Design of a Pembrolizumab Manufacturing Plant Using Continuous Bioprocess Technology and Single-Use Bioreactors

A Technical Report submitted to the Department of Chemical Engineering

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On my honor as a University Student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

Signature _____ Date_____

Approved_____ Date_____
Eric W. Anderson, Department of Chemical Engineering

Letter of Transmittal

Under the advisement of Professor Eric Anderson, we have designed a facility for the manufacturing of Keytruda or pembrolizumab. The following document has been prepared to communicate the design specifications of the capstone design project.

2. Summary

The purpose of this capstone design project was to develop a continuous manufacturing facility that uses single-use bioprocessing technology in Ireland to produce pembrolizumab. Pembrolizumab is a monoclonal antibody used in the treatment of many types of cancer, such as melanoma, lung cancer, and cervical cancer (Merck & Co., 2019a). Pembrolizumab is currently manufactured by Merck & Company and is known as the immunotherapy drug Keytruda®. Keytruda continues to receive regulatory approvals and is set to be one of the world's top-selling drugs (Keown, 2019).

Continuous bioprocessing technology, a major component of this design, proves to have many advantages, such as enhanced product quality, greater efficiency, and rapid scalability. Other advantages include lower equipment costs and smaller quantities of cell media necessary (Kakes, 2018). Likewise, single-use technology is incorporated into the design as it proves to have many advantages such as easy disposal and a reduction in time, costs related to CIP/SIP procedures, and labor necessary (Saxena, 2019).

The capacity of the proposed facility is to produce 226 kg Keytruda per year, or approximately 2.3 million doses per year. The total capital investment in the facility is $16,434,686. It was estimated that 2.5 years of validation and construction would occur prior to production, where construction would take 1.5 years, and validation would take 1 year. Economic analysis performed on this capstone design project demonstrates its financial ability with an internal rate of return of 69.7% and net present value of $401 billion based on 20 years of operation. Overall, the economic analysis predicts high levels of profitability for this pembrolizumab manufacturing facility.

3. Introduction

3.1 Motivation and Background

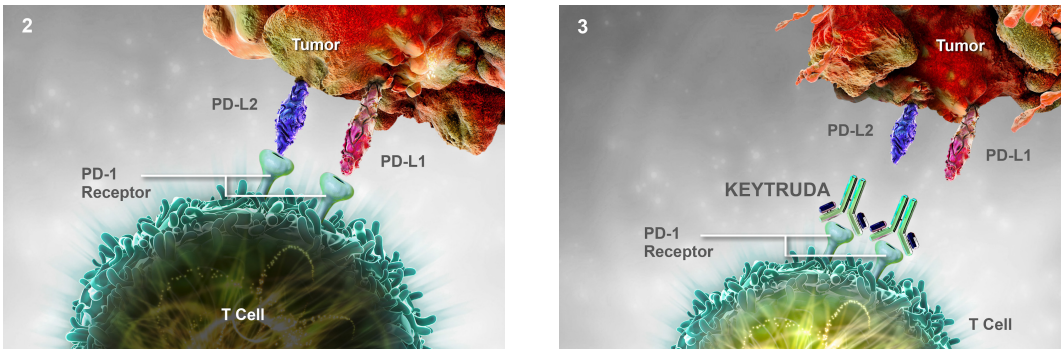
Cancer is the second leading cause of death in the U.S., with the annual number of cancer cases expected to rise from 14.1 million in 2012 to 23.6 million in 2030 (National Cancer Institute, 2015). Corresponding to this increase in disease rates is the rise of new technology in the pharmaceutical industry, specifically through antibody-based drugs, which have risen as the fastest growing class of protein therapeutics due to their multiple benefits such as decreased immunogenicity, improved deliverability, and decreased potential to adversely affect normal biological processes compared to standard chemotherapy procedures (Awwad & Angkawinitwong, 2018).

The cancer immunotherapy drug Keytruda, also known as pembrolizumab, is a checkpoint inhibitor monoclonal antibody (mAb) manufactured by Merck. Keytruda was first approved by the U.S. Food and Drug Administration (FDA) in 2014 for advanced melanoma. Since then, Keytruda has been approved 22 times for further indications (FDA, 2014). Due to increasing global demand, Merck announced that it will invest $300 million into a Keytruda manufacturing facility in Dublin, Ireland, estimated to begin manufacturing operations in 2022 (The Irish Times, 2018).

We plan to design the new Merck Keytruda production facility with a continuous integrated bioprocess and single-use bags. Incorporating single-use reactor bags will decrease the need for extensive cleaning protocols, save time, reduce employment costs, improve compliance with environmental regulations, improve the modularity of the manufacturing facility, and ensure product purity between batches (Jacquemart et al., 2016). Using a perfusion reactor will allow

for near-continuous production of Keytruda, rather than the production of the drug in batches, which will increase product yields and subsequently decrease production costs. As continuous bioprocessing gains increasing attention for possible benefits, we are interested in examining the feasibility of a fully integrated bioprocess.

3.2 Pharmacology



Figures 3a and 3b. Cancer cell silencing of immune response via the PD-1/PD-L1 signaling pathway and mechanism of Keytruda action, respectively (Merck & Co., 2019c).

Keytruda is a cancer medicine that interferes with the growth and spread of cancer cells in the body (Stewart, 2019). The Keytruda drug, an injectable that is given to the patient every three weeks, is comprised of the active drug substance, pembrolizumab, a humanized monoclonal antibody that blocks the interaction between PD-1 and its ligands, PD-1 and PD-2 (Merck & Co., 2019). Through this, the drug is able to prevent cancer cells from hiding in the PD-1 pathway (see Figure 3b) (Merck & Co., 2019b).

Keytruda (pembrolizumab) is a highly selective IgG4-kappa humanized monoclonal antibody against PD-1 receptors. Keytruda has an approximate molecular weight of 149 kDa and isoelectric point (pI) of 6.8-6.9. It is packaged with inactive ingredients L-histidine, polysorbate 80, and sucrose. (“Pembrolizumab,” 2019).

3.3 Treatment and Dosage

Keytruda is used to treat advanced skin cancer, lung cancer, small cell lung cancer, classic Hodgkin's lymphoma, and many other cancers. Keytruda is often given when cancer has spread to other parts of the body, cannot be surgically removed, or when other cancer treatments did not work or have stopped working. It is given to the patient every three weeks. As for the success rate of Keytruda, studies conducted by Merck show that Keytruda demonstrated a five-year overall survival (OS) rate of 23.2% in treatment-naive patients and 15.5% in previously-treated patients. The five-year OS rate among patients whose tumors expressed PD-L1 was 29.6% in treatment-naive patients and 25.0% in previously treated patients (Merck & Co., 2019a).

The Keytruda drug exists in two forms: Keytruda for injection, a sterile, preservative-free lyophilized powder in single-dose vials, and Keytruda injection, a sterile, preservative-free solution for intravenous infusion. As with most parenteral injectables, Keytruda end purity must exceed 99% to be safely injected (Kelley, 2009).

3.4 Plant Capacity

As of 2019, the sales of Keytruda total to $10.8 billion (Nathan-Kazis, 2019). The sales of Keytruda worldwide are projected to be $22.5 billion by 2025 (GlobalData Healthcare, 2019). This represents a 48% increase in the total sales of Keytruda from 2019 to 2025. The typical delivery mechanism of Keytruda is an intravenous infusion every three weeks (Huntington, 2018). The average cost of Keytruda is around $51.79 per mg (Andrews, 2015). Taking into account the average cost of Keytruda per mg, approximately 226 kg of Keytruda will be needed in order to satiate the increased demand projected for the year 2025. It is important to note that

all of our analyses are dependent on whether the cost of Keytruda per mg stays relatively constant at $51.79 per mg. However, with the increasing demand for the drug, we expect the wholesale price of the drug to either stay constant or increase, leading to slight overestimation in the amount needed by 2025. The slightly overestimated scope for our plant is therefore acceptable and will ensure an adequate surplus for the future.

4. Discussion

4.1 Upstream Process

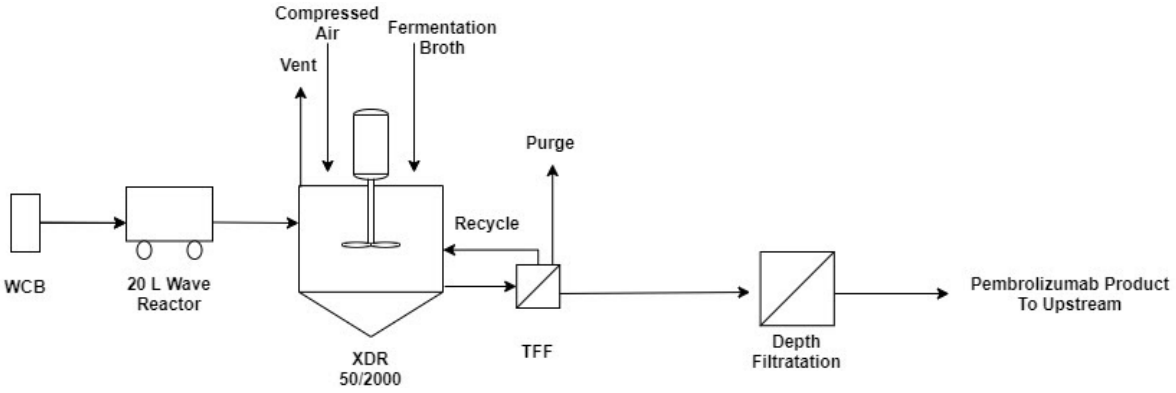


Figure 4.1: Basic process flow diagram for upstream process.

4.1.1 Cell Line Acquisition and Storage

Chinese hamster ovary (CHO) cells are often used for the production of therapeutic proteins. These mammalian cells allow for consistent glycosylation profiles which are necessary for regulatory purposes and therapeutic efficacy (Omasa, Onitsuka, & Kim, 2010). CHO cells are mammalian cells that can be genetically modified to produce a specific antibody with exceptionally high titers (Trill et al., 1995). We will utilize recombinantly modified CHO cells to produce pembrolizumab. Additionally, in order to avoid FDA approval, regulatory requirements, and clinical trials associated with the creation of a new master cell bank, we will obtain a portion of the master cell bank from Merck & Co.'s current facility in West Point, PA. According to Professor King, this can be done by maintaining strict documentation of the shipping process overseas and ensuring that no major excursions during this process. These vials will be stored in-flight and at the manufacturing facility at -87^{o}C in a VIP Series Model MDF-U76VC-PA Freezer. We will purchase two of these freezers with one being used as a backup on-site in case of a malfunction of the first freezer system.

The WCB cell vials will be held as high-density vials consisting of a cell density of $50x10^6$ cell/mL with each vial containing 4.5 mL. This high-density cell bank design was proposed by GE. Studies performed at GE indicated a significant reduction in scale-up time using these vials while still meeting GMP requirements (GE Healthcare, 2020c).

4.1.2 Inoculum Train

By implementing the high density working cell bank vials, the scale-up process can be significantly reduced with respect to time and the pieces of equipment required. The overall scale-up process is demonstrated in Figure 4.2.

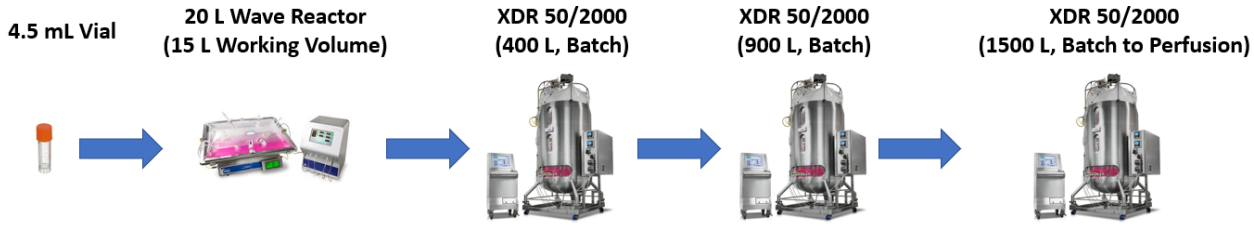


Figure 4.2. Scale-up equipment and working volume requirements from 4.5 mL vial to 1500 L perfusion reactor (*Xcellerex™ XDR Cell Culture Bioreactor Systems*, 2020).

The initial volume will be placed into the 20L Wave bioreactor with a working volume of 15 L. The rocking speed of this reactor will initially be set to 20 RPM and subsequently increased to 25 RPM after 10 hours of processing as recommended by the vendor (GE Healthcare, 2019). We will purchase two of these Wave reactors with one being held as a backup. The fermentation broth will then be sent to an XDR 50/2000. The XDR bioreactor systems provide the performance and flexibility needed from process development to large-scale biopharmaceutical manufacturing. XDR bioreactor systems can be operated in batch, fed-batch, and perfusion mode. This reactor has a minimum working volume of 400 L and a maximum working volume of 2000 L (*Xcellerex™ XDR Cell Culture Bioreactor Systems*, 2020). Therefore,

the first scale-up will be from the 15L Wave reactor to a 400 L working volume in a reactor bag. The broth will then be transferred to a 900 L XDR reactor bag followed by a 1500 L reactor bag. We will have two XDR 50/2000 reactors to accommodate the necessary maintenance. Additionally, the subsequent inoculations between campaigns must start early to ensure continuity of the process.

The kinetics data used for these steps was obtained from a study using an infliximab monoclonal antibody derived from CHO cells. Infliximab is also an IgG monoclonal antibody with a molecular weight of 149 kDa, the same as pembrolizumab (López-Meza et al., 2016). The Monod model was used to calculate the growth constant. The equations used to model the kinetics are shown below.

$$\frac{dX}{dt} = \mu X \qquad \frac{dP}{dt} = \frac{1}{Y_{X/P}}\mu X$$

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}}\mu X \qquad \mu = \frac{\mu_{max}[S]}{K_s+[S]}$$

Equations 4.1a-1d. Three ordinary differential equations to model cell (X), substrate (S), and product (P) growth. Additionally, the Monod Model is shown.

The constants utilized for the various scale-up reactors are as follows. μ_{max} was 0.039 h^{-1}, K_s was 0.664 g/L, $Y_{x/s}$ was 1.436 g cell/g substrate, and $Y_{P/S}$ was 0.25 g media/g substrate. Using these constants, the time required to scale up and respective component concentrations were calculated (see Figure 4.3 below).

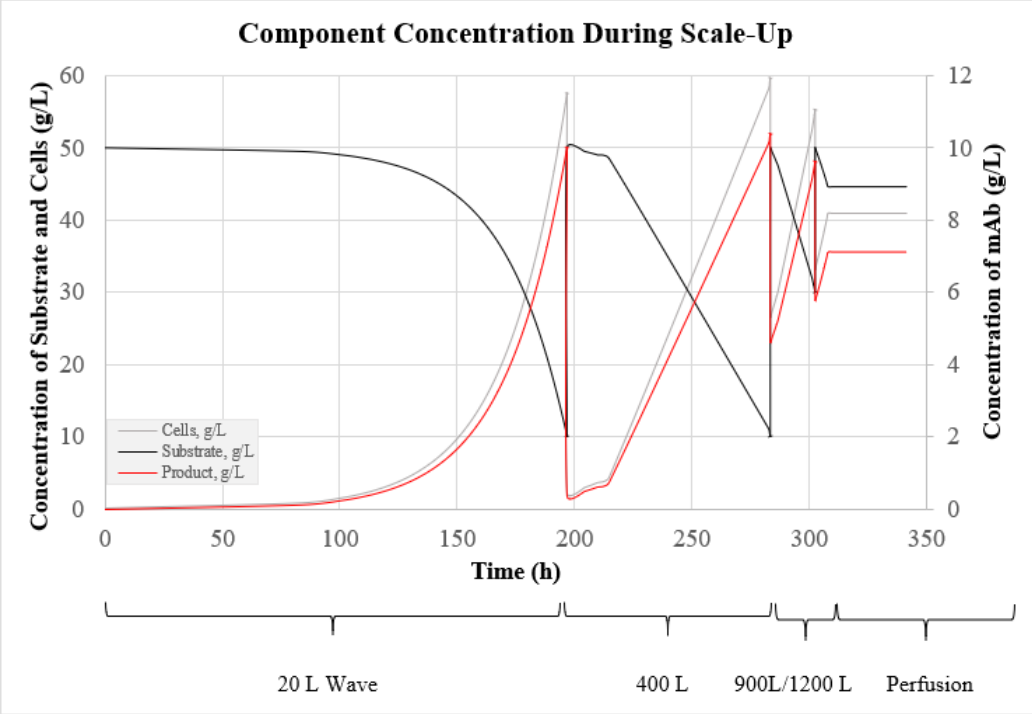


Figure 4.3. Time scale for inoculation scale-up with concentrations of product (red), substrate (black), and cells (grey), in g/L.

