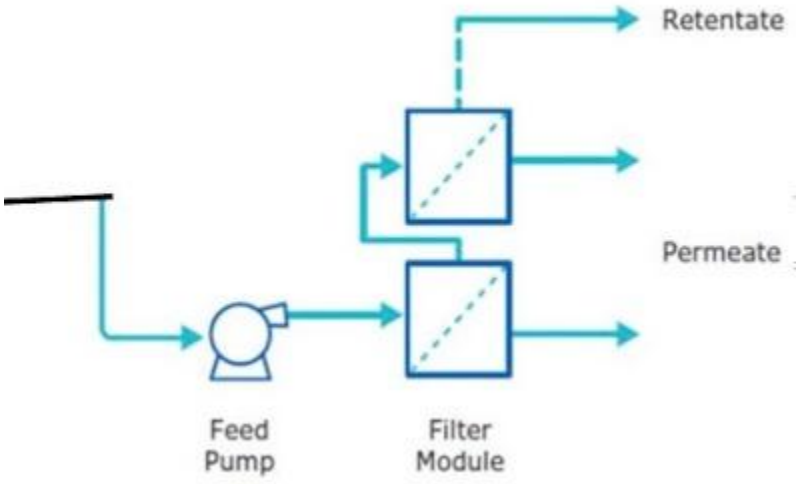
The overarching goal of a bioreactor is to grow certain types of cells under specific conditions. In this case, mice myeloma cells are cultured at a physiological pH of 7.3-7.4, a temperature of 37°C in a serum-free medium. The temperature and pH are critical to the process, as they control the efficiency of the reaction. Since myeloma cells are being used, oxygen does not need to be pumped into the reactor. The growth happens quickly, 6 days, because of the nature of the myeloma cells. Once inside the bioreactor, the reactants are constantly stirred in order to mix everything together. The pressure of the bioreactor is controlled very strictly in order to ensure safe and efficient mixing of our cells. With a stainless steel bioreactor, and without the need of oxygen gassing, we can maintain a high pressure in our bioreactor in order to speed up the process.



[14] Allman, T. (2020, September 4). *What is a bioreactor and how does it work?* EN Blog. Retrieved December 8, 2021

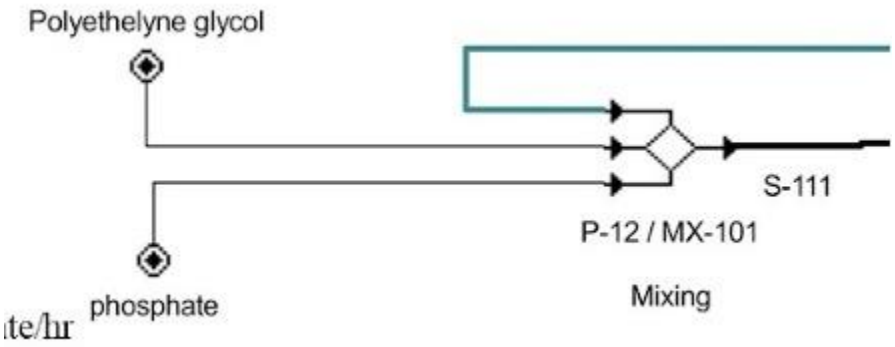
Tangential Flow Filtration Perfusion System:[15]

This process is used for cell retention to separate the cell-free filtrate from the suspended cells. To get these cells, the liquid broth from the bioreactor is pumped through a fibrous filtration (perfusion) which allows the liquid to pass through but stops the cells. The system is tangential rather than orthogonal in order to minimize filter fouling and increase the efficiency of the continuous system. Additionally, the system allows culturing cells to remain in their growth phase far longer than other flow systems, which contributes to the overall efficacy of the process by developing more cells in a shorter period of time.

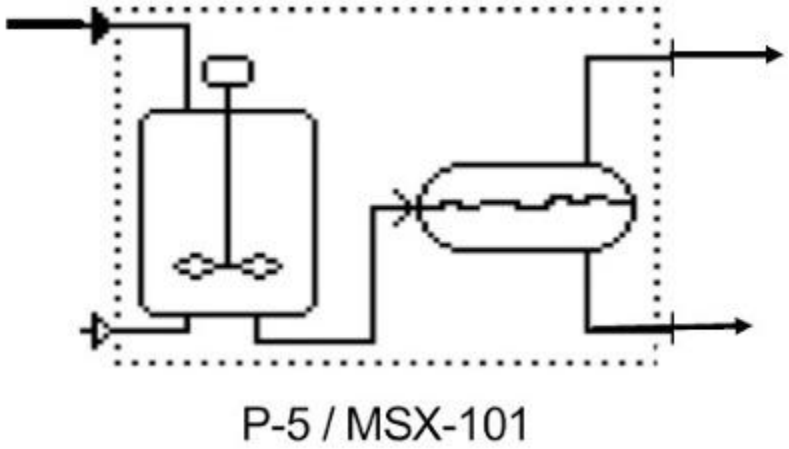


[15] *Tangential flow filtration (TFF) and alternating tangential flow filtration (ATF)*. Ebrary. (n.d.). Retrieved December 8, 2021

Mixer:[16]
The cells from the tangential flow filtration perfusion system will then enter the mixer, where they will be combined with a mixture of PEG and phosphate buffer solution. This weakens the membranes of the murine myeloma cells and the immunized B cells allowing them to fuse into their final, antibody-synthesizing hybridoma state. Once fused, the cells will pass on to extract the antibodies out of the hybridomas.