To begin the initial Wave reactor process, 15 L of WFI will be added in addition to the 4.5 mL vial. The initial cell concentration will be 0.03 g/L, and 750 g of solid media will be added to bring the initial substrate concentration to 50 g/L. After 197 hours, the broth will be transferred to the 400 L bag in the XDR reactor. The outlet concentrations of CHO cells, media, and pembrolizumab leaving the 15 L process will be 53.9 g/L, 14.5 g/L, and 8.88 g/L, respectively. After the addition of 19,800 g of media and dilution with 385 L of WFI, the starting substrate concentration will return to 50 g/L. The starting concentrations of CHO cells, media, and pembrolizumab will be 11.8 g/L, 50 g/L, and 2.05 g/L, respectively. After 284 cumulative hours, the broth will be transferred to the 900 L bag in the XDR reactor. The outlet

concentrations of CHO cells, media, and pembrolizumab leaving the 400 L process will be 57.5 g/L, 10.0 g/L, and 10.0 g/L, respectively.

After the addition of 41,000 g of media and dilution with 500 L of WFI, the starting substrate concentration will return to 50 g/L. The starting concentrations of CHO cells, media, and pembrolizumab for the 900 L process will be 19.9 g/L, 50 g/L, and 3.46 g/L, respectively. After 303 cumulative hours, the broth will be transferred to the 1500 L bag in the XDR reactor. The outlet concentrations of CHO cells, media, and pembrolizumab leaving the 900 L process will be 59.6 g/L, 10.0 g/L, and 10.4 g/L, respectively.

After the addition of 48,000 g of media and dilution with 600 L of WFI, the starting substrate concentration will return to 50 g/L. The starting concentrations of CHO cells, media, and pembrolizumab for the 1500 L batch process will be 33.6 g/L, 50 g/L, and 2.28 g/L, respectively. After 308 cumulative hours, the 1500 L reactor will be directed to begin perfusion operations. The outlet concentrations of pembrolizumab leaving the perfusion process will be 7.12 g/L.

4.1.3 Perfusion Reactor

The bioreactor that will be used for this is the Xcellerex XDR 2000 Single-use stirred-tank bioreactor operating in perfusion mode. The inner tank and impeller dimensions are given in Table 4.1 and diagrammed in Figure 4.4. The working volume will be 1500 L of solution which will be maintained over a 30-day perfusion campaign. The 30-day perfusion length was determined as it is in the median range of perfusion lengths. We chose the median perfusion run time as shorter perfusion times do not fully utilize the advantages of continuous processing, and longer perfusion times cause the cells to be recycled too many times, which

results in excessive cell damage and cell aging. The relevant geometric ratios are the height of the tank to the height of the impeller (defined as the distance from the bottom of the tank to the bottom of the impeller) and the Impeller Diameter to the height of the tank. The values of these are 3:1 and 4.35:1, respectively.

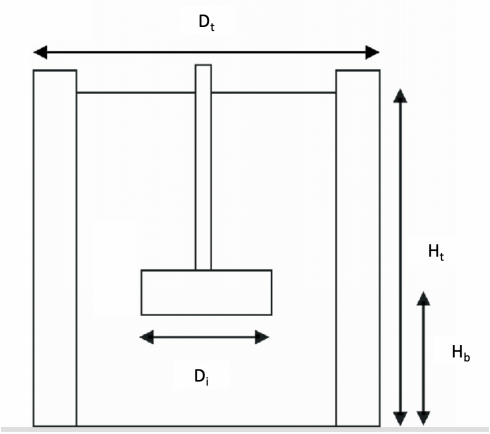


Figure 4.4. Xcellerex XDR 2000 Single-use stirred-tank bioreactor Dimension Diagram

The XDR bioreactor bag is constructed with a contact layer of USP class VI-compliant low-density polyethylene (LDPE) plastic making the bioreactor bags robust to withstand process conditions. All bioreactor bag assemblies incorporate a seal-less, bottom-mounted, impeller and sparger assembly with a centrally positioned integral magnet. The impeller and spargers are also single-used designs that couples with the magnetic drive head, creating a powerful and robust agitation system with minimal risk of seal leakage. All bioreactor bags for cell culture include a magnetically coupled, M40e 45°, pitched-blade impeller with ceramic bearings. Ceramic bearings, originally developed for aggressive mixing applications and fermentation, provide excellent overall performance. The medical-grade ceramic bearings meet the relevant industry requirements for leached material, extracted material, and particulates according to the bag validation guide (*Xcellerex^{TM} XDR Cell Culture Bioreactor Systems*, 2020). Dissolved oxygen

(DO) and pH can be measured using conventional polarographic sensors and glass electrodes, respectively. These sensors will be autoclaved prior to use in a specially designed probe sheath (*Xcellerex™ XDR Cell Culture Bioreactor Systems*, 2020)

Table 4.1. Xcellerex XDR 2000 Single-use stirred-tank Dimensions

Parameter	Value	Unit
D_t	122	cm
H_t	183	cm
V	2000	L
D_i	42	cm
H_b	61	cm

Oxygen requirements are satisfied by feeding compressed air with atmospheric concentrations of oxygen and nitrogen to the reactor through custom sparger. The bioreactor is also equipped with stripping spargers to remove excess CO2 & Nitrogen. The bioreactor is equipped with a custom oxygenation system that allows additional spargers to triple the air providing capability of the default system. The oxygen demands are calculated using a steady-state approximation that the rate of oxygen transferred to the cells is equal to the rate of oxygen consumed (Davis & Davis, 2013). This approximation yields Equation 4.2 which cell density (X) multiplied by cell oxygen consumption rate (q_{O2}) is equated to mass transfer coefficient ($K_L a$) multiplied by the difference between the minimum concentration of oxygen (C_{O2}) and the solubility of oxygen in water (C^*_{O2}). The cell density, volume, and cell oxygen consumption rate define the oxygen uptake rate (OUR) as 16,354 g/day.

$$q_{O2}X = K_L a\,(C^*_{O2} - C_{O2})$$

Equation 4.2. Steady-State Approximation of Consumption of Oxygen to Transfer of Oxygen.

Table 4.2. Oxygen Demand Parameters for Xcellerex XDR 2000 Single-use stirred-tank

Parameter	Value	Unit
q_{o2}	5.46 *10^{-12}	mol/day/cell
X	6.24*10^{8}	cell/mL
OUR	16,354	g/day
C_{o2}	6.71	g/L
C_{o2}^{*}	2.68	g/L
$K_L a$	102.1	hr^{-1}

Using Equation 4.2, the required $K_L a$ was determined using values of a cell oxygen consumption rate obtained literature for CHO cells and the designed cell density of 6.24*10^{8} cell/mL (Goudar et al., 2011). The values for these concentrations were determined by utilizing solubility tables for water at the operating temperature of 37°C (Walczyńska & Sobczyk, 2017). The approximate minimum concentration of oxygen is 40% of the solubility (Garcia-Ochoa & Gomez, 2009).

$$Re = ND_i^2 \varrho/\mu$$

Equation 4.3. Reynolds Number for Flow Regime Identification.

$$N_a = Q_{air}/ND_i^3$$

Equation 4.4. Aeration Number for use in Blanchard & Clark Correlation.

$$P = N_p \varrho N^3 D_t^5$$

Equation 4.5. Power Calculations using Power Number.

The design parameters of impeller speed and airflow rate were determined based on the required $K_L a$ value determined by the steady-state approximation. A maximum amount of compressed air flow was chosen to minimize the impeller speed required and thus minimize shear imposed on the cell broth. This airflow rate is 0.032 vvm or approximately 63 L/min. Equation 4.3 and 4.4

were used to calculate the aeration number and Reynolds number for a given impeller speed. For all reasonable impeller speeds, Reynolds number exceeded 4000 into the turbulent zone which correlates to a constant power number for a 4-blade 45° pitch blade impeller of 1.27 (Major-Godlewska & Karcz, 2018). Using a correlation Blanch & Clark, the gassing factor was determined using the aeration number then multiplied by power (P), found using Equation 4.5, to find P_g (Clark & Blanch, 1995). This value is used in Equation 4.6 from Professor George Prpich's CHE 3347 to calculate $K_L a$ for the chosen parameter. An impeller speed of 547 revolutions per second is the minimum impeller speed to achieve the required $K_L a$. For CHO cells, shear should not cause damage under these operating conditions with a pitch blade turbine but to protect the cells from damage caused by bubble gas bruising, Pluronic F-68 will be added as a protectant. (Velez, 2013) This protectant has been shown to protect CHO cells up to 600 RPM. (Hu & Chalmers, 2011)

$$K_L a = 0.026(P_g/V)^{0.4} V_s^{0.5}$$

Equation 4.6. Correlation for Mass Transfer Coefficient for Oxygen using Impeller/Aeration Design Specifications.

Table 4.3. Design Parameters for Xcellerex XDR 2000 Single-use stirred-tank

Parameter	Value	Unit
V	1500	L
T	37	C
ρ	997	kg/m^3
μ	$3.41 * 10^{-2}$	Pa*S
N	547	min^{-1}
Q_{air}	0.032	vvm
$K_L a$	102.4	hr^{-1}

In order to properly calculate the material balances around the reactor, we first set the dilution rate of the system based on Ks and a specified substrate concentration S_0 which was set to be 50 g/L. The equations to calculate the dilution rate are as follows:

$$D_{opt} = \mu_{max}\left(1 - \frac{K_s}{K_s + S_o}\right)$$

Equation 4.7. Correlation between D_{opt} and K_s and S_o.

With this equation, we found the optimum dilution rate for the system to be 0.00354 hr^{-1}. With this we were able to use the equation:

$$F_o = D_{opt} V$$

Equation 4.8. Equation relating D_{opt} to the inlet flow rate of new media and the volume of the reactor.

For our system, the F_o was 0.89 L/min. The cell density was calculated by using the equation:

$$Productivity * Cell\ Density * Working\ Volume = Production\ Rate$$

Equation 4.9. Correlation between D_{opt} and K_s and S_o.

The cell density inside of the reactor and exiting the reactor was 6.24 x 10^8 cell/mL. With this current information, we could use the following equation and the goal seek function within excel to solve for the recycle flow rate:

$$D = \frac{\mu}{1 - a(b-1)}$$

Equation 4.10. Equation relating the dilution rate to a and b constants.

$$a = \frac{F_R}{F_o}$$

Equation 4.11. Equation for a constant in terms of the recycle flow rate and the inlet flow rate of new media.

$$b = \frac{\rho(0.99)(F_o + F_R - F_p)}{F_R(X_1)}$$

Equation 4.12. Equation for b constants related to the recycle flow rate and cell concentrations leaving the TFF.

From goal seek, we calculated a recycle flow rate of 1.03 L/min. With a total mass balance around the reactor, we were able to get a purge stream flow rate of 0.79 L/min.

4.1.4 Tangential Flow Filtration

Tangential Flow Filtration (TFF) is used for the harvest of pembrolizumab from the CHO cell culture. In TFF, a recirculation pump pushes the cell culture through a tangential flow filter cartridge that consists of many spiral hollow-fiber membranes. The cell culture is pumped through the hollow-fiber membranes, and the cell-free mABs and particulate matter smaller than the pore size of the membrane permeate out into the downstream process. The retentate, consisting of cells, is recirculated back into the perfusion reactor.

$$\mathrm{Re} = \frac{4Q}{\pi d \nu} < 2000$$

$$k_f = 1.62 \frac{D_0}{d} (\mathrm{Re})^{0.33} \left(\frac{\nu}{D_0} \right)^{0.33} \left(\frac{d}{L} \right)^{0.33}$$

$$\frac{\Delta P}{L} = \frac{2 f \rho u^2}{d} = \frac{32}{\pi^2} \frac{f \rho Q^2}{d^5}$$

$$f = \frac{16}{\mathrm{Re}}$$

Equations 4.13a-13d. Laminar Flow Equations Used to Calculate Pressure Drop in TFF System

For TFF, the Repligen KrosFlo KR2i TFF system will be used, consisting of a filter cartridge containing 12,500 MidiKros polyethersulfone spiral hollow fiber filters each with a pore size of 0.2 μm, the KR2i recirculation pump, and an automatic back pressure valve. This system can handle volumes from 2 mL to 15 L (Repligen, 2019). The filter cartridge is a single-use cartridge that can reduce product contamination and eliminate the need for additional sterilization procedures. The surface area of the filter cartridge is 81 cm^2. This was found through the use of Equations 4.13a-13d, which correlated laminar cross-flow velocity to filter cartridge

area length and pressure drop (Carta, 2019). Through the manipulation of these equations, the transmembrane pressure was found to be 1.15 psi, with a cross-flow velocity of 48 cm/min and a filter cartridge length of 20 cm. Although all cells should be recycled, an assumption is made that 1% of cells passing through the TFF system will be lysed and found in the permeate. A stream will be purged from the recycle stream, mainly to remove excess water added to the cell culture. This waste stream will be collected into a 5000 L waste holding tank along with other waste streams.

CHO cell cultures contain impurities such as host cell proteins, DNA, lipids, and endotoxins. Impurity estimates for the aforementioned were approximated using values from literature; all approximations were conservative overestimates based on these values. Studies have found that approximately 100 mg host cell proteins (HCPs) are secreted into the culture medium for every gram of product produced (Lavoie et al., 2019). DNA and lipids are also released into the cell culture when cells are lysed. Studies have shown that mammalian cells are reported to consist of 75% proteins, 12% nucleic acids, and 10% lipids by dry mass (Carta & Jungbauer, 2010). In order to obtain the impurity estimate of DNA and lipids contributed by lysed cells, the dry mass of CHO cells was estimated to be 2000 pg (Pan et al., 2017). It was also assumed that half of the lysed cells permeate out of the TFF system as cell debris, whereas the other half permeate out as DNA, lipids and other impurities. Endotoxins, shed by bacteria, are also found in mammalian cell cultures (Ryan, 2008). It was found that similar murine cell cultures can produce approximately 9.7×10^{-3} mg/L of endotoxins, so we conservatively estimated that 9.9×10^{-3} mg/L of endotoxins were present in the cell culture (Carta & Jungbauer,

2010). As advised by Professor Carta, it was assumed that only whole cells are recycled back into the reactor. It was assumed that cell debris would only continue in the permeate.

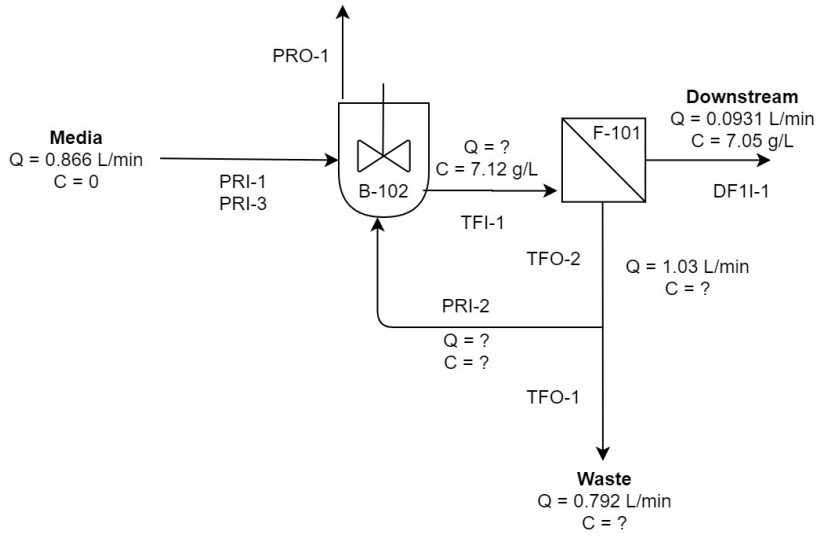


Figure 4.5. Unknown reactor and tangential flow filtration values

$$Q_{TFI-1} = Q_{PRI-1} + (Q_{TFO-2} - Q_{TFO-1}) = 0.886 + (1.03 - 0.792) = 1.13\ L/min$$
$$Q_{PRI-2} = Q_{TFO-2} - Q_{TFO-1} = 1.03 - 0.792 = 0.242\ L/min$$
$$C_{TFI-1}Q_{TFI-1} = C_{DF1I-1}Q_{DF1I-1} + C_{TFO-2}Q_{TFO-2}$$
$$(7.12\ g/L)(1.13\ L/min) = (7.05\ g/L)(0.0931\ L/min) + C_{TFO-2}(1.03)$$
$$C_{TFO-2} = C_{TFO-1} = C_{PRI-2} = 7.12\ g/L$$

4.1.5 Media Selection and Campaign Requirements

Cell growth constants were obtained from a study in infliximab producer CHO cells (López-Meza et al., 2016). In order to make the use of these numbers as accurate as possible, we are using the same nutrients used in this study. We will be feeding the cells Invitrogen CD OptiCHO AGT media diluted to 50 g/L, supplemented with 8 mM Invitrogen L-glutamine. This requires 2022 kg of AGT medium and 49.3 kg of L-Glutamine per 30-day campaign. Including the fermentation seed train and the continuous media inlet to the perfusion reactor, each campaign will require 39800 L of WFI.

4.2 Downstream Process

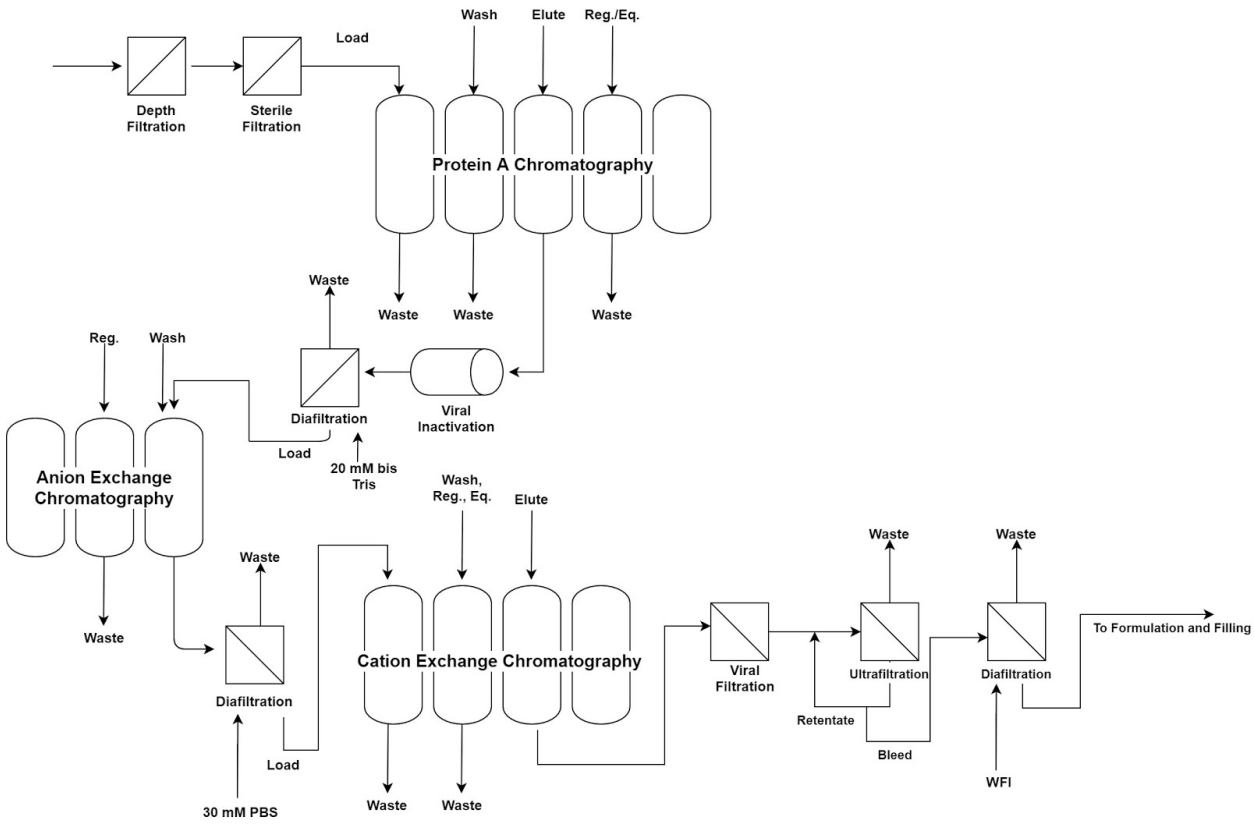


Figure 4.6. Basic process flow diagram for downstream process.

4.2.1 Depth Filtration

The depth filter is the first filter present in the downstream process. This filter has no defined pore size or structure. The filters separate impurities by having the particles entrapped or absorbed within the filter due to the random pore matrix found within the filter that creates a tortuous path through the filter.

The depth filter used in the experiment will be Millistak+® HC Pod Depth Filter, A1HC media series. The filter area will be 0.11 m^2 with a length of 62 cm. Based on literature the maximum operating pressure is 30.5 psi at 25^{o}C.

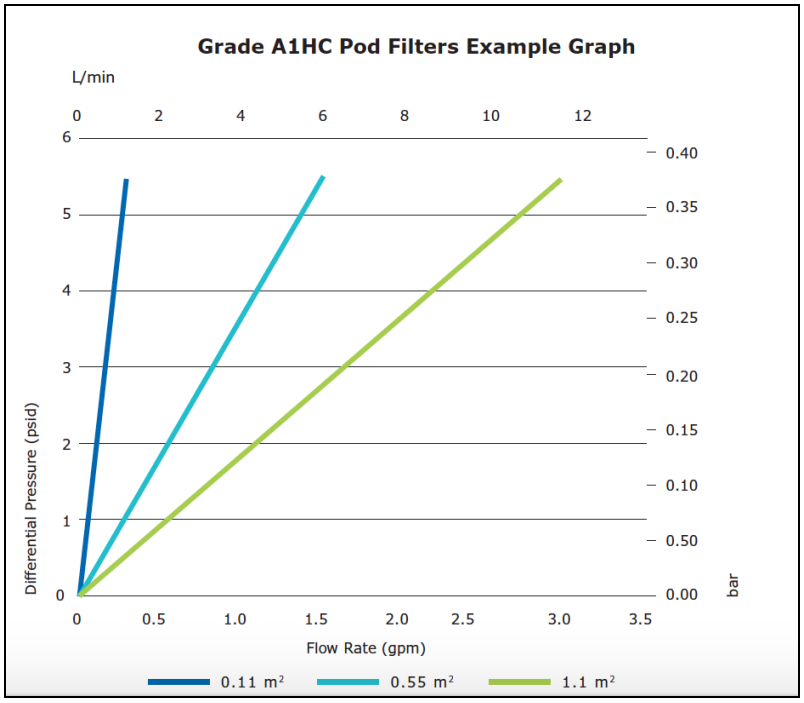


Figure 4.7. Experimental data relating flow rate to differential pressure in the depth filter system (Millipore, 2019).

From this graph, it can be seen that the flow rate across the depth filter at 0.093 L/min would lead to a pressure differential of approximately 0.4 psi or 0.028 bar. This is well below the maximum operating pressure of the system (Millipore, 2019). The depth filter will remove most of the cell debris from the product stream. Other components such as HCPs, lipids, and DNA were removed in fewer amounts through the depth filter due to their smaller size.

4.2.2 Sterile Filtration

Sterile filtration is used in order to prevent microbial contamination and to ensure patient safety. This part of the process only removes microorganisms from the product stream. Sterile filtration filters usually come in 0.20 µm or 0.22 µm sterilizing grade pore sizes. The maximum differential pressure of the system is 60 psi at 25 degrees Celsius.

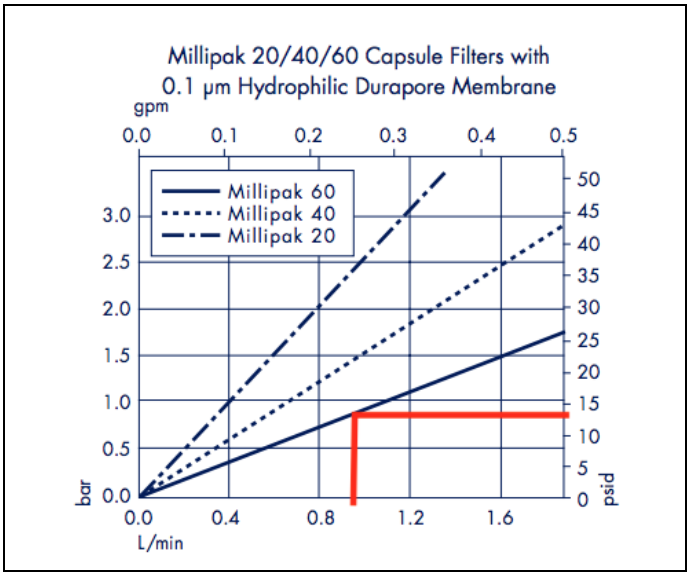


Figure 4.7. Experimental data relating flow rate to differential pressure in the sterile filter system (EMD Millipore, 2015).

The type of sterile filtration system used has a pore size of 0.1 µm and a length of 9.4 cm. The diameter of the sterile filter used is 76 mm and length of 9.4 cm. The filter area will be 300 cm^2. Through the experimental data in Figure 4.7, the differential pressure of the system at 0.093 L/min would be approximately 14 psi, falling well below the maximum differential pressure that the system can handle.

Due to the smaller pore size of 0.1 µm compared to other filters previously in the process such as the TFF we assumed that an even small percentage of lipids, DNA, and host cell proteins would be removed from the product stream.

4.2.3 Protein A Chromatography

Protein A chromatography is the reversible binding of antibodies to protein A ligands, effectively mobilizing the antibody on the stationary phase of the column. The ligands are derived from a strain of *staphylococcus aurenus* and are composed of five binding domains that are able to capture antibodies due to the attraction to mammalian species proteins, especially

immunoglobulin G (IgG). This IgG protein contains a region known as the Fc region which is targeted by the ligands of protein A with great specificity (Choe et al., 2016) Positive interactions between a component and ligand will result in retention of the component; otherwise, the component will pass through to waste. This process retains the mAb protein and is used to remove host cell proteins, DNA, and waste aggregates (Carta & Jungbauer, 2010). Protein A chromatography with high performing resins typically archives mAb protein purity greater than 99%. As the protein binds to the resin, eventually the ligands all become filled. When the resin's capacity, known as the dynamic binding capacity, has been reached an elution phase is implemented. By manipulating pH, which plays a role in determining the interaction strength between substances and the ligands, we can alter the interactions happening at the ligands. By using a low pH buffer in the elution phase the mAb protein is released and collected. The resin is then subjected to a regeneration protocol which removes any leftover substances and primes the ligands to once again accept mAb protein solution (Carta & Jungbauer, 2010).

We will perform this step using ThermoFisher science Poros MabCapture A Select Resin. This resin is a premier high throughput resin which has a high dynamic binding capacity at both low and high linear flow rates in addition to reduced bottlenecking compared to competitors (ThermoFischer, 2019)). This resin has an average particle size of 45 μm and a well defined dynamic binding capacity of 42.5 g/L resin at the desired residence time of 6 minutes seen in Figure 4.9. The Poros MabCapture A Select resin has a stable operating pH range of 2-10 (Thermo Scientific, 2019b).

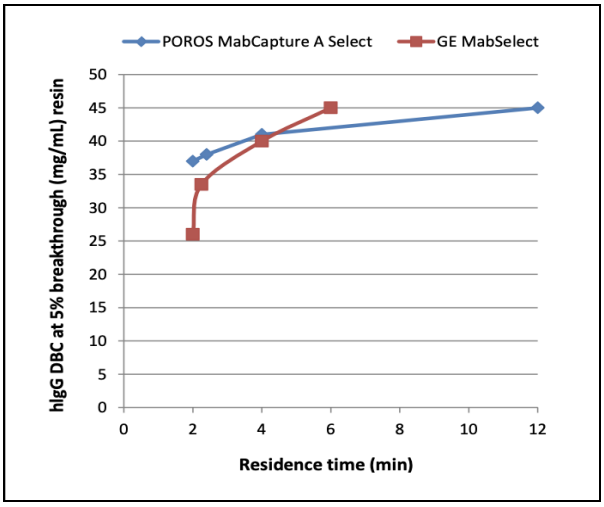


Figure 4.9. Dynamic Binding Capacity of Poros MabCapture A Select Resin by residence time (Thermo Scientific, 2019a)

The five distinct operational phases of the column in order are loading, washing, eluting, cleaning, and regeneration. Each of these uses a specific buffer based nature of the phase and identified in Table 4.4. The loading phase uses the incoming solution from previous separations when capturing of mAb onto ligand occurs. Washing and equilibrating will use a 20 mM bis Tris buffer. Elution utilizes a low pH to interfere with mAb-ligand interactions and release the captured protein for collection. Regeneration is an extension of this going to an even lower pH using 0.02 M phosphoric acid to remove all substances which remain interacting with resin ligands. Finally, the cleaning step utilizes a 0.1 M NaOH solution as recommended by the vendor to prolong the dynamic binding capacity lifetime of the resin (Thermo Scientific, 2019b).

Table 4.4. Buffers and respective column volumes for a given column process for Protein A

Phase	Buffer	Amount
Load	Filtrate from Sterile Filtration	6 CV
Wash	20 mM bis Tris buffer at pH of 5.8	5 CV
Elute	0.1 M Glycine HCl @ pH of 3.0	6 CV
Regenerate	0.02 M Phosphoric Acid @ pH of 2.05	5 CV
Equilibrate	20 mM bis Tris buffer at pH of 5.8	5 CV
Clean	0.1 M NaOH	5 CV

The inlet flow concentration was calculated using the required outlet concentration by integrating the resin yield by the number of column runs, the relationship of which is given in Figure 4.10. By using the inlet and outlet concentrations, the flow rate through the column required to maintain our production schedule was calculated and a linear flow rate that the resin is capable of handling, 0.06 m/min, was chosen. This allowed the calculation of the diameter of the column required as 4.45 cm. Using this diameter and a chosen residence time of 6 minutes, dynamic binding capacity, length, and volume of the column were calculated as 45 g/L, 36 cm, and 0.56 L. With the volume of resin and dynamic binding now known the total amount of incoming protein solution, at a concentration of 6.91 g/L, that is processed by the column in one load phase was found to be 10.9 L. The chosen column to purchase for this process is the XK 50/60 column. The column will be slightly modified to fit the exact dimensions of our process and a conservative increase in cost will be used to account for the modifications.

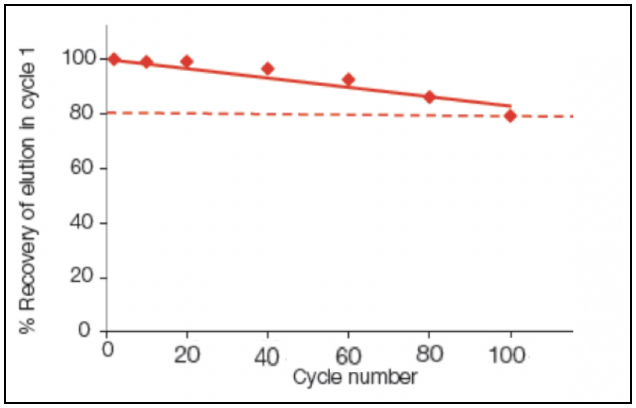


Figure 4.10. Effect of Cycle Number on Recovery of Monoclonal Antibody for Poros MabCapture A Select Resin (Thermo Scientific, 2019a).

$$Residence\ Time\ =\ L * U$$

Equation 4.14. Calculation for length (L) of a column required for chosen linear velocity (U) & residence time for desired dynamic binding capacity.

Assuming an extraparticular porosity of 0.3, the column pressure was approximated as 26.0 psi using the Carman-Kozeny correlation shown in Equation 4.15. This also falls within the pressure constraints of the XK 50/60 Column which can sustain pressures up to 72.5 psi (GE Healthcare, 2016).

$$\Delta P = \frac{150\,(1-\varepsilon)^2}{d_p^2 \varepsilon^3} L u \eta$$

Equation 4.15. The Carman-Kozeny equation is shown above to calculate column pressure.

To maintain continuous operations, five columns will be simultaneously operated with inlet and outlet concentrations being 6.91 g/L and 6.24 g/L, respectively seen Figure 4.11. The columns were designed to have one column continuously loading while another column continuously elutes. The other columns will be undergoing other steps including washing, regeneration, equilibrating or a wait time. A sixth prepared column will be held on standby for long-term cleaning or operating column malfunction needs. The proposed column diagrams are shown in Figure 4.11. The timing of the column process is diagrammed in an operating schedule below in Table 4.5.

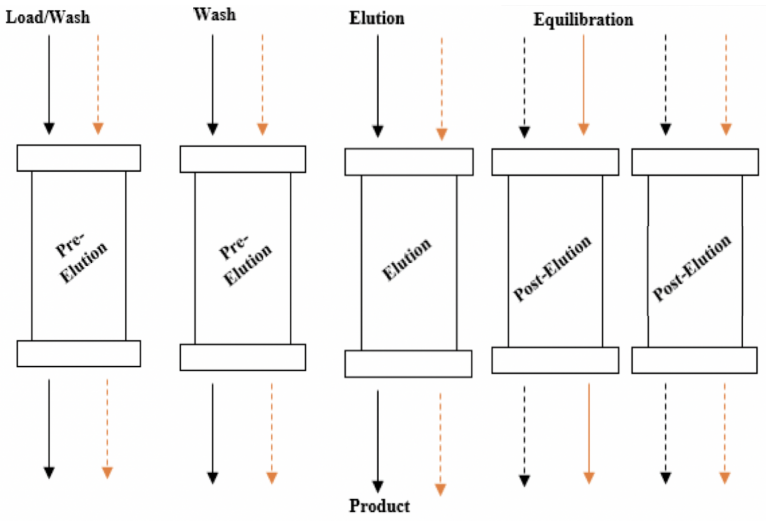


Figure 4.11. Proposed flow-through design for Protein A columns

Table 4.5. Proposed flow-through schedule for Protein A columns.