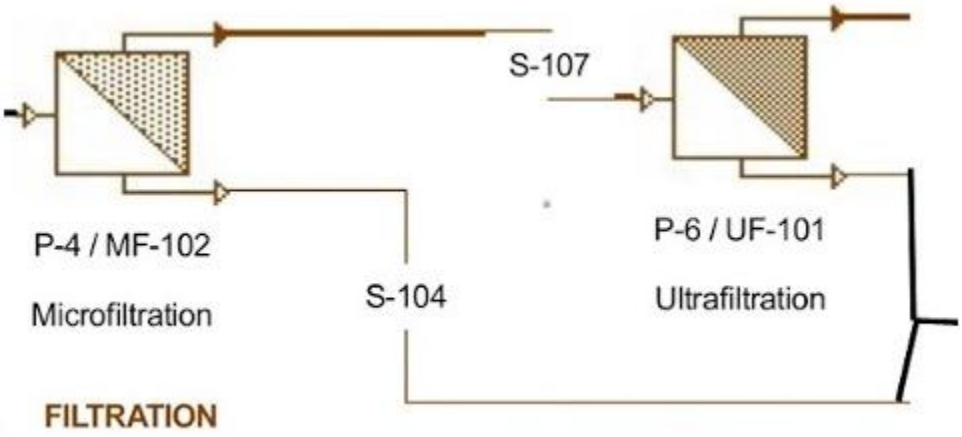
Extraction Column:[17]
The filtrate is sent through an extraction column to separate the light phase from the heavy phase. The light phase contains the dispersed antibodies, which will remain in the flask while the heavy phase is extracted. This works through differences in boiling points. The heavy phase has a lower boiling point than the light phase, so it will be boiled off while the antibodies remain in the flask.



[16] *Chemical Industry Mixers*. Dynamix Agitators Inc. (2020, November 19). Retrieved December 8, 2021
[17] *Solid liquid extraction*. Solid Liquid Extraction - an overview | ScienceDirect Topics. (n.d.). Retrieved December 8, 2021

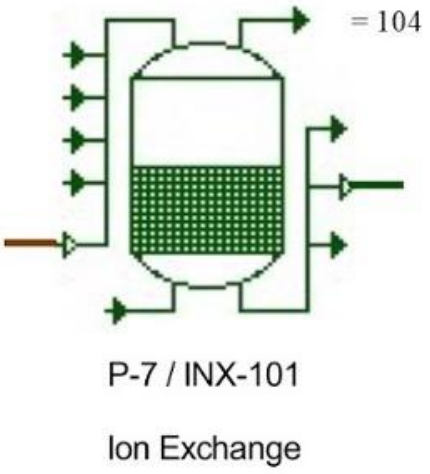
Micro and Ultrafiltration:[18]

The remaining light phase must go through a micro and ultrafiltration. A microfiltration is a membrane separation process that works to separate particles, in this case antibodies from a solution. It works by passing the solution through a porous membrane. Ultra-filtration is a similar membrane separation process where the filtrate passes through a semipermeable membrane that blocks anything larger than 0.2 microns.



Ion Exchange Chromatography:[19]

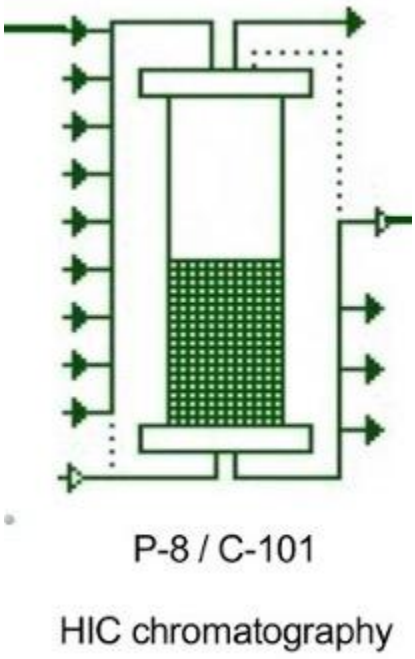
Ion exchange chromatography works to separate ions and molecules from a filtrate based on their ionization and affinity to the ion exchanger. mAb's have a net negative charge, so they will separate from the impurities.



[18] *Microfiltration systems by Met-Chem: Learn more about microfiltration*. Metchem. (2019, August 27). Retrieved December 8, 2021

[19] *Ion Exchange chromatography*. Bio. (n.d.). Retrieved December 8, 2021

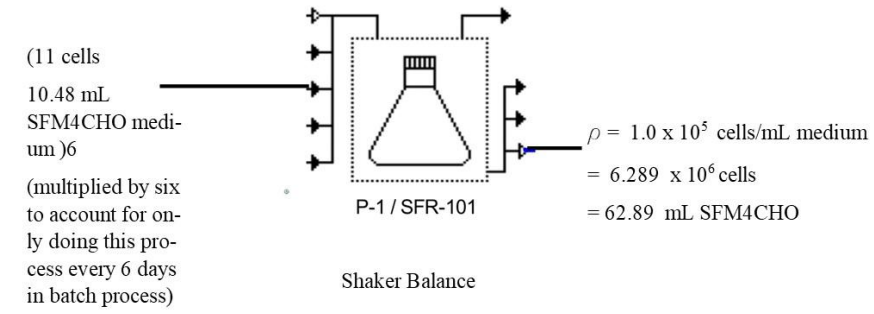
Hydrophobic Interaction Chromatography:[20]
Hydrophobic interaction chromatography separates molecules in a solution based on how hydrophobic they are. mAb's are very hydrophobic, which makes their separation from hydrophilic impurities. Through both chromatographies, the antibodies are separated from the filtrate and can undergo solidification and be lyophilized.



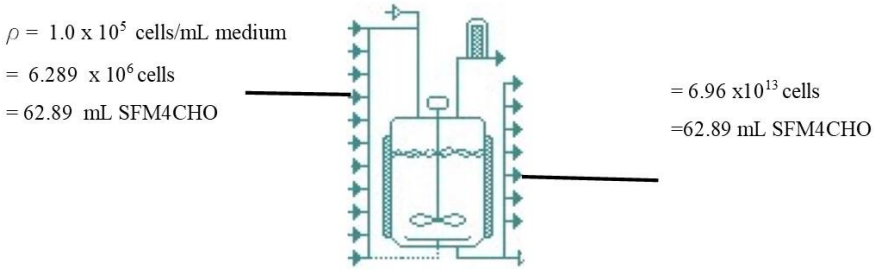
Lyophization:[21]
Long term storage of antibodies is integral to our process. To do this, the purified, liquid, antibodies are kept at 2 degrees celsius in a large scale freeze dryer. Antibodies enter the freeze dryer as a liquid. They freeze due to low temperatures, then the pressure is dropped, so that when reheated, any liquid in the antibodies immediately evaporates.

[20] *Hydrophobic interaction chromatography*. Hydrophobic Interaction Chromatography - an overview | ScienceDirect Topics. (n.d.). Retrieved December 8, 2021
[21] Johnson, M. (2021, May 23). *Antibody storage and antibody shelf life*. Materials and Methods. Retrieved December 8, 2021

Process Calculations



Shaker Balance



Bioreactor Balance

[22] Feng, Y., & Dimitrov, D. S. (2009). Scaling-up and production of therapeutic antibodies for preclinical studies. *Methods in molecular biology (Clifton, N.J.)*, *525*, 499–xiii.

[23] Petrides, D., Carmichael, D., Siletti, C., & Koulouris, A. (2014). Biopharmaceutical process optimization with simulation and scheduling tools. *Bioengineering*, *1*(4), 154–187.

= 6.96 x10^{13} cells

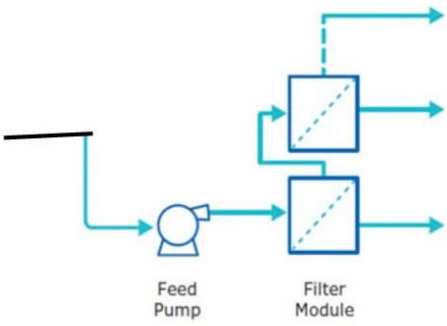
=62.89 mL
SFM4CHO

Now the process is continuous and send only

1.160x10^{13} cells/hr

10.48 mL
SPM4CHO

At 37 ℃



W= 0.96 Mab product

= 1.114x10^{13}cells/hr

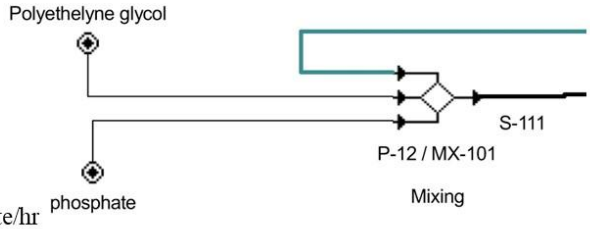
= 300.67 g cells/ hr

w= 0.04 Mab product

= 4.64x10^{11}cells/hr

Tangential Flow Filtration Balance

= 40 mL PEG/hr



=60 mL phosphate/hr

= 1.114x10^{13}cells/hr

= 300.67 g cells/ hr

= 300.67 g cells/ hr

= 110.48 mL/hr

= 40 mL PEG/hr

=60 mL phosphate/ hr

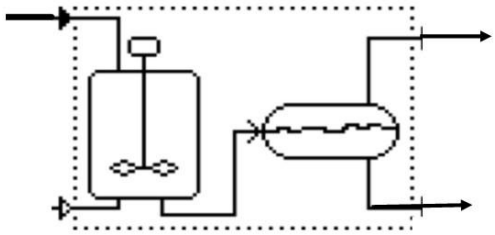
Mixing Balance

= 300.67 g cells/ hr

= 110.48 mL/hr

= 40 mL PEG/hr

=60 mL phosphate/hr



Light phase:

w= 0.60 mAb (containing cells)