Column 1	100 Minutes					83.33 Minutes					100 Minutes					69.44 Minutes					69.44 Minutes					77.78 Minutes				
	Load					Wash					Elute					Regen					Equil					Wait				
Column 2	22.21 M	77.87 Minutes				100 Minutes					83.33 Minutes					100 Minutes					69.44 Minutes					47.23 Minutes				
	Equil	Wait				Load					Wash					Elute					Regen					Equil				
Column 3	52.97 M	69.44 Minutes				77.87 Minutes					100 Minutes					83.33 Minutes					100 Minutes					16.67 M				
	Regen	Equil				Wait					Load					Wash					Elute					Regen				
Column 4	83.33 Minutes					69.44 Minutes					69.44 Minutes					77.87 Minutes					100 Minutes					83.33 Minutes				16.67 M
	Elute					Regen					Equil					Wait					Load					Wash				Elute
Column 5	83.33 Minutes					100 Minutes					69.44 Minutes					69.44 Minutes					77.87 Minutes					100 Minutes				
	Wash					Elute					Regen					Equil					Wait					Load				

Only the eluting phase will be taken as a product, the washing phase will be discarded despite having a dilute amount of product in the stream. By not including the washing phase into the product collection, dilution of the product from its required concentration for downstream unit operations is avoided. Additionally, the processing time for a given column will be 500 minutes per column broken down into the following intervals: 100 minutes for loading, 83 minutes for washing, 79 minutes for regeneration, 70 minutes for equilibration, 100 minutes for elution, and 77 minutes for waiting. The total product collection time will be 500 minutes per column.

4.2.4 Viral Inactivation

Following the capture and elution of the mAb product through protein A chromatography, the product stream is subjected to a low pH for a period of 14.5 minutes. The purpose of this is to inactivate large enveloped viruses. Since the pH of the elution stream from protein A chromatography is approximately 3, a coiled flow inverter PFR with a minimum residence time of 15 minutes is the method that will be used to facilitate this step in a continuous manner. This 15 minutes will be sufficient according to a study done by Laura David that indicated that “Complete viral inactivation took place within the first 14.5 min for both

continuous and batch studies" (David et al., 2020). It has been suggested that a spiral design for the plug flow reactor would minimize the volume taken up by the PFR.

The coiled flow inverter accounts for axial dispersion and creates a nonuniform flow pattern seen in laminar flow. The stainless steel PFR will have dimensions of 570 cm linear length and 2.5 cm inner diameter. The flow rate through the tubes will be approximately 0.01 L/min yielding a Reynolds number of 30, well below the turbulent zone for flow through a pipe.

$$E(t) = \begin{cases} 0 & t < \frac{\tau}{2} \\ \frac{\tau^2}{2t^3} & t \geq \frac{\tau}{2} \end{cases}$$

Equation 4.16. The Residence Time Distribution Function for Laminar Flow Reactor.

Using this flow rate can cause uneven flow which would result in product heterogeneity (David et al., 2020). By using Equation 4.16 which gives the residence time distribution function for laminar flow through a pipe, the risk can be eliminated. The distribution function indicates that no liquid will have residence time less than half the mean residence time. Using Equation 4.17, it can be seen that the mean residence time for this case is just the spacetime, the volume divided by the volumetric flow rate. Given this, the mean linear velocity and length of the pipe cause the mean residence time to be 30 minutes and minimum residence time of 15 minutes.

$$t_m = \int_{\tau/2}^{\infty} tE(t)\,dt = \frac{\tau^2}{2}\int_{\tau/2}^{\infty} \frac{dt}{t^2}$$
$$= \frac{\tau^2}{2}\left[-\frac{1}{t}\right]_{\tau/2}^{\infty} = \tau$$

Equation 4.17. Solution for Mean Residence Time for Laminar Flow Reactor

4.2.5 Diafiltration for Anion Exchange Chromatography

Diafiltration is a technique used to replace the buffer species in which the protein of interest is suspended. The process uses a diafiltration membrane which allows the buffer species to pass through and retains the protein of interest. As the buffer species is drawn off in the permeate, a fresh replacement buffer is added (Millipore, 2003). Our process will use continuous diafiltration, also known as constant volume diafiltration, in which fresh buffer is added at the same rate as filtrate is produced. Discontinuous diafiltration, in which a fixed volume of fresh buffer is added, requires a larger volume of buffer to achieve the same purity when compared to continuous diafiltration. Seen in Figure 4.12, continuous diafiltration only requires 7 diavolumes to reach 99.9% buffer replacement, whereas discontinuous diafiltration requires 9 diavolumes to reach the same purity (Millipore, 2003). Thus, both production and waste costs will be reduced by employing continuous diafiltration. In continuous diafiltration, a diavolume is the ratio of the buffer flow rate to the feed flow rate. Therefore, in order to achieve 99.9% purity, the buffer flow rate must be 7 times that of the feed flow rate.

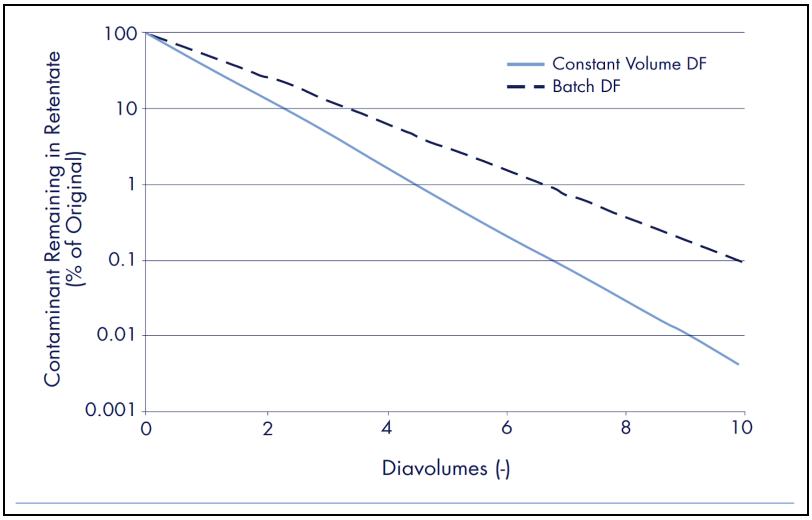


Figure 4.12. Retentate volume and buffer exchange during batch and constant-volume diafiltration (Millipore, 2003)

For all diafiltration unit operations, we will be using the Cadence Inline Diafiltration Module (Pall, 2020), which will be fitted with the appropriate Pall Delta 30 kDa T-series TFF Cassettes (Pall, 2019). This ensures that the 149 kDa protein will be retained with at least 99.9% purity, as confirmed by Professor Giorgio Carta. This module includes a manual control to dial in the number of diavolumes, and also allows for removal factors greater than 99.9%, thus giving the desired purities. Additionally, this diafiltration module includes smaller system hold-up volume and single-pass technology, both of which increase process yield due to minimized losses due to dead volume and shear. All filter calculations were made assuming no flux decay, no membrane caking, and no concentration polarization. These assumptions are valid as the flow rate is very slow, and thus the retained protein has sufficient time to diffuse back into the bulk fluid. These will also be held true for the remaining filtration steps of viral filtration and ultrafiltration.

Diafiltration will be utilized before anion exchange chromatography, before cation exchange chromatography, and before final formulation, for a total of 3 diafiltration modules, each of which is fitted with 12 filter cassettes. Each filter membrane will be exchanged every campaign. Although the membranes may be reused with proper cleaning and maintenance, we have opted to completely replace the membranes in order to ensure maximum product purity. This then requires 36 membranes to be purchased for each 30-day campaign, or 396 membranes per year. The details of the other diafiltration unit operations will be outlined later.

Prior to anion exchange chromatography, diafiltration will be used for buffer exchange such that the protein is suspended in a pH 5.8, 20 mM bis Tris buffer. The diafiltration module

will be fitted with twelve 186 cm^2 filter cassettes, for a total membrane area of 0.22 m^2. It will be operated at a pressure drop of 38 psi in order to produce 0.65 L/min of permeate. This pressure drop was selected in order to operate at 7 diavolumes for 99.9% buffer replacement. The conditions into diafiltration are 0.093 L/min and 6.24 g/L. The outlet conditions are 0.093 L/min and 6.20 g/L. For each 30-day campaign, a total of 28200 L of WFI and 80.0 kg of solid bis Tris will be needed, resulting in 28200 L of waste. The filters will result in 3.21 kg of waste. After this unit operation, the solution will enter anion exchange chromatography.

Anion Exchange Chromatography Diafiltration Calculations

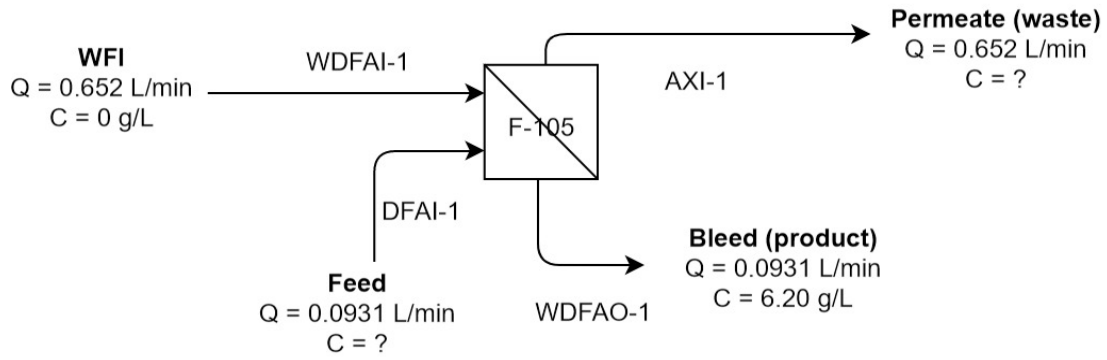


Figure 4.13. Unknown values for diafiltration prior to anion exchange chromatography

$$\sigma = 0.999$$

$$C_P = (1 - \sigma)C_B = (1 - 0.999)(6.201\ g/L) = 0.005\ g/L$$

$$C_F Q_F = C_P Q_P + C_B Q_B$$

$$C_F(0.0931\ L/min) = (0.005\ g/L)(0.6517) + (6.201\ g/L)(0.0931\ L/min)$$

$$C_F = 6.236\ g/L$$

4.2.6 Anion Exchange Chromatography

Anion exchange chromatography (AEX) is a form of ion-exchange chromatography that uses positively charged resins to bind negatively charged contaminants. These contaminants often include DNA, virus fragments, and host cell proteins (Carta, 2019). Processes involving

CHO cells are known to produce endogenous retroviruses or retrovirus-like particles (RVLPs). Additionally, the product has the potential to be infected by adventitious viruses during mAb production (Strauss et al., 2010). Whether or not a substance will bind to the resin is largely dependent on the ionic strength of the inlet solution and the pH of the inlet solution. For the purposes of mAb processing, pH is incredibly important. Proteins such as mAbs have isoelectric points (pI) which vary based on the combination of amino acids that form the protein. The basic and acidic amino acids work to produce an overall net charge on the protein. When the pH of the buffer is above the pI, the protein will have a net positive charge, and when the pH of the protein is below the pI, the protein will have a net negative charge. Many of the contaminants present at the AEX polishing stage are highly acidic with low pI's, and will, therefore, bind to the resin at a neutral pH. Contaminants at this stage often have a pI between 2 and 5 (Mayer et al., 2015) Pembrolizumab, however, has a pI between 6.8 and 6.9. In order to separate the slightly acidic mAb from the acidic contaminants, the AEX series will be operated in a flow-through mode. The buffer will have a pH below the pI of pembrolizumab but above the pI of common contaminants.

We will perform this polishing step using GE's Capto Q Resin. This resin is widely used in the polishing of mAbs, and it is the only strong anion exchange resin sold in bulk for the highly-productive purification of mAbs. We chose this resin based on its high inlet flow rate capacity, high volumetric throughput, and high dynamic binding capacity (DBC) (GE Healthcare, 2018b). This resin is composed of a rigid, high-flow agarose matrix modified with dextran surface extenders and a strong quaternary ammonium (Q) anion exchanger. This chemical structure allows the resin to maintain its positive charge over a wide variety of pH's (GE Healthcare, 2018b). This resin has a particle size of 90 μm, and we conservatively

approximated the dynamic binding capacity to be 124 g/mL for bovine serum albumin (see Figure 4.14). This resin can operate at pressures up to 58 psi and flow rates up to 0.23 m/min, whereas GE's previous Sepharose 6 Fast Flow Resin had a maximum velocity of 0.063 m/min (GE Healthcare, 2018a).

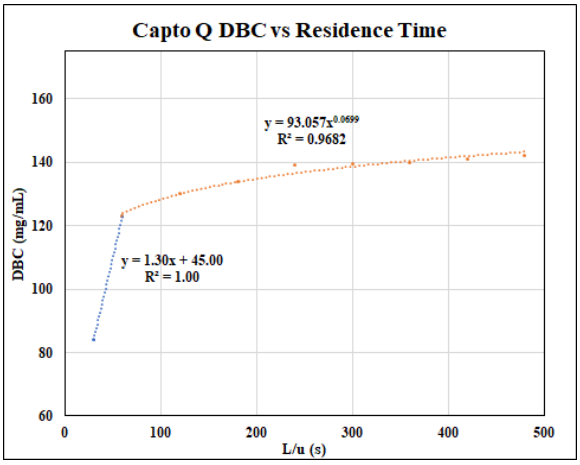


Figure 4.14. Dynamic binding capacity vs residence time (L/u) of BSA for GE Capto Q Resin.

In order to operate in flow-through mode, 20 mM bis Tris buffer at a pH of 5.8 was chosen based on recommendations from Professor Carta and literature (Carta & Jungbauer, 2010). This pH was chosen because it is 1 pH unit below the pI of the desired mAb whereas the convention for flow-through capability is typically 0.5 pH units below the pI (Carta & Jungbauer, 2010). The proposed buffers for the various column processing stages are shown below in Table 4.6.

Table 4.6. Buffers and respective column volumes for a given column process for AEX.

Phase	Buffer	Amount
Load	Product stream in 20 mM bis-Tris buffer at pH of 5.8	21 CV
Wash	20 mM bis Tris buffer at pH of 5.8	2 CV
Regenerate	20 mM bis Tris buffer at pH of 5.8 + 2M NaCl	5 CV
Equilibrate	20 mM bis Tris buffer at pH of 5.8	5 CV
Clean	0.1 M NaOH	5 CV

By assuming the DBC and choosing a linear velocity of 0.176 m/min, we calculated the column diameter, length, and volume to be 2.6 cm, 18 cm, and 0.09 L, respectively. The chosen column is the XK 26/20 Column provided by GE Healthcare. This column has an inner diameter of 2.6 cm with an adjustable bed height with a maximum height of 20 cm. Additionally, the load volume required was determined to be 11.1 L using Equation 4.18.

$$DBC_{10} = \frac{V_{load} C_F}{V_{col}}$$

Equation 4.18. Calculation for loading volume from dynamic binding capacity and column volume.

Assuming an extraparticular porosity of 0.3, the column pressure was approximated as 21.5 psi using the Carman-Kozeny correlation shown in Equation 4.15. This also falls within the pressure constraints of the XK 26/20 Column which can sustain pressures up to 72.5 psi (GE Healthcare, 2020d).

To maintain continuous operations, two columns will be simultaneously operated with inlet and outlet concentrations being 6.20 g/L and 5.81 g/L, respectively (see Figure 4.15). The columns were designed to have one column continuously loading/producing product while one column is regenerated to maintain process continuity. The section of the proposed flow-through schedule is shown in Table 4.7.

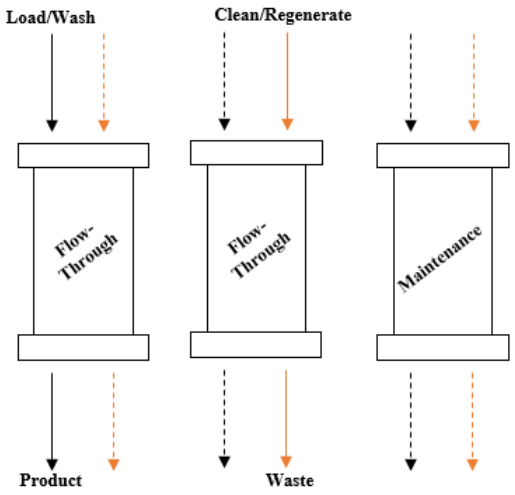


Figure 4.15. Proposed flow-through design for AEX columns

Table 4.7. Proposed flow-through schedule for AEX columns.

COL 1	5 CV	5 CV	WAIT		21 CV		2 CV
	u = 0.176 m/min				u = 0.176 m/min		
	CLEAN	REGEN			LOAD/COLLECT		WASH
COL 2	21 CV		2 CV	5 CV	5 CV	WAIT	
	u = 0.176 m/min						
	LOAD/COLLECT		WASH	CLEAN	REGEN		

Both the loading and the washing solutions will be collected as product, and as a result, the mAb concentration leaving the wash stage will be somewhat diluted. To account for this, we designed the AEX system such that the loading outlet concentration will be 6.20 g/L, and the washing concentration will be 3.04 g/L. By assuming ideal mixing during the subsequent diafiltration stages, the outlet concentration will be the desired 5.81 g/L. Additionally, the processing time for a given column will be 186 minutes per column broken down into the following intervals: 118 minutes for loading, 28 minutes for cleaning, 28 minutes for regeneration, and 11 minutes for washing. The product collection time will be 129 minutes per column.

4.2.7 Diafiltration for Cation Exchange Chromatography

Prior to cation exchange chromatography, diafiltration will be used for buffer exchange of bis Tris for pH 6, 30 mM PBS buffer. This unit operation will be carried out with the same assumptions as previously mentioned. The diafiltration module has a pressure drop of 38 psi and is fitted with 12 186 cm^2 filter cassettes, for a total membrane area of 0.22 m^2. This pressure drop was selected in order to operate at 7 diavolumes for 99.9% buffer replacement. The conditions into the first diafiltration filter are 0.093 L/min and 5.81 g/L. The outlet conditions are 0.0931 L/min and 5.78 g/L. For each 30-day campaign, a total of 28200 L of WFI, 21.0 kg of Na_2HPO_4-$7H_2O$, and 68.3 g of $NaH_2PO_4H_2O$ will be needed, resulting in 28150 L of waste. The filters will result in 3.21 kg of waste. After this unit operation the solution will enter cation exchange chromatography.

Cation Exchange Chromatography Diafiltration Calculations

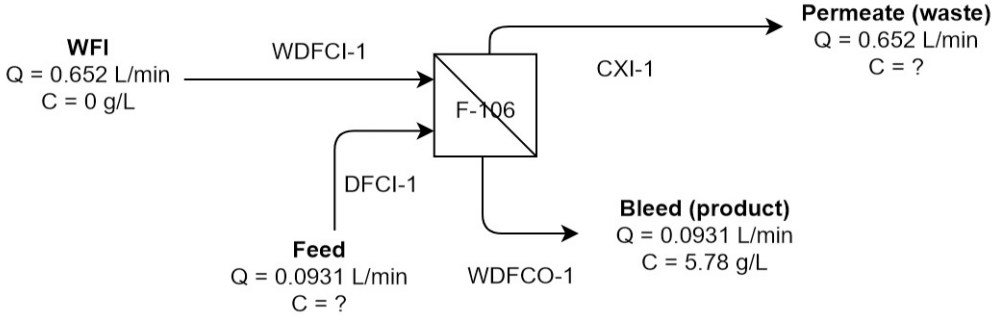


Figure 4.16. Unknown values for diafiltration prior to cation exchange chromatography

$$\sigma = 0.999$$

$$C_P = (1 - \sigma)C_B = (1 - 0.999)(5.781\ g/L) = 0.0044\ g/L$$

$$C_F Q_F = C_P Q_P + C_B Q_B$$

$$C_F(0.0931\ L/min) = (0.0044\ g/L)(0.6517) + (5.781\ g/L)(0.0931\ L/min)$$

$$C_F = 5.812\ g/L$$

4.2.8 Cation Exchange Chromatography

Cation exchange chromatography (CEX) is a form of ion-exchange chromatography that uses negatively charged resins to bind positively charged contaminants. These contaminants often include host cell proteins (HCPs), leached protein A resin, and waste aggregates (Carta, 2019). By using a buffer with a pH below the isoelectric point of pembrolizumab, the mAb will have a net positive charge and will then bind to the resin. As a result, gradient elution can be performed to separate the desired monomer from aggregates and other contaminants. Additionally, by implementing a buffer such as PBS, this design will minimize the risk of denaturing the mAb during the gradient salt elution.

We will perform this polishing step using GE's Capto S Impact Resin. This resin has proven very successful in the downstream purification of mAbs due to its high binding capacity, efficient aggregate removal, and small particle size (GE Healthcare, 2014). This resin has a particle size of 50 µm, and we conservatively approximated the dynamic binding capacity to be 88 g/mL for bovine serum albumin (see Figure 4.17). The Capto S Impact resin contains -SO_3 ligands, and it is operationally stable within the pH range of 4-12 (GE Healthcare, 2020b).

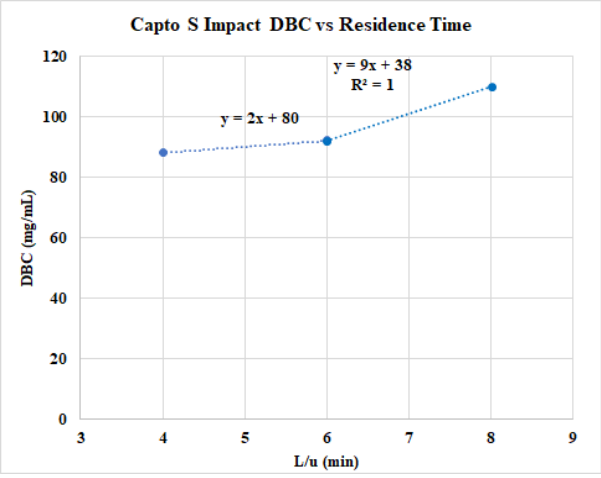


Figure 4.17. Dynamic binding capacity vs residence time for Capto S Impact resin.

Phosphate buffer was chosen for this step due to recommendations from Professor Carta as well as proven success in literature (Carta & Jungbauer, 2010). The specific buffers and the respective volumetric amounts required for a given step are shown below in Table 4.8.

Table 4.8. Buffers and respective column volumes for a given column process for CEX.

Phase	Buffer	Amount
Load	30 mM PBS Buffer @ pH of 6.0 + mAb	15 CV
Wash	30 mM PBS Buffer @ pH of 6.0	5 CV
Elute	Gradient with 30 mM PBS Buffer @ pH of 7.3	15 CV
Regenerate	30 mM PBS Buffer @ pH of 6.0 + 2 M NaCl	5 CV
Equilibrate	30 mM PBS Buffer @ pH of 6.0	5 CV
Clean	0.1 M NaOH	5 CV

By assuming the DBC and choosing a linear loading velocity of 0.047 m/min, we calculated the column diameter, length, and volume to be 5 cm, 19 cm, and 0.37 L, respectively. The chosen column is the XK 50/20 Column provided by GE Healthcare. This column has an inner diameter of 5.0 cm with an adjustable bed height with a maximum height of 20 cm. Additionally, the load volume required was determined to be 29.9 L using a similar approach to the AEX design.

$$DBC_{10} = \frac{V_{load} C_F}{V_{col}}$$

Equation 4.18. Calculation for loading volume from dynamic binding capacity and column volume.

Assuming an extraparticular porosity of 0.3, the column pressure was approximated as 19.7 psi using the Carman-Kozeny correlation shown in Equation 4.15. This also falls within the pressure constraints of the XK 50/20 Column which can sustain pressures up to 72.5 psi (GE Healthcare, 2020d).

To maintain continuous operations, three columns will be simultaneously operated with inlet and outlet concentrations being 5.78 g/L and 5.38 g/L, respectively (see Figure 4.18). The

columns were designed to have one column continuously loading while another column continuously elutes. The third column will be regenerating/equilibrating, and a fourth prepared column will be held on standby for long-term cleaning needs. The section of the proposed flow-through schedule is shown in Table 4.9. The inlet linear velocities were altered for the equilibration and washing stages in order to maintain the proposed continuous schedule.

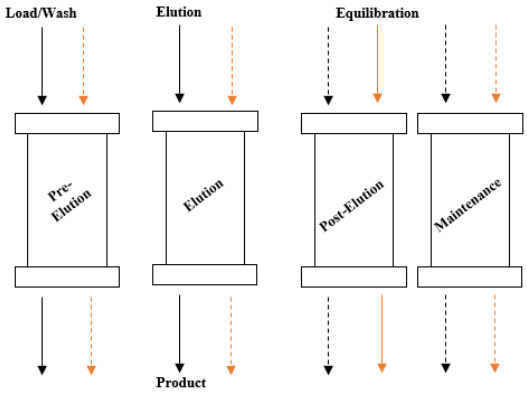


Figure 4.18. Proposed flow-through design for CEX columns.

Table 4.9. Proposed flow-through schedule for CEX columns.

COL 1	15 CV					5 CV	15 CV					5 CV	5 CV									WAIT
	u=0.06 m/min					u=0.095	u=0.06 m/min					u = 0.047 m/min	u=0.072									
	LOAD					WASH	ELUTE					REGEN	EQUIL									
COL 2	15 CV	5 CV		5 CV	WAIT	15 CV							5 CV	15 CV								
		u = 0.047 m/min		u=0.0569		u = 0.047 m/min							u=0.095	u = 0.047 m/min								
	ELUTE	REGEN		EQUIL		LOAD							WASH	ELUTE								
COL 3	5 CV	15 CV							5 CV	5 CV	WAIT	15 CV										
	u=0.095	u = 0.047 m/min							u = 0.047 m/min	u=0.0569		u = 0.047 m/min										
	WASH	ELUTE							REGEN	EQUIL		LOAD										

Additionally, the processing time for a given column will be 953 minutes per column broken down into the following intervals: 320 minutes for loading, 53 minutes for washing, 105 minutes for regeneration, 88 minutes for equilibration, 317 minutes for elution, 28 minutes for cleaning, and 70 minutes for waiting. The total product collection time will be 317 minutes per column.

4.2.9 Viral Filtration

Viral filtration is the final step in the viral clearance process. This operation utilizes a size-exclusion membrane that can then separate any remaining viral contaminants from the desired protein solution. This step is crucial to meet FDA regulatory requirements; however, after the robust chromatography stages, there are rarely viruses present at this point in the process. We chose to utilize a Virosart HF Mid-Scale Module Filter with an area of 200 cm^2. The assumptions made are that the flux is constant during processing time and that there is no change in protein concentration or yield loss. Additionally, using data provided by the vendor, the transmembrane pressure was found to be approximately 25.6 psi (see Figure 4.19). This was determined by using the inlet volumetric flow-rate of 0.093 L/min. The inlet and outlet concentrations of pembrolizumab are 5.37 g/L.

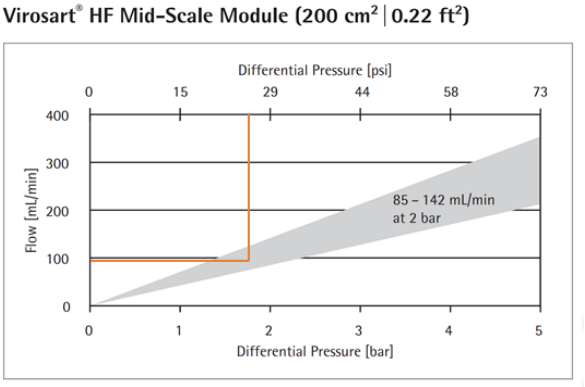


Figure 4.19. Flowrate and pressure information for the Virosart HF Mid-Scale Module Filter (Sartorius, 2019).

4.2.10 Final Ultrafiltration and Diafiltration

Ultrafiltration is used to concentrate a protein to the desired concentration by allowing the buffer to pass through the filter membrane while retaining the protein of interest. As the dilute feed flows into the ultrafiltration module, permeate is drawn off as waste, thus concentrating the retentate product. Typically, the retentate must pass over the filter several times

to obtain the desired protein concentration (Millipore, 2003). We will be using the GE Healthcare AKTA flux filtration hardware (GE Healthcare, 2020a), installed with the 177 cubic centimeters 30 kDa PLTK MilliporeSigma Ultracel Ultrafiltration Disc (Fischer Scientific, 2020). Since these are the same filters used for diafiltration, we can again assume 99.9% retention of the protein, since the protein is 149 kDa, and the membrane nominal molecular weight limit is 30 kDa. This ultrafiltration membrane will produce 0.074 L/min of permeate at the operating pressure of 10 psi. This operating pressure was selected as it is 25% of the maximum operating pressure, thus ensuring low membrane fatigue and shear. Each filter membrane will be exchanged every campaign. Although the membranes may be reused with proper cleaning and maintenance, we have opted to completely replace the membranes in order to ensure maximum product purity. This unit operation, therefore, requires 11 ultrafiltration membranes per year. The inlet conditions are 0.093 L/min and 5.38 g/L. The outlet conditions are 0.019 L/min and 25.7 g/L. After this unit operation, the solution will proceed to final diafiltration.

Ultrafiltration Calculations

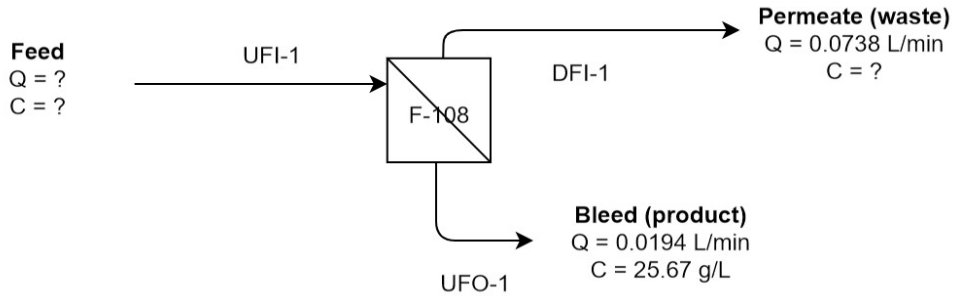


Figure 4.20. Unknown ultrafiltration values.

$$Q_F = Q_P + Q_B = 0.0738 \ + \ 0.0194 \ L/min \ = \ 0.0931 \ L/min$$
$$C_P = (1 - \sigma)C_B = (1 - 0.999)(25.67 \ g/L) \ = \ 0.0257 \ g/L$$
$$C_F Q_F = C_P Q_P + C_B Q_B$$
$$C_F(0.0931 \ L/min) = (0.0257 \ g/L)(0.0738 \ L/min) + (25.67 \ g/L)(0.0194 \ L/min)$$
$$C_F = 5.37 \ g/L$$

For final diafiltration, we will be exchanging the PBS buffer for WFI, which will be the final injection solution. This unit operation will be carried out with the same assumptions as previously mentioned. The diafiltration module has a pressure drop of 15.9 psi and is fitted with 12 93 cm^2 filter cassettes, for a total membrane area of 0.11 m^2. This pressure was selected in order to ensure that 7 diavolumes are being achieved for 99.9% buffer replacement. The conditions into the first diafiltration filter are 0.0194 L/min and 25.67 g/L. The outlet conditions are 0.0194 L/min and 25.0047 g/L. For each 30-day campaign, a total of 5900 L of WFI will be needed, resulting in 5900 L of waste. After this unit operation, the solution is pure enough for human injection and only needs some stabilizing additives.

Final Diafiltration Calculations

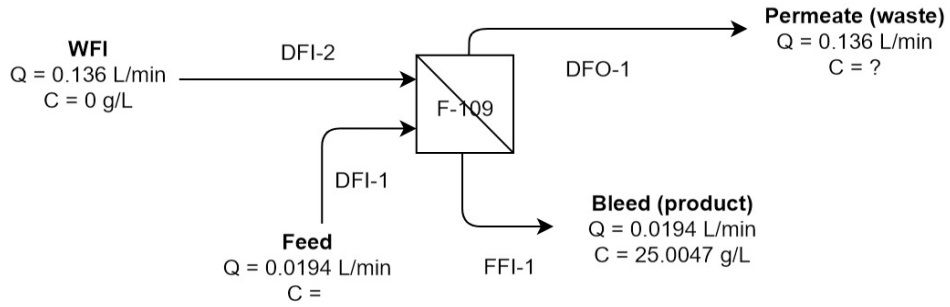


Figure 4.21. Unknown final diafiltration values

$$\sigma = 0.996$$

$$C_P = (1 - \sigma)C_B = (1 - 0.996)(25.0047\ g/L) = 0.095\ g/L$$

$$C_F Q_F = C_P Q_P + C_B Q_b$$

$$C_F(0.0194\ L/min) = (0.095\ g/L)(0.1358) + (25.0047\ g/L)(0.0194\ L/min)$$

$$C_F = 25.67\ g/L$$

4.2.11 Formulation and Filling

The final formulation will include 25 mg pembrolizumab, 70 mg sucrose, 1.55 mg L-histidine, and 0.2 mg polysorbate 80 per mL of solution, for a final protein concentration of 25 g/L (Merck & Co., 2019b). This solution is directly injectible to the patient. The final injection is formulated with these additives such that it can be preserved appropriately, and matches the osmolarity of human blood for safe injection. The sucrose, L-histidine, and polysorbate 80 additives will be added to the stream exiting diafiltration. As polysorbate 80 is a liquid with a density of 1.08 g/cm^3, the process outlet must have a slightly higher concentration of Keytruda at 25.0047 g/L (VWR, 2019).

The annual production rate has been determined to be 226 kg. Upon deciding 30-day campaigns, this leaves sufficient time for 11 full campaigns per year, in addition to a 2-week shutdown period, and 21 extra days in case of production malfunctions. In order to achieve 226 kg per year, each campaign must produce 20.55 kg of Keytruda, which we rounded to 21 kg per campaign in order to account for production losses and inefficiencies. Therefore, in order to produce 21 kg of Keytruda per 30-day campaign, the process requires 1302 g L-histidine, 168 g polysorbate 80f, and 58,800 g sucrose per campaign for final formulation. This production rate also indicates an outlet Keytruda mass flow rate of 0.49 g/min and a volumetric flow rate of 0.019 L/min prior to the addition of the final formulation additives.