= 180.40 g cells with mAb/hr

= 110.48 mL/hr

Heavy Phase

=0.40

=44.18 g cells/hr

P-5 / MSX-101

Mixer-Settler Extraction

Light phase:

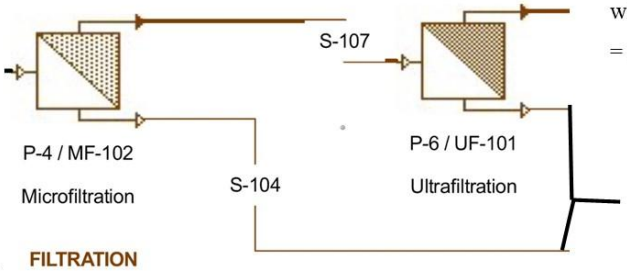
w= 0.60 mAb (containing cells)

= 180.40 g cells with mAb/hr

= 110.48 mL/hr

= 40 mL PEG/hr

=60 mL phosphate/hr



FILTRATION

w= 0.65 light

=117.26 g cells with mAb/hr

= 110.48 mL/hr

w= 0.35 heavy

=63.14 g cells/hr

[24] Petrides, D., Carmichael, D., Siletti, C., & Koulouris, A. (2014). Biopharmaceutical process optimization with simulation and scheduling tools. *Bioengineering*, *1*(4), 154–187. https://doi.org/10.3390/bioengineering1040154

Light phase:

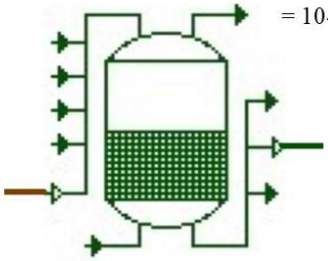
w= 0.60 mAb (containing cells)

= 117.26 g cells with mAb/hr

= 110.48 mL/hr

= 40 mL PEG/hr

=60 mL phosphate/hr



w=0.95 buffer solution

= 104.95 mL/hr

= 117.26 g cells with mAb/hr

w=0.05 buffer

= 5.524 mL/hr

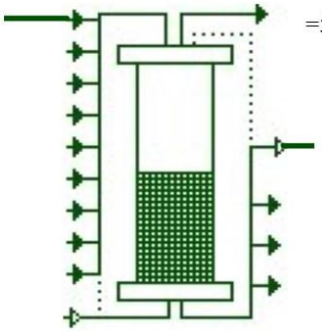
P-7 / INX-101

Ion Exchange

= 117.26 g cells with mAb/ hr

w=0.05 buffer

= 5.524 mL/hr



=buffer solution

=5.524 mL/hr

= 117.26 g cells with mAb/hr

P-8 / C-101

HIC chromatography

Health, Safety, and Environmental Concerns

We will develop a procedure for incoming inspection of raw materials. This will ensure that all reagents and components used to manufacture the monoclonal antibodies are consistent in composition, activity, and purity. This procedure will include processes for auditing all vendors based on level of risk by having a team research into the history of our vendors and take into consideration how critical the reagent is before purchasing. We also have a procedure for all incoming materials to review the safety data sheet to ensure that we maintain the current versions in our files and which are easily accessible

Following FDA regulations[25], we will include characterization of the parent cells, donor history for human cells, immunogen, immortalization procedures, screening, and cell cloning procedures. We will describe specifications and analytical methods used for release testing, shelf life, and distribution as well as include certificates of analysis and analytical results for our product. We will test for an impurity profile of our product. As our product is intended to be sterile, we will provide evidence of container and closure integrity for the duration of 1 year expiry period.

Following the Occupational Safety and Health Act, 29 U.S.C. et seq. (1970)[26], we as employers will provide a place of employment free from recognized hazards to safety and health. We will also provide personal protective equipment and training.

Following the Toxic Substances Control Act (TSCA), 15 U.S.C. 2601 et seq. (1976)[27], we will report information about new or alleged health or environmental effects caused by a chemical. We will submit a premanufacture notice to the EPA 90 days before manufacturing our product and importing our reagents. We understand the authority of the EPA.

Following the Emergency Planning and Community Right-to-Know Act (EPCRA),42 U.S.C. 11011 et seq. (1986)[28], we will make plans for major incidents and publicly disclose these plans.

We will follow all FDA regulations for submitting an application to obtain approval for a new monoclonal antibody for use in humans. This will include submitting the chemistry manufacturing and controls (CMC) information and a new biologic license application (BLA). We will request FDA inspection of our facility to ensure compliance with good manufacturing practices.

We will comply with all FDA regulations under 21 CFR 600-680.