Formulation and Filling Calculations

$$\frac{25\ g\ Keytruda}{L} = \frac{0.025\ kg}{L} = \frac{21\ kg}{x}\ solve\ for\ x = 840\ L\ injectable\ solution\ per\ campaign$$

$$\frac{168\ g\ polysorbate}{campaign} * \frac{mL}{1.064\ g} * \frac{L}{1000\ mL} = 0.158\ L\ polysorbate\ per\ campaign$$

$$\frac{21{,}000\ g\ Keytruda}{840 - 0.158\ L} = 25.0047\ g/L\ exiting\ final\ diafiltration$$

4.3 Ancillary Equipment

4.3.1 Pump Design

As all flow rates are significantly less than 1 L/s, we will only be employing the use of peristaltic pumps throughout the entire production facility. All intermediate and waste streams will only require the use of 1 pump. Media and buffer streams will require 3 pumps, 1 required to pump WFI to the initial mixing tank, 1 required to pump from the initial mixing tank to the main holding tank, and 1 required to pump the solution into the appropriate unit operation. Each unit operation requiring a pressure differential will also require an additional pump. These unit operations include all filtration and chromatography modules. In total, this process requires 66 pumps. We will have 20 pumps on standby in case of system pump failure.

Each pump will be assumed to have a 0.5 atm or 7.25 psi loss through pipes. Additionally, since all pipe lengths are unknown, and the flow rates are so small, each flow stream will be assumed to require a 1 atm, or 14.7 psi pressure in order to create flow from one unit operation to another. This pressure was selected as it is low enough to prevent high shear effects on the product streams, but it is high enough to provide movement for the small volumetric flows. Although it may seem peculiar that the pressure to create flow is higher than the pressure drop across some unit operations, this is because we overestimated the pressure needed to create flow in order to remain conservative in our calculations. Each pump will be assumed to have a 70% shaft efficiency and 70% electrical driver efficiency due to the small power requirements of each pump.

Table 4.12. Power requirements for all pressure pumps.

Pressure Pumps					
Module	**Flow rate (L/min)**	**Pressure (PSI)**	**Power (Watts)**	**70% Shaft Efficiency (W)**	**70% Electrical Driver Efficiency (Watts)**
TFF	1.13	1.15	0.149	0.213	0.304
Depth	0.093	15.1	0.162	0.231	0.33
Sterile	0.093	14	0.15	0.214	0.306
Protein A	0.42	26	1.25	1.79	2.56
AEX DF	0.745	38	3.25	4.65	6.64
AEX	0.134	25.1	0.385	0.551	0.787
CEX DF	0.745	38	3.25	4.65	6.64
CEX	0.247	19.7	0.559	0.799	1.14
VF	0.093	25.7	0.275	0.393	0.561
UF	0.093	10	0.107	0.153	0.218
Final DF	0.019	15.9	0.035	0.0506	0.0723
Air filter	63	2.9	21	30	42.9
				Total (W) =	**62.4**

Table 4.13. Power requirements for all flow pumps.

Flow Pumps						
Stream	**Flow rate (L/min)**	**Pressure (PSI)**	**Pumps Needed**	**Power (Watts)**	**70% Shaft Efficiency (W)**	**70% Electrical Driver Efficiency (W)**
1	0.886	22	3	6.73	9.61	13.73
2	63	22	2	319	456	651
3	1.13	22	1	2.86	4.08	5.83
4	1.03	22	1	2.62	3.74	5.35
5	0.792	22	1	2.01	2.87	4.1
6	0.242	22	1	0.613	0.876	1.25
7	0.0932	22	1	0.236	0.337	0.482

8	0.0932	22	1	0.236	0.337	0.482
9	0.0932	22	1	0.236	0.337	0.482
10-13	0.326	22	12	9.92	14.2	20.3
14	0.326	22	1	0.827	1.18	1.69
15	0.0932	22	1	0.236	0.337	0.482
16	0.0932	22	1	0.236	0.337	0.482
17	0.652	22	3	4.95	7.07	10.11
18	0.652	22	1	1.65	2.36	3.37
19	0.0932	22	1	0.236	0.337	0.482
20-23	0.0405	22	12	1.23	1.76	2.51
24	0.0405	22	1	0.103	0.147	0.209
25	0.0932	22	1	0.236	0.337	0.482
26	0.652	22	3	4.95	7.07	10.11
27	0.652	22	1	1.65	2.36	3.37
28	0.0932	22	1	0.236	0.337	0.482
29-31	0.154	22	9	3.5	5	7.15
32	0.154	22	1	0.389	0.556	0.794
33	0.0932	22	1	0.236	0.337	0.482
34	0.0932	22	1	0.236	0.337	0.482
35	0.0737	22	1	0.187	0.267	0.381
36	0.0194	22	1	0.0491	0.0702	0.1
37	0.136	22	1	0.344	0.491	0.702
38	0.136	22	1	0.344	0.491	0.702
39	0.0194	22	1	0.0491	0.0702	0.1
					Total (W) =	**748**

$$Power\ (Watts)\ =\ Flow\ rate\ (m^3/s) * Pressure\ (Pa) * Pump\ quantity$$

Equation 4.19. Pump power requirement

4.3.2 Tank Design

Holding tanks will be used in the process to store and mix material which will be added to the process, primarily buffers, as well as provide an emergency holding location for any

unforeseen complications in the continuous bioprocessing chain of unit operations. The holding tanks will be designed to hold the amount of buffer equal to ~1/4th of the amount (depending on commercial availability of holding tank sizes) of material needed for an entire 30 day campaign. As the buffer is drained over time, it will be replaced by a CSTR which mixes the solid additive with WFI to create more buffer. The CSTR is designed to be ~1/10th the size of the holding tank it feeds. A system of 5 waste tanks will be used to collect and denature the liquid waste from various unit operations in the continuous biomanufacturing process. All tanks used will be the ThermoFisher HyPerforma Single-Use Mixers, which are stainless steel tanks with single-use bags (ThermoFischer, 2020). Each tank will be 5000 L in order to accommodate the approximate volume of 3786 L of waste per day collected and held for a sufficient period of time. The comprehensive list of holding and mixing tanks can be seen in Table 4.10:

Table 4.10.Holding tank descriptions for pembrolizumab manufacturing facility.

Tank Identifier	Volume (L)	Type	Contents	Purpose
Upstream, Waste, & Maintenance				
T1	1600	Storage	Content from Bioreactor	Upstream Emergency Holding
T2	300	Mixing	0.1 M NaOH	Column Cleaning
T3	5000	Storage	Waste Content	Waste Removal
T4	5000	Storage	Waste Content	Waste Removal
T5	5000	Storage	Waste Content	Waste Removal
T6	5000	Storage	Waste Content	Waste Removal
T7	5000	Storage	Waste Content	Waste Removal
Protein A Chromatography				
T8	1000	Storage	0.1 M Glycine HCL Buffer	Elution Main Tank

T9	1700	Storage	20 mM Bis Tris Buffer	Washing/Equilibration Main Tank
T10	850	Storage	0.02 M Phosphoric Acid	Regeneration Main Tank
T11	100	CSTR	0.1 M Glycine HCL Buffer	Elution Mixing Tank
T12	170	CSTR	20 mM Bis Tris Buffer	Washing/Equilibration Mixing Tank
T13	85	CSTR	0.02 M Phosphoric Acid	Regeneration Mixing Tank
T14	50	Storage	Empty	Emergency Holding
Diafiltration for Anion Exchange Chromatography				
T15	5000	Storage	20 mM Bis Tris Buffer	Buffer Exchange Main Tank
T16	500	CSTR	20 mM Bis Tris Buffer + WFI	Buffer Mixer
Anion Exchange Chromatography				
T17	350	Storage	20 mM Bis Tris Buffer	Washing/Equilibration Main Tank
T18	250	Storage	20 mM Bis Tris Buffer + 2.0 M NaCl Mixture	Regeneration Main Tank
T19	35	CSTR	20 mM Bis Tris Buffer + WFI	Washing/Equilibration CSTR
T20	25	CSTR	20 mM Bis Tris Buffer + 2.0 M NaCl Mixture + WFI	Regeneration Main CSTR
T21	50	Storage	Content from AEX	Emergency Holding
Diafiltration for Cation Exchange Chromatography				
T22	5000	Storage	30 mM PBS Buffer	Buffer Exchange Main Tank
T23	500	CSTR	30 mM PBS Buffer	Buffer Mixer
Cation Exchange Chromatography				
T24	550	Storage	30 mM PBS Buffer	Elution Main Tank

T25	1850	Storage	30 mM PBS Buffer	Washing/Equilibration Main Tank
T26	350	Storage	30 mM PBS Buffer + 2.0 M NaCl Mixture	Regeneration Main Tank
T27	55	CSTR	30 mM PBS Buffer	Elution Mixing Tank
T28	185	CSTR	30 mM PBS Buffer	Washing/Equilibration Mixing Tank
T29	35	CSTR	30 mM PBS Buffer + 2.0 M NaCl Mixture	Regeneration Mixing Tank
T30	50	Storage	Content from CEX	Emergency Holding
Final Ultrafiltration & Diafiltration				
T31	5000	Storage	WFI	Buffer Exchange Main Tank

4.4 Water for Injection (WFI) System Design

All unit operations requiring water must use water-for-injection (WFI), as the final product will be a direct intravenous injection into humans. Therefore, all inlets into the process must be of at least 99% purity, and federal regulations enforce that all water entering the system must comply with WFI standards. WFI is a sterile, nonpyrogenic, solute-free preparation of water. It is most commonly used in laboratory benchwork experiments, as a parenteral injection dilution solution, or for use in pharmaceutical processes to ensure process purity (Baxter Corporation, 2014). By using WFI in our Keytruda manufacturing process, we can further control the impurities within the process, and therefore better ensure final product quality. Table 4.11 below shows the requirement by unit operation. This pharmaceutical facility will require 3 L/min of constant WFI or 132,000 L WFI per 30-day campaign.

Table 4.11. WFI requirements per campaign

WFI requirements	
Module	**L water**
Fermentation	39800
Protein A	14100
AEX DF	28150
AEX	5290
CEX DF	28150
CEX	10800
Final DF	5870
Total	132160

We will be utilizing the Meco Vapor Compression Distillation Unit with a storage capacity of 6000 L (MECO, 2019). The total WFI flow rate into our system is an average of 3.00 L/min. Although we were not able to obtain the specific power requirement of this water purification system, similar systems typically require 8 kWh per metric tonne of water (Lokiec & Ophir, 2007). This storage unit will be employed should there be any shortages of WFI or additional requirements due to process letdown. Should there be a problem with the water source, such as higher than manageable metal or endotoxin contamination, the storage tank should keep the process supplied with WFI for as long as 1.39 days, which should be sufficient time to shut down the process if the water supply does not normalize within a reasonable time frame.

This unit operation utilizes vapor compression distillation technology. Vapor compression distillation condenses and compresses evaporated water in order to form distillate. It is the oldest and most commonly used form of creating water for injection. The process begins when water is

heated by the distillate cooler, blowdown cooler, and feed heater via heat exchange. This heated water is then sprayed downwards in the decarbonator tank onto rising vent vapor, which strips non-condensable components of the water. Water then evaporates as it passes over steam-heating coils within the evaporator. Some liquid water is purged at this stage for process control. Within the compressor, a de-mister then removes entrained water droplets from the vapor. The vapor then condenses while simultaneously vaporizing new water with its latent heat, giving this apparatus high energy efficiency. The condensate, or distillate, is collected in the distillate collection box and pumped through a cooler and out of the module for use (MECO, 2019). It is important to note that this system comes with all necessary system pumps, and we will therefore not perform pump design regarding the WFI module.

4.5 Air Filtration Design

The air sparged into the bioreactor must be purified in order to reduce the chance of contamination in the final product. Therefore, at a flow rate of 63 L/min of air into the bioreactor, the Sartorius Sartopore size 4 module air filter must be operated at a pressure of 200 mbar, or 2.9 PSI, as can be seen in Figure 4.22 below. Each filter has an area of 0.016 m^2. The filter will be replaced once every 30-day campaign (Sartorius, 2019).

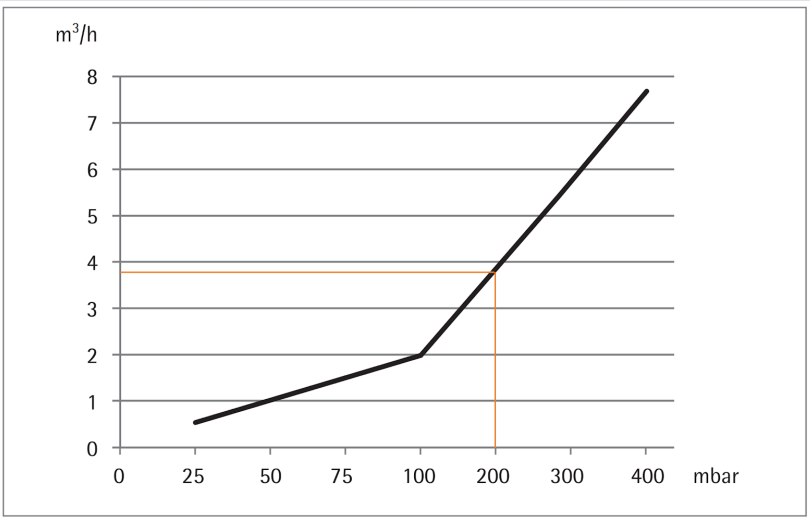


Figure 4.22. Flowrate and pressure information for the Sartopore Size 4 Capsule Air Filter (Sartorius, 2019).

4.6 Disposal

4.6.1 Liquid Waste

Liquid waste from each process will be collected in a 5000 L holding tank until the tank is 90% full, then treated with solid NaOH to denature any viruses present. Another 5000 L tank will be swapped into the process, to collect the waste stream. After waiting 5 days for all viruses to be denatured through the addition of NaOH, HCl will be added to neutralize the solution in the treated holding tank. This holding tank containing the final treated waste solution will be emptied into an existing local wastewater treatment system. 90% of the tank will be filled with waste solution, and the remaining 10% will be filled with equal parts NaOH and HCl. The total amount of liquid waste produced from this plant is detailed in Section 5.3.1. 5 5000 L tanks will be used and cycled through this process so that each tank can be treated for 5 days. Stream flow rates are detailed in Sections 5.6.1 and 5.6.2. All liquid waste will be treated by the Veolia Ireland waste management services in Dublin, Ireland.

4.6.2 Solid Waste

Solid waste includes used bioreactor bags, impellers, resins, filters, and filter cartridges. It is estimated that this plant will produce 567 kg of solid waste produced per campaign. Nonhuman cell lines are classified as biohazardous, so laboratory waste items that come into contact with such biohazardous material must also be disposed of as biohazardous waste as per regulation (University of Washington, 2020). This includes used bioreactor bags and impellers, filters, resin, and TFF cartridges. This waste, along with other single-use components that do not come in contact with such biohazardous material will be outsourced to a local autoclave cost center in Carlow, Ireland for autoclaving and sterilization procedures necessary. All solid waste will be treated by the Veolia Ireland waste management services in Dublin, Ireland.

4.7 Plant Scale Market Calculations

4.7.1 Market Analysis

Keytruda is a cancer treatment that works as a checkpoint inhibitor targeting the PD-1/PD-L1 pathway. Right now, there are 2 other checkpoint inhibitors targeting the PD-1/PD-L1 pathway, which are Opdivo manufactured by Bristol-Myers Squibb, and Libtayo manufactured by Regeneron. The prices of Keytruda, Opdivo, and Libtayo, when compared across the same website, were found to be $51.79, $28.86, and $27.17 per mg, respectively (Drugs.com, 2019). These prices will be assumed to be uniform throughout the world, as they are only accessible in developed countries. Despite being nearly double the price of competing drugs, Keytruda currently dominates the market over these other checkpoint inhibitors. From the third quarter of 2018 to the third quarter of 2019, Keytruda revenue grew 67%, reaching sales of $3.2 billion, whereas Opdivo sales only increased by 1%, reaching $1.8 billion (Erman, 2019).

Libtayo is several years behind both Keytruda and Obdivo (Biopharma Dive, 2019). As Keytruda continues to dominate the market despite being significantly more expensive, it can be expected to remain competitive when the patent expires in 2028 and biosimilars hit the market (Carven et al., 2013). Additionally, a risk analysis case study performed later in this paper will demonstrate that Keytruda sales will remain extremely profitable despite changes in cost, further proving that this is a sound project. Not only does Keytruda dominate the other checkpoint inhibitors on the market right now, but Keytruda is set to become the world's top-selling drug by 2025 as it is approved for increasing oncology indications and as worldwide cancer cases rise (Keown, 2019). It is also important to mention other forms of cancer therapy, such as radiation therapy, chemotherapy, hormone therapy, stem cell transplant, and surgery. Thus far, Keytruda has been approved for over 20 oncology indications, however, each type of cancer is extremely unique, and Keytruda may therefore not be the right treatment for patients with the appropriate cancer. Although there is market competition for Keytruda, it's recent successes and market projections indicate that sales should remain strong, even past the patent expiration.

5. Final Design

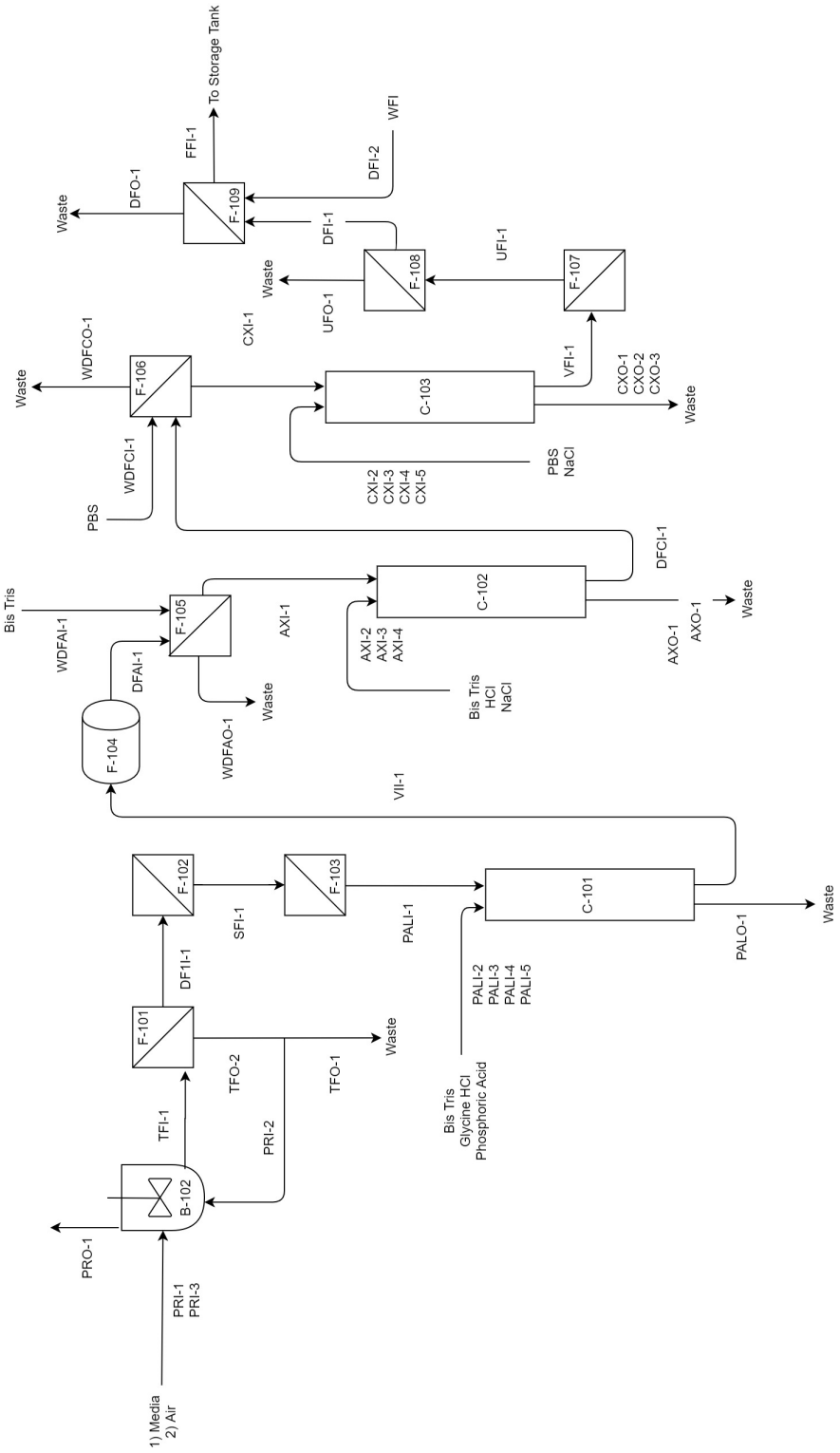


Figure 5.1. Process flow diagram of Keytruda manufacture.
*Indicates process streams are not added continuously.

5.1 Upstream Process

5.1.1 Cell Line Acquisition and Storage

The CHO cell line will be obtained from Merck's facility in West Point, PA. This will be used to develop our own master cell bank and subsequent working cell banks. These vials will be stored in-flight and at the manufacturing facility at -87°C in a VIP Series Model MDF-U76VC-PA Freezer (F 100). The WCB cell vials will be held as high-density vials consisting of a cell density of 50 x 10^6 cell/mL with each vial containing 4.5 mL.

5.1.2 Inoculum Train

This scale-up will utilize a 20 L Wave reactor (B 101) and an XDR 50/2000 bioreactor (B 102) with variable single-use bags. To begin the initial Wave reactor process, 15 L of WFI will be added in addition to the 4.5 mL vial. The initial cell concentration will be 0.03 g/L, and 750 g of solid media will be added to bring the initial substrate concentration to 50 g/L. After 197 hours, the broth will be transferred to the 400 L bag in the XDR reactor. The outlet concentrations of CHO cells, media, and pembrolizumab leaving the 15 L process will be 53.9 g/L, 14.5 g/L, and 8.88 g/L, respectively. After the addition of 19,800 g of media and dilution with 385 L of WFI, the starting substrate concentration will return to 50 g/L. The starting concentrations of CHO cells, media, and pembrolizumab will be 11.8 g/L, 50 g/L, and 2.05 g/L, respectively. After 284 cumulative hours, the broth will be transferred to the 900 L bag in the XDR reactor. The outlet concentrations of CHO cells, media, and pembrolizumab leaving the 400 L process will be 57.5 g/L, 10.0 g/L, and 10.0 g/L, respectively.

After the addition of 41,000 g of media and dilution with 500 L of WFI, the starting substrate concentration will return to 50 g/L. The starting concentrations of CHO cells, media,

and pembrolizumab for the 900 L process will be 19.9 g/L, 50 g/L, and 3.46 g/L, respectively. After 303 cumulative hours, the broth will be transferred to the 1500 L bag in the XDR reactor. The outlet concentrations of CHO cells, media, and pembrolizumab leaving the 900 L process will be 59.6 g/L, 10.0 g/L, and 10.4 g/L, respectively.

After the addition of 48,000 g of media and dilution with 600 L of WFI, the starting substrate concentration will return to 50 g/L. The starting concentrations of CHO cells, media, and pembrolizumab for the 1500 L batch process will be 33.6 g/L, 50 g/L, and 2.28 g/L, respectively. After 308 cumulative hours, the 1500 L reactor will be directed to begin perfusion operations. A perfusion run will then run for a cumulative 30 days for a given campaign. The outlet concentrations of pembrolizumab leaving the perfusion process will be 7.12 g/L.

5.1.3 Perfusion Reactor

The bioreactor that will be used for this is the Xcellerex XDR 2000 Single-use stirred-tank bioreactor (B 102) operating in perfusion mode. The reactor has a diameter of 122 cm and height 183 cm giving a total volume of 2000 L. The perfusion campaigns will be run with a 1500 L constant working volume. Single-use components include the bag linings and impeller which magnetically seal. The impeller diameter and height are 42 cm and 61 cm respectively. The impeller will be run at 550 revolutions per minute in conjunction with a 0.042 vvm (or 63 L/min) airflow rate to achieve the required $K_L a$ of 102.4 hr^{-1}. Excess CO2 and Nitrogen will be removed with striping spargers. A single perfusion reactor will be used for a given 30-day campaign. As per the Production Schedule, the subsequent inoculation train for the next campaign will start prior to the end of the current campaign so that after a 30-day period,

one perfusion reactor may be shut down, and the second perfusion reactor may start at the same time to provide complete continuity.

5.1.4 Tangential Flow Filtration

The Repligen KrosFlo KR2i TFF system (F 101) will be used. This system is capable of handling process volumes of 2 mL-15 L. Based on the kinetics of the bioreactor, the recycle flow rate leaving the TFF and entering the perfusion reactor was designed to be 1.03 L/min. The permeate flow rate out of the TFF was designed to be 0.093 L/min. Pembrolizumab enters the TFF system at a concentration of 7.12 g mAb/L and exits at 7.05 g mAb/L. The mAb is recycled back into the perfusion reactor at a concentration of 7.12 g mAb/L. The disposable TFF cartridge will be replaced after every campaign (approximately 30 days).

5.2 Downstream Process

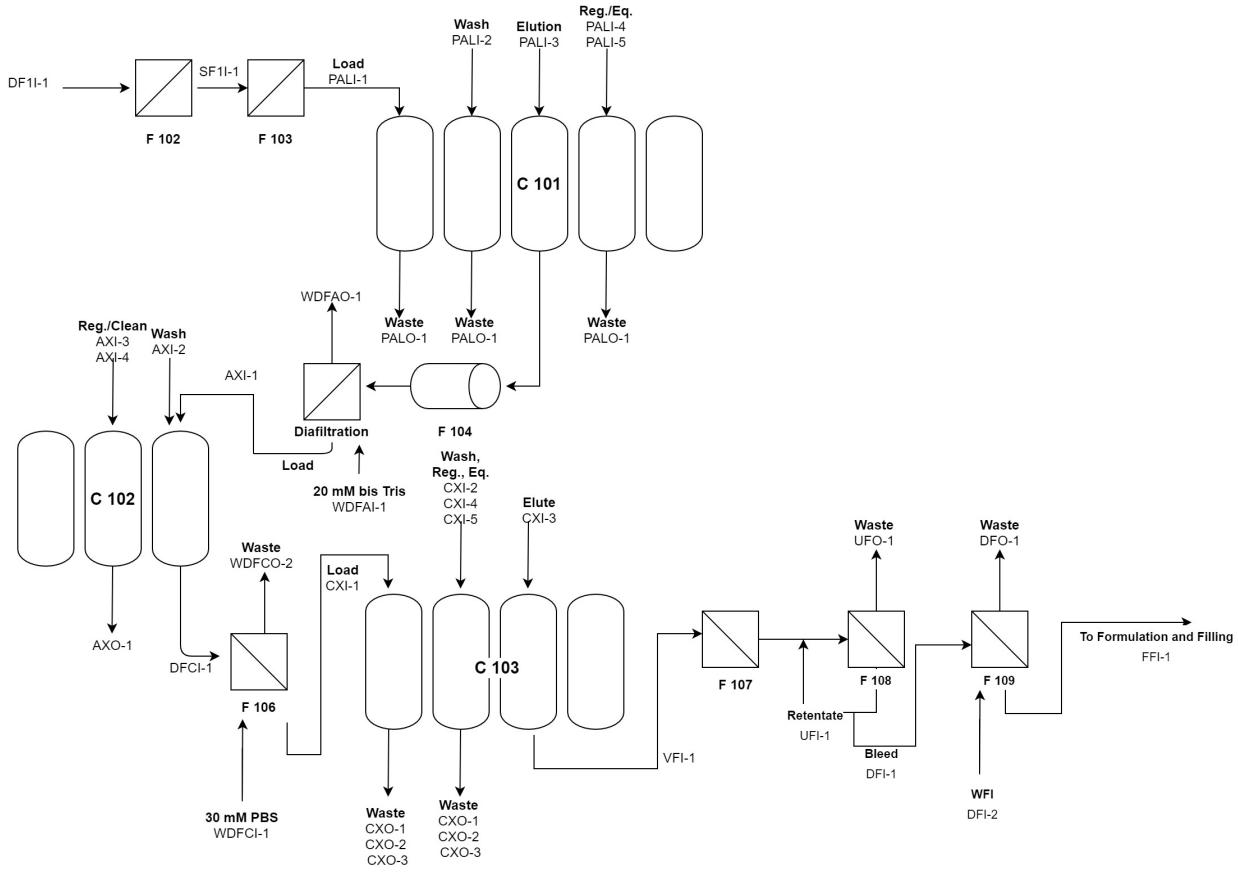


Figure 5.2. Final downstream process flow diagram with superimposed stream and equipment labels. See section 5.6.2 for stream compositions.

5.2.1 Depth Filtration

The depth filter used in our process will be the disposable Millistak+® HC Pod Depth Filter (F 102), A1HC media series. The filter is 0.11 m^2 and the length is 62 cm. The filter will be changed after every 30-day campaign and will have a product stream flowing in and out of the filter at 0.093 L/min.

5.2.2 Sterile Filtration

The sterile filter that will be used is the Millipak 20 Disposable Filter Units (F 103). The filter will be replaced after every campaign. The dimensions of the filter used will be a filter area of 300 cm^2 with a length of 9.4 cm. The diameter of the filter will be 7.6 cm. The pore size of this filter is 0.1 μm. The product stream flowing through the filter will be at 0.093 L/min.

5.2.3 Protein A Chromatography

This cation exchange chromatography step will utilize 5 modified XK 50/60 columns which will be purchased from GE Healthcare. GE Healthcare provides the XK columns in a variety of sizes and the XK 50/60 is the closest in specification requiring only a 0.5 cm reduction in diameter and 24 cm reduction in length. An increase in pricing has accounted for the custom nature of the column. The column (C 101) will operate at a pressure of 26.0 psi with a diameter of 4.5 cm and a height of 36 cm. The chosen resin will be the Poros MabCapture A Select purchased from ThermoFischer science. This process will operate in a bind and elute mode with an inlet and outlet volumetric flow rate of 0.093 L/min. The inlet and outlet concentrations of pembrolizumab will be 6.91 g/L and 6.24 g/L, respectively, with a step yield of 90.3%. The load phase will accept 6 CVs (column volumes) of incoming harvested and clarified solution from the bioreactor. The elution steps will also require 6 CV's but use 0.1 M Glycine HCL buffer at pH of 3.0. Washing and Equilibration will both utilize 5 CV's of a 20 mM bis-Tris buffer at pH of 5.98 while regeneration will utilize 5 CV's of 0.02 M phosphoric acid at a pH of 2.0. Cleaning will occur between 30-day campaigns with a 0.1 M NaCl salt solution.

5.2.4 Viral Inactivation

The low pH viral inactivation will utilize a standard coiled flow inverter with four 90 coiled sections at 90º angles. The custom made plug flow reactor (F 104) will be constructed of stainless steel and purchased from DPM solutions. The linear length and diameter will be 570 cm and 2.5 cm respectively. The reactor will accept the elution buffer leaving the network of protein A columns at a pH of 3.0 and a volumetric flow rate of 0.093 L/min. At this flow rate, the flow is laminar and the residence time distribution ensures no material spends less than half the mean residence time in the reactor. For this flow, the minimum period equates to l5 minutes, which exceeds the required 14.5 minutes to entirely deactivate the viral material. The inlet and outlet concentrations remain the same as leaving protein A chromatography at 6.24 g/L.

5.2.5 Diafiltration for Anion Exchange Chromatography

This diafiltration step will replace the buffer with pH 5.8 20 mM bis Tris buffer. It will be carried out with the Pall Delta 30 kDa Cadence inline diafiltration module (F 105) , fitted with 12 186 cm^2 Pall Delta T02 Centramate regenerated cellulose membrane cassettes for a total membrane area of 0.22 m^2 and a pressure drop of 38 PSI. All 12 filter cassettes will be replaced every 30-day campaign. The inlet feed flow rate and Keytruda concentration are 0.093 L/min and 6.24 g/L, respectively. The inlet bis Tris buffer flow rate is 0.65 L/min. The outlet bleed product flow rate and Keytruda concentration is 0.093 L/min and 6.20 g/L, respectively. The outlet permeate waste flow rate is 0.652 L/min. This unit operation will produce 28200 L of liquid waste and 3.21 kg of solid waste per 30-day campaign.

5.2.6 Anion Exchange Chromatography

This anion exchange chromatography step will utilize 3 XK 26/20 columns (C 102) which will be purchased from GE Healthcare. The column will operate at a pressure of 25.1 psi with a diameter of 2.6 cm and a height of 18 cm. The chosen resin will be the Capto Q Resin, also purchased from GE Healthcare. This process will operate in a flow-through mode with an inlet and outlet volumetric flow rate of 0.093 L/min. The inlet and outlet concentrations of pembrolizumab will be 6.20 g/L and 5.81 g/L, respectively, with a step yield of 98%. The load, wash, and equilibration steps will utilize 20 mM bis-Tris buffer at a pH of 5.8. These steps will require 21 CV, 2 CV, and 5 CV, respectively. Regeneration will utilize 5 CV's of a 20 mM bis-Tris buffer at a pH of 5.8 with a 2 M NaCl salt solution. Off-process cleaning will utilize 5 CVs of 0.1 M NaOH.

5.2.7 Diafiltration for Cation Exchange Chromatography

This diafiltration step will replace the bis Tris buffer with pH 6, 30 mM PBS buffer. It will be carried out with the Pall Delta 30 kDa Cadence inline diafiltration module (F 106), fitted with 12 186 cm^2 Pall Delta T02 Centramate regenerated cellulose membrane cassettes for a total membrane area of 0.22 m^2, and a pressure drop of 38 PSI. All 12 filter cassettes will be replaced every 30-day campaign. The inlet feed flow rate and Keytruda concentration is 0.093 L/min and 5.81 g/L, respectively. The inlet PBS buffer flow rate is 0.65 L/min. The outlet bleed product flow rate and Keytruda concentration is 0.093 L/min and 5.78 g/L, respectively. The outlet permeate waste flow rate is 0.65 L/min. This unit operation will produce 28200 L of liquid waste and 3.21 kg of solid waste per 30-day campaign.