Operation hazard statement:

If we cannot reduce hazards and the data shows risks still remain higher than we anticipated, then we need to go back and reevaluate procedures and may have to redesign the process to reduce risk to as reasonable as possible.

We will follow the Code of Ethics for Engineers[29]:

Engineers, in the fulfillment of their professional duties, shall:

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[25] FDA. (1996, August). *Guidance for industry*. Retrieved December 7, 2021

[26] *United States Department of Labor*. OSH Act of 1970 | Occupational Safety and Health Administration. (n.d.). Retrieved December 7, 2021

[27] Environmental Protection Agency. (n.d.). EPA. Retrieved December 7, 2021

[28] Environmental Protection Agency. (n.d.). EPA. Retrieved December 7, 2021.

[29] *Code of ethics*. Code of Ethics | National Society of Professional Engineers. (n.d.). Retrieved December 7, 2021.

1. Hold paramount the safety, health, and welfare of the public.
2. Perform services only in areas of their competence.
3. Issue public statements only in an objective and truthful manner.
4. Act for each employer or client as faithful agents or trustees.
5. Avoid deceptive acts.
6. Conduct themselves honorably, responsibly, ethically, and lawfully so as to enhance the honor, reputation, and usefulness of the profession.

To dispose of our waste, we partnered with Clean Management Environmental Group Inc[30], a professional waste disposal service, to ensure proper disposal in accordance with federal guidelines. Doing so prevents harming surrounding ecosystems.

Ethically, some may disagree with the production of these antibodies as we will be purchasing cells from mice. While that is true and these mice are bred to be killed for science, it is understood that these mice are respected and treated as living conscious beings. With the use of these mice, we are able to save countless human and animal lives.

Polyethylene glycol, while considered harmless, can be toxic if ingested. Phosphate buffer solution may be irritating to the mucous membranes and upper respiratory tract and may be harmful by inhalation, ingestion, or skin absorption. We will educate our workers on these risks and make sure they have proper equipment when working.

We have developed a cross-functional HAZOP team to identify all elements of the required systems and consider all variations in operation parameters. They will identify all potential hazards and failure points. The team will perform the HAZOP analysis to reduce all residual risks to as low as reasonably possible. If we cannot reduce hazards and the data shows risks still remain higher than we anticipated, then we need to go back and reevaluate procedures and may have to redesign the process to reduce risk to as reasonable as possible

[30] Group, C. M. E. (n.d.). *Environmental Waste Disposal Company: Clean Management*. Clean Management Environmental Group, Inc. Retrieved December 8, 2021

HAZOP Analysis of Units:

Vessel- **Bioreactor** Intention: Culture mice-ovarian cells, 1 atm, 37° C				
Guide Word	**Deviation**	**Causes**	**Consequences**	**Action**
Line S-101 Intention: Transfers pre-cultures into reactor				
NO	Flow	Valve fully closed	Fall in culture rate	Alarm on $\pi 2$
LESS	Flow	Valve partially closed	Same as NO	Same as NO
MORE	Flow	Valve too open	Increase in	Same as NO
CONTAMINATION	Contamination of vessel	Improper Maintenance	Contaminated Product	Proper maintenance and operator alert

Vessel- **Pump**
Intention: Powers transfer of cells and filtrate out of bioreactor to filtration, 1 atm, 37°C

Guide Word	Deviation	Causes	Consequences	Action
Line S-102 Intention: Removes mixture from bioreactor				
NO	Flow	Valve fully closed	Lines overpressure and pump overheating	Alarm on 𝜋2
NO	Flow	No power to pump	No transfer out of bioreactor	Alarm on 𝜋2
LESS	Flow	Valve partially closed, Less power to pump	Less transfer out of bioreactor	Alarm on 𝜋2
MORE	Flow	Too much power to pump	Lines overpressure and pump overheating	Alarm on 𝜋2
Line S-103 Intention: Transfers mixture into filtration system				
NO	Flow	Valve fully closed	Lines overpressure	Alarm on 𝜋3
NO	Flow	No power to pump	No transfer into filtration system	Alarm on 𝜋3
LESS	Flow	Valve partially closed, Less power to pump	Less transfer into filtration system	Alarm on 𝜋3
MORE	Flow	Too much power to pump	Lines overpressure and pump overheating	Alarm on 𝜋3

Vessel- **Mixer-Settler**
Intention: Mixes cell-free filtrate with poly ethylene glycol (PEG) and phosphate buffer saline (PBS), and extracts the light phase and heavy phase of the filtrate, 1 atm , 100°C, 250°C

Guide Word	**Deviation**	**Causes**	**Consequences**	**Action**
Line S-111 Intention: Mixes cell-free filtrate with poly ethylene glycol and phosphate buffer solution, 37°C				
NO	Flow	Stream Valve Malfunction	No flow into vessel	Install valve check
LESS	Flow	Stream Valve Malfunction	Less flow into vessel	Install check valve
MORE	Flow	Stream Valve Malfunction	More flow into vessel	Install check valve
NO	No mixing	Motor malfunction	Less reaction	Install component to make sure liquid is spinning
Vessel, 1 atm, 100°C to boil off PBS , 250°C to boil off PEG				
LESS	Less Heat	Less or no power to heater	Improper extraction	Alarm on 𝜋4
MORE	More heat	Too much power to heater	Improper extraction	Alarm on 𝜋4
CONTAMINATION	Contamination of machine	Improper Maintenance	Contaminated Product	Proper maintenance and operator alert
LOSS	Loss of containment	Pressure build up, too much heat	Mixture leaks, Damage to vessel, Loss of product	Alarm on 𝜋4
Line dis antibodies Intention: Removes distilled antibodies from vessel, 1 atm, 37°C				
NO	Flow	Stream Valve Malfunction	No flow from vessel	Install valve check

LESS	Flow	Stream Valve Malfunction	Less flow from vessel	Install check valve
MORE	Flow	Stream Valve Malfunction	More flow into vessel	Install check valve

Vessel- **Freeze Dryer**
Intention: Solidifies liquid antibodies, 1 atm, -20°C

Guide Word	**Deviation**	**Causes**	**Consequences**	**Action**
Line liquid antibodies Intention: Brings liquid antibodies into freeze dryer, 1 atm, 37°C				
NO	Flow	Stream Valve Malfunction	No flow into vessel	Install valve check
LESS	Flow	Stream Valve Malfunction	Less flow into vessel	Install check valve
MORE	Flow	Stream Valve Malfunction	More flow into vessel	Install check valve
Vessel, 1 atm, -20°C				
MORE	Heating product too high	Too much power to heater	Melt-back, product collapse	Install alarm at π5
CHOKING	Vapor choking	Vapor produced too fast	Increase in chamber pressure	Install alarm at π5
Line: S-115 Intention: Brings solidified antibodies to electric cooler				
MORE	Heat	Insulation Problems	Melt-back or product collapse on line	Install alarm at π5

Cost Estimate for the Process

The process includes 3 different reactants:

- Buffer Solution: $20/L * 365 days = $7,300 Annually[31]
  - We require 1 liter per day of buffer solution
- Mice Cells: $600/day * 365 days = $219,000 Annually[32]
  - We require one vial of murine myeloma cells per day
- B Cells: 2 Vials/day @ $660/vial * 365 Days = $481,000 Annually[33]
  - We require 2 vials per day of B cells for our process

The process also includes units of operation:

- Pump = $300[34]
- Glass Spinner Flasks = $400[35]
- Magnetic Stirrers = $3,000[36]
- 5L Bioreactor = $14,000[37]
- Biological Safety Cabinet = $5,000[38]
- Incubator = $1,000[39]
- Microcentrifuge = $6,000[40]
- Microcentrifuge Bottles = $30[41]
- Chromatography Machines = 2 * $5,000 = $10,000[42]
- Extraction Column = $200[43]

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[31] *Propylene glycol USP (99.9%) - 1 gallon*. www.ChemWorld.com. (n.d.). Retrieved December 8, 2021

[32] *NS0 cell line from murine myeloma mouse myeloma, 85110503: Sigma-aldrich*. Sigma. (n.d.). Retrieved December 8, 2021

[33] *Buy total B cells, buy human total B lymphocytes*. Cellero. (n.d.). Retrieved December 8, 2021

[34] MSC Industrial Supply - metalworking tools and MRO supplies. (n.d.). Retrieved December 8, 2021

[35] *Double sidearm Celstir Spinner flask*. The Lab Depot. (n.d.). Retrieved December 8, 2021

[36] *Fisherbrand magnetic stirrers - hotplates and stirrers, magnetic stirrers*. Fisherbrand Magnetic Stirrers:Hotplates and Stirrers:Magnetic Stirrers | Fisher Scientific. (n.d.). Retrieved December 8, 2021

[37] *5l glass mechanical stirring fermenter bioreactor*. Toolots, Inc. – Reliable Equipment Fast. (n.d.). Retrieved December 8, 2021

[38] *2 foot class II type A2 biological safety cabinet with stand and CE certificate (ships in 8-10 weeks aro)*. Government Lab Enterprises. (n.d.). Retrieved December 8, 2021

[39] *Thermo Scientific™ Compact Microbiological Incubator*. The Lab Depot. (n.d.). Retrieved December 8, 2021

[40] *Sorvall-Legend-MICRO21R-Microcentrifuge for sale*. Acme Revival. (2021, November 17). Retrieved December 8, 2021

[41] *1.5/1.7 ml wide cap microcentrifuge tubes, clear, polypropylene, 500 tubes, lot traceable, certified DNase & RNase Free*. Premium Vials. (n.d.). Retrieved December 8, 2021

[42] *Dionex ICS-5000 DC-5 Ion Chromatography*. Acme Revival. (2021, December 3). Retrieved December 8, 2021

[43] *2" standard closed column extractor 115-200g*. BVV. (n.d.). Retrieved December 8, 2021