5.2.8 Cation Exchange Chromatography

This cation exchange chromatography step will utilize 4 XK 50/20 columns (C 103) which will be purchased from GE Healthcare. The column will operate at a pressure of 19.7 psi with a diameter of 5 cm and a height of 19 cm. The chosen resin will be the Capto S Impact, also purchased from GE Healthcare. This process will operate in a bind and elute mode with an inlet and outlet volumetric flow rate of 0.093 L/min. The inlet and outlet concentrations of pembrolizumab will be 5.78 g/L and 5.38 g/L, respectively, with a step yield of 93%. The load, wash, and equilibration steps will utilize a 30 mM phosphate buffer at a pH of 6.0. These steps will require 21 CV, 5 CV, and 5 CV, respectively. Regeneration will utilize 5 CVs of a 30 mM phosphate buffer at a pH of 6.0 with a 2 M NaCl salt solution. Elution will utilize 5 CVs of a 30 mM phosphate buffer at a pH of 7.3. Off-process cleaning will utilize 5 CVs of 0.1 M NaOH.

5.2.9 Viral Filtration

This viral filtration unit operation will utilize the 200 cm^2 Sartorius Virosart HF Mid-Scale Module (F 107), operating at a 25.6 psi pressure drop. The flow rate and Keyturda concentration through the filter are maintained at 0.093 L/min and 5.38 g/L, respectively. The filter membrane will be replaced every 8 hours at the maintenance shift change. This will result in 2.6 kg of solid waste per 30-day campaign.

5.2.10 Final Ultrafiltration and Diafiltration

For ultrafiltration, we are using the GE Healthcare Life Sciences AKTA Flux Tangential Flow Filtration System (F 108) fitted with 30 kDa 177 cm^2 regenerated cellulose PLTK Millipore Sigma Ultracel Ultrafiltration cassettes, operating at a 10 psi pressure drop. The inlet flow rate and Keytruda concentration is 0.093 L/min and 5.38 g/L, respectively. The outlet bleed product

flow rate and Keytruda concentration is 0.019 L/min and 25.7 g/L, respectively. The outlet permeate waste flow rate and concentration is 0.074 L/min and 0.109 g/L, respectively. The filter membrane will be replaced every campaign, resulting in a solid waste of 0.025 kg from ultrafiltration per campaign.

This final diafiltration step will replace the PBS with WFI. It will be carried out with the Pall Delta 30 kDa Cadence inline diafiltration module (F 109), fitted with 12 93 cm^2 Pall Delta T01 Centramate regenerated cellulose membrane cassettes for a total membrane area of 0.11 m^2 and a pressure drop of 15.9 PSI. All 12 filter cassettes will be replaced every 30-day campaign. The inlet feed flow rate and Keytruda concentration are 0.019 L/min and 25.7 g/L, respectively. The inlet PBS buffer flow rate is 0.136 L/min. The outlet bleed product flow rate and Keytruda concentration is 0.019 L/min and 25.0047 g/L, respectively. The outlet permeate waste flow rate is 0.136 L/min. This unit operation will produce 5900 L of liquid waste and 1.61 kg of solid waste per 30-day campaign.

5.2.11 Formulation and Filling

Each 30-day campaign produces 21 kg of Keytruda at a concentration of 25 g/L. This results in 840 L of product solution, which will be stored in a 1000 L Thermo Fisher HyPerforma Single-Use Mixer. Additives of 58,800 g sucrose, 1302 g L-histidine, and 168 g polysorbate 80 will be added to each tank. These storage tanks will then proceed to filling and packaging, which is beyond the scope of this project.

5.3 Disposal

5.3.1 Liquid Waste

The liquid waste produced during manufacturing production will be treated as detailed in section 4.5.1. The total liquid waste produced per campaign is shown in Table 5.1 below.

Table 5.1. Liquid waste production

Unit Operation	**Liquid Waste Produced Per Campaign (L)**
Fermentation	0
Tangential Flow Filtration	36,500
Depth Filtration	0
Sterile Filtration	0
Protein A Chromatography	14,100
Viral Inactivation	0
Diafiltration for Anion Exchange Chromatography	28,150
Anion Exchange Chromatography	1,750
Diafiltration for Cation Exchange Chromatography	28,150
Cation Exchange Chromatography	6,640
Viral Filtration	0
Final Ultrafiltration and Diafiltration	5,870
Total Waste	121,160

5.3.2 Solid Waste

The solid waste produced during manufacturing production will be treated as biohazardous waste. The total solid waste produced per campaign is shown in Table 5.2 below.

Table 5.2. Solid waste production. IEC resin density obtained from (Cheremisinoff, 2002)

Item	Quantity/campaign	Mass/unit	Total Mass/campaign	Total Mass/campaign Unit
WCB Cryovials	1 Vial	10.0 g	10.0	g
TFF Filters	1 Filter	0.91 kg	0.91	kg
Depth Filters	1 Filter	0.84 kg	0.84	kg
Sterile filter module	1 Module	1.0 kg	1.00	kg
Sterile Filters	1 Filter	0.23 kg	0.23	kg
20 L bags	1 Bag	2.5 kg	2.50	kg
500 L bags	1 Bag	62.5 kg	62.5	kg
1000 L bags	1 Bag	125 kg	125.0	kg
2000 L bags	1 Bag	250 kg	250.0	kg
XK columns	12	1 kg	12.0	kg
Protein A Resin	2.8 L	1000 kg/L	2.80	kg
CEX Resin	0.37 L	800 g/L	300.0	g
AEX Resin	0.09 L	620 g/L	56.0	g
Viral Filters	90 Filters	0.288 kg	25.9	kg
Intermediate DF filters	24 Filters	0.268 kg	6.43	kg
Final DF Filters	12 Filters	0.134 kg	1.61	kg
Ultrafiltration Filters	1 Filter	0.25 kg	0.25	kg
Peristaltic Tubing	3000 ft	25.2 g/ft	75.6	kg
Total Waste			567.0	kg

5.4 Production Schedule

As previously described, approximately two weeks are required to scale-up the initial CHO cell concentration to the required cell density necessary to produce an adequate titer of monoclonal antibodies. Our proposed production schedule will consist of 11 annual campaigns that are entirely integrated and continuous. The production schedule for a single campaign is shown below in Table 5.4, and the annual production schedule is shown below in Table 5.4. In order to accommodate this continuous process, we have implemented a two-week facility shutdown into the production schedule. During this time prior to the first campaign, the initial scale-up will occur. During subsequent facility shutdowns, the scale-up will occur as well to ensure that the 11 campaigns can begin and end as planned. We have implemented the necessary safeguards to ensure that the process can maintain continuity, both during cleaning protocols and unpredicted process excursions. Each campaign was designed to last 30 days. There will, therefore, be an additional two weeks to accommodate for any unexpected process shutdowns or excursions. The duration of any given campaign can, therefore, be altered if needed. In the event that this extra time goes unused, we will add this time to the facility shut down in June.

Table 5.3. Production shcedule for a single campagaign for the pembrolizumab manufacturing facility.

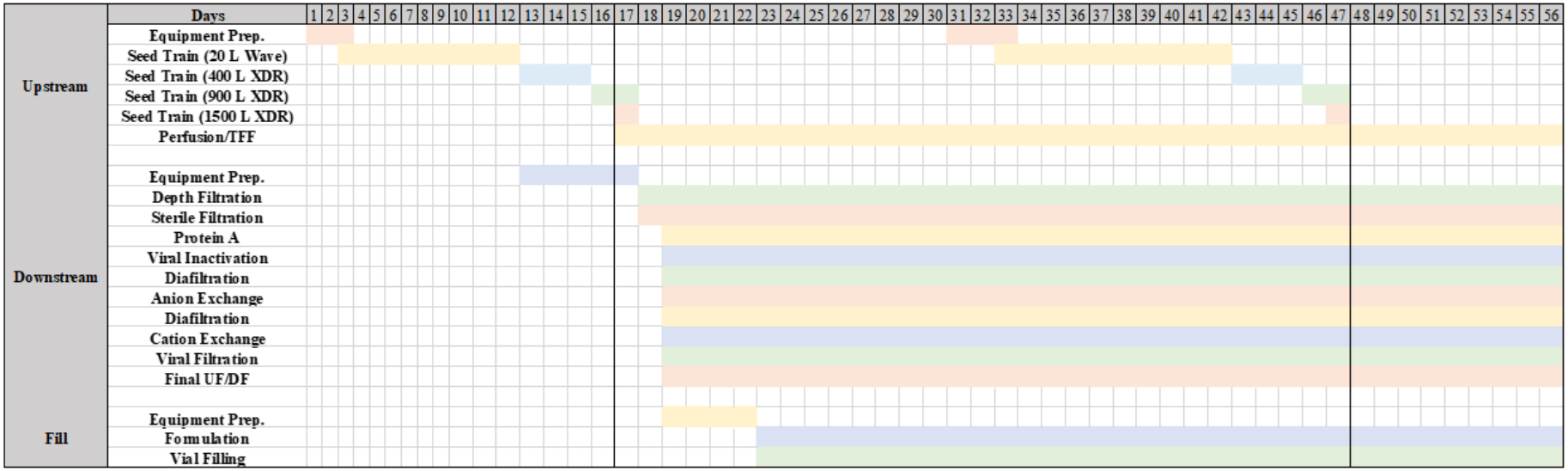
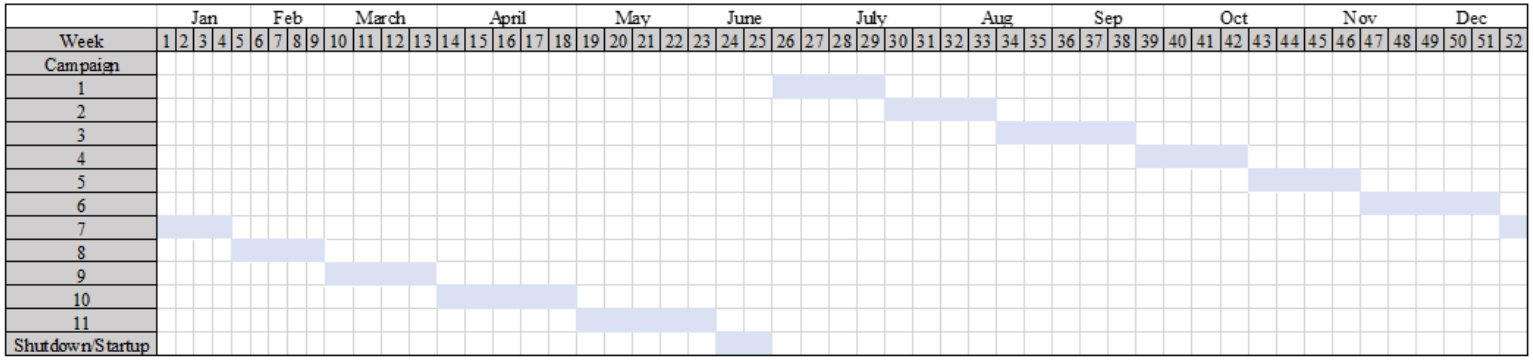


Table 5.4. Production shcedule for a year for the pembrolizumab manufacturing facility.



5.5 Equipment Tables and Specifications

5.5.1 Upstream Equipment Table

Table 5.5. Upstream equipment table.

Operation	Unit	Unit No.	Quantity	Temperature (^{O}C)	Pressure Drop (psi)	Size
MCB/WCB Storage	Freezers	F 100	2	-87	n/a	n/a
Fermentation	20 L Wave Bioreactor	B 101	2	37	n/a	20 L
	XDR 50/2000	B 102	2	37	n/a	2000 L
TFF	Repligen KrosFlo KR2i	F 101	2	37	1.15	A= 81 cm^2 L= 20 cm

5.5.2 Downstream Equipment Table

Table 5.6. Downstream equipment table.

Operation	Unit	Unit No.	Quantity	Temperature (^{O}C)	Pressure Drop (psi)	Size
Depth Filtration	Millistak+® HC Pod Depth Filters	F 102	2	n/a	15.1	A=0.11 m^2, L = 62 cm
Sterile Filtration	Millipak 20 Disposable Filter Units	F 103	2	n/a	14.0	D = 76 mm L = 9.4 cm
Protein A Chromatography	Modified XK 50/60	C 101	6	n/a	26.0	D = 4.5 cm L = 36 cm
Viral Inactivation	Custom built Stainless Steel CFI	F 104	1	n/a	n/a	D = 2.5 cm L = 570 cm
Diafiltration	Pall Delta Cadence Inline Diafiltration Module	F 105	2	RT	38.0	
Anion Exchange	XK 26/20	C 102	3	n/a	25.1	D = 2.6 cm

Chromatography						h = 16 cm
Diafiltration	Pall Delta Cadence Inline Diafiltration Module	F 106	2	RT	38.0	
Cation Exchange Chromatography	XK 50/20	C 103	4	n/a	19.7	D = 5 cm h = 19 cm
Viral Filtration	Sartorius Virosart HF	F 107	2	RT	25.6	
UF	GE AKTA crossflow tangential flow filtration system	F 108	2	RT	10.0	
Final DF	Pall Delta Cadence Inline Diafiltration Module	F 109	2	RT	15.9	
Formulation and Filling	Weinas VD 160	FF 101	2	RT	--	

5.5.3 Miscellaneous Equipment Table

Table 5.7. Miscellaneous equipment table.

Operation	Unit	Unit No.	Quantity	Temperature (^{O}C)	Pressure Drop (psi)	Size (L)
Misc.	Peristaltic Pump	P1-66	66	RT	See 4.3.1	n/a
	MECO Vapor Compression Distillation WFI module	WFI-1	1	n/a	n/a	6000
	Holding Tanks	T1 T2 T3 T4 T5	1 1 1 1 1	RT RT RT RT RT	n/a n/a n/a n/a n/a	1600 300 5000 5000 5000

		T6	1	RT	n/a	5000
		T7	1	RT	n/a	5000
		T8	1	RT	n/a	1000
		T9	1	RT	n/a	1700
		T10	1	RT	n/a	850
		T11	1	RT	n/a	100
		T12	1	RT	n/a	170
		T13	1	RT	n/a	85
		T14	1	RT	n/a	50
		T15	1	RT	n/a	5000
		T16	1	RT	n/a	500
		T17	1	RT	n/a	350
		T18	1	RT	n/a	250
		T19	1	RT	n/a	35
		T20	1	RT	n/a	25
		T21	1	RT	n/a	50
		T22	1	RT	n/a	5000
		T23	1	RT	n/a	500
		T24	1	RT	n/a	550
		T25	1	RT	n/a	1850
		T26	1	RT	n/a	350
		T27	1	RT	n/a	55
		T28	1	RT	n/a	185
		T29	1	RT	n/a	35
		T30	1	RT	n/a	50
		T31	1	RT	n/a	5000

5.6 Material and Energy Balances Table

5.6.1 Upstream Material Balances

Table 5.8. Upstream material balance table.
Note: Mass of CHO cells in Section 4.1.4, explanation for impurities in Discussion Section

Description	Inlet/Outlet	Stream	Stream Flow Rate (L/min)	Material	Concentration	Units
Reactor	Inlet	PRI-1	0.89	Media	50	g/L
	Recycle (Incoming after purge)	PRI-2	0.242	Pembrolizumab HCPs DNA Endotoxins Lipids Cell Debris Media Cells	7.12 52.5 23 $1.0x10^{-4}$ 30 62.0 48.2 44.7	g/L mg/L mg/L mg/L mg/L mg/L g/L g/L
	Outlet	TFI-1	1.13	Pembrolizumab HCPs DNA	7.12 705 744	g/L mg/L mg/L

				Endotoxins Lipids Cell Debris Media Cells	$9.70x10^{-3}$ 620 62.0 44.6 40.9	mg/L mg/L mg/L g/L g/L
	Air Inlet	PRI-3	63	Oxygen Nitrogen CO2	0.3 0.98 0.66	g/L g/L mg/L
	Vent	PRO-1	63	Oxygen CO2 Nitrogen	0.12 0.19 0.98	g/L g/L g/L
TFF System	Inlet	TFI-1	1.13	Pembrolizumab HCPs DNA Endotoxins Lipids Cell Debris Media Cells	7.12 705 744 $9.70x10^{-3}$ 620 62.0 44.6 40.9	g/L mg/L mg/L mg/L mg/L mg/L g/L g/L
	Outlet	DF1I-1	0.093	Pembrolizumab HCPs DNA Endotoxins Lipids Cell Debris Cells	7.05 705 744 9^{-3} 620 62.0 0	g/L mg/L mg/L mg/L mg/L mg/L g/L
	Purge	TFO-1	0.792	Pembrolizumab HCPs DNA Endotoxins Lipids Cell Debris Cells Media	7.12 52.5 23 $1.0x10^{-4}$ 30 0 44.7 48.9	g/L mg/L mg/L mg/L mg/L mg/L g/L g/L
	Recycle (Outgoing before purge)	TFO-2	1.03	Pembrolizumab HCPs DNA Endotoxins Lipids Cell Debris Cells Media	7.12 52.5 23 $1.0x10^{-4}$ 30 0 44.7 48.9	g/L mg/L mg/L mg/L mg/L mg/L g/L g/L

5.6.2 Downstream Material Balances

Table 5.9. Downstream material balance table.

Description	Inlet/Outlet	Stream	Stream Flow Rate (L/min)	Material	Concentration	Units
Depth Filtration	Inlet	DF1I-1	0.093	Pembrolizumab HCPs DNA Endotoxins Lipids Cell Debris	7.05 705 744 $9.70x10^{-3}$ 620 62.0	g/L mg/L mg