- Tangential Perfusion System = $4,000[44]
- Freeze Dryer = $4,000[45]
- Instrumentation = $10,000[46]
- Piping and Valves = $2,000[47]

Total cost for the process C_p, n = years of operation:

$C_p = \$59,930 + \$707,300n$

Economic Analysis for the Project

These estimates were made in regard to the competitor's pricing, which is estimated to be about $960 per vial sold to the patient.[48]

CCOP = VCOP + FCOP

VCOP =

Raw Materials:

- Buffer Solution: $20/L * 365 days = $7,300 Annually
- Mice Cells: $600/day * 365 days = $219,000 Annually
- B Cells: 2 Vials/day @ $660/vial * 365 Days = $481,000 Annually

Utilities: $24,000 Annually

Consumables: $20,000 Annually

Waste Disposal:

- Wastewater: 100,000 gal * $6/1000 gallons = $600 Annually
- Solid Waste: 10 tons * $50/ton = $500 Annually

VCOP = $752,800

FCOP =

Labor Costs:

- 10 technicians @ $60,000/year = $600,000 Annually
- 1 CEO @ $150,000/year = $150,000 Annually
- 1 COO @ $140,000/year = $140,000 Annually
- 1 CMO @ $120,000/year = $120,000 Annually
    - 2 Marketing assistants @ $70,000/year = $140,000 Annually

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[44] *Pall Minim II tangential flow filtration system for sale*. Acme Revival. (2021, November 12). Retrieved December 8, 2021

[45] *Harvest right premium medium pharmaceutical freeze dryer*. Rightbud. (n.d.). Retrieved December 8, 2021

[46] Jean-Francois Denault Agnes Coquet Vincent Dodelet. (2014, June 11). *Construction and start-up costs for biomanufacturing plants*. BioProcess International. Retrieved December 8, 2021

[47] Google. (n.d.). *Piping for Manufacturing Facilities*. Google Shopping. Retrieved December 8, 2021

[48] U.S. National Library of Medicine. (n.d.). *Cost comparison table*. Pharmacoeconomic Report: Eculizumab (Soliris): Alexion Pharma Canada Corp. Indication: Neuromyelitis optica spectrum disorder [Internet]. Retrieved December 8, 2021.

- 1 Project Manager @ $110,000/year = $110,000 Annually
- 1 HR Manager @ $80,000 = $80,000 Annually

Maintenance = $10,000 Annually
Taxes = $75,000 Annually
Insurance = $15,000 Annually
Overhead = $100,000 Annually

FCOP = $1,540,000
CCOP = $752,800 + $1,540,000
CCOP = $2,022,200 Annually

ISBL =

Major Equipment:

- Pump = $300
- Glass Spinner Flasks = $400
- Magnetic Stirrers = $3,000
- 5L Bioreactor = $14,000
- Biological Safety Cabinet = $5,000
- Incubator = $1,000
- Microcentrifuge and Bottles = $6,030
- Chromatography Machines = 2 * $5,000 = $10,000
- Extraction Column = $200
- Tangential Perfusion System = $4,000
- Freeze Dryer = $4,000

Bulk Items:

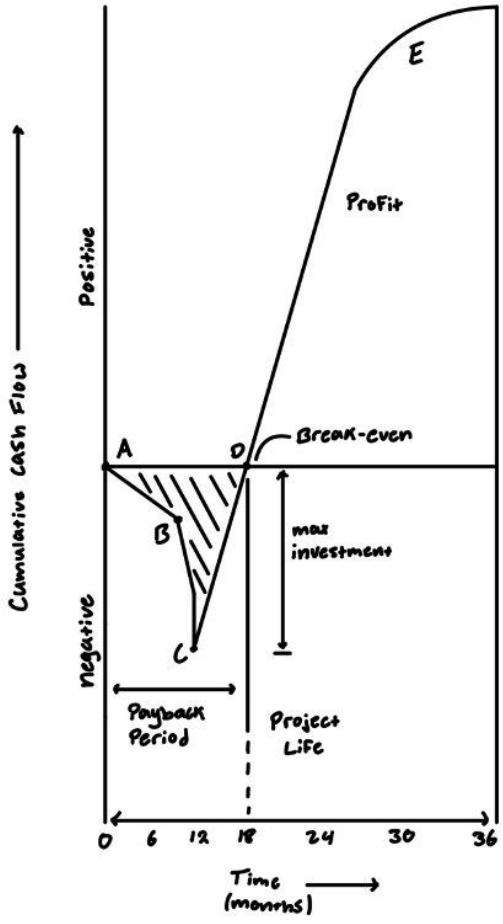
- Instrumentation = $10,000
- Piping and Valves = $2,000

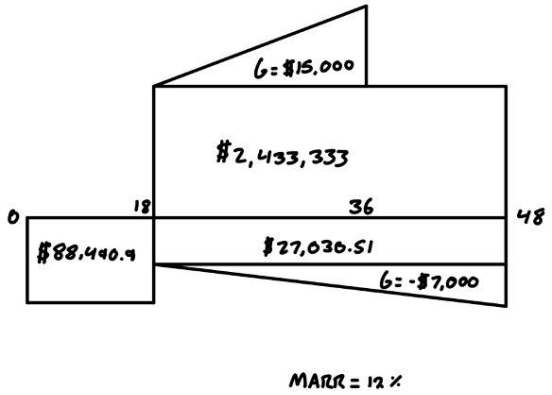
Construction = $4,000,000
ISBL = $4,047,330

OSBL =

Engineering = $400,000
Expenses = $100,000
Bonding = $100,000
Procurement = $50,000
OSBL = $650,000

Contingency = $500,000
Working Capital = $600,000





- All initial cost is spread through the first 18 months
  - A = (7,819,524/12 months)*(A/P, 12%, 19) = $88,490.95
- The next 30 months do not have any initial costs
  - A = (2,622,200/12 months)*(A/P, 12%, 31) = $27,030.51
- Present worth of the company through 4 years
  - PW = -651627 – 218,516.67*(P/F, 12%, 18) – 7000*(P/G, 12%, 31)*(P/F,12%,18) + 24,333,333*(P/A, 12%, 31)*(P/F, 12%, 18) + 15,000*(P/G, 12%, 19)*(P/F, 12%, 18)
  - PW = $1,907,061.42

Marketing and Business Partnership Strategy

Marketing strategies will proceed in a variety of ways with a focus on wide scale advertising using the appropriate channels, as well as developing mutually beneficial relationships with vendors and business partners.

For advertising, campaigns will launch in appropriate publications, such as medical magazines and journals frequently distributed amongst the medical community. Ads will also be featured on heavily trafficked medical websites, in physical mail, brochures, and pamphlets to best reach the consumers. In addition to these more traditional methods, peer-to-peer marketing will also be utilized in the form of doctor and patient testimonials and interviews when available; this will heavily increase the amount of consumers reached, and it will improve the content of the information by ensuring it comes from a reliable source.

The company itself will have its own website where relevant purchasing information will be found as well as linking the testimonials. On this website, the company's LinkedIn and Twitter can be found so consumers can see product updates and speak with the company directly, plus many doctors and consumers are getting their information from these social media platforms, so the presence of our company on these sites is beneficial.

Moreover, the company will develop relationships with vendors by consistently buying their products in bulk; for example, purchasing the cell lines on a consistent basis and in bulk will be suitable for both the company and the vendor. These sales channels will be forged by reaching out to companies prior to construction via our sales team and signing contracts with them. This will be done predominantly through the raw materials manufacturers.

In addition to the company's purchasing channels, it will also forge selling channels with wholesalers that deal with hospitals nationwide, as well as other medical companies that may sell the monoclonal antibodies as well. This will be done using the same contractual agreements as the purchasing channels.

Last, partnerships will be developed for the maintenance aspect of the project; this will include forging partnerships with third party inspections teams to maintain the company's facilities, test both the product and the feedstock material, and ensure that the machinery is up to date and does not have any issues.

Conclusion

Olizumab is a top of the line drug of monoclonal antibodies developed using a continuous process after pre-culture batch procedures. These antibodies prevent the formation of the membrane attack complex, thus preventing the patient from eliciting an immune response to the donor kidney cells. This medication has an increasingly high demand as life expectancy across humans increases, and the ability to transplant kidneys becomes more available. Already, over 35,000 people a year undergo kidney transplantation, and every single one of these patients requires an immunosuppressant to ensure the transplantation is successful. In order to achieve the goal of successful immunosuppression, the monoclonal antibodies must have the highest purity possible, and the culturing procedures must be maximized for optimal output.

This is done using the aforementioned processes to maximize output and minimize cost; this will best meet the client's needs since the project will be constructed and running within a timely manner at a low cost. Additionally, the long shelf life of the antibodies contributes drastically to the maximization of output and increase in profit.

The overall construction of the project will take roughly 45 days to complete, and once the other steps are factored in, the overall project will be finished in about 60 days. With clear distributions of projects, the procedure will be completed by 27 employees varying in skill level with extensive training in order to properly handle the materials and avoid factory issues.

As for the cost to employ each member, it amounts to roughly $1.34 million annually. In addition to these employment costs, other amounts include the ISBL amounting to about $4.1 million, the OSBL amounting to about $650,000, the contingency costs at about $500,000, working capital at about $600,000, insurance and overhead at about $115,000, and maintenance at about $10,000 annually.

For profit, the company should bring in about $28 million annually by selling the monoclonal antibodies for about $800 per gram. This estimate is about 20% less than the competitor who sells their products to medical facilities for about $960. The profit margin is significantly increased by purchasing from sellers in bulk at lower prices, ensuring that the process operates on a small scale, and that quality is maintained.

Factors that may impact production are inspections, errors in handling chemicals, temporary factory shutdowns pending damage to machinery, accidents within the lab, or prolonged, unforeseen circumstances that may occur during construction.

Overall, if the project proceeds to completion in a timely manner, and culturing procedures are underway, the biochemical process of creating these antibodies should be a dramatic success that will contribute to the longevity and health of patients across the nation.

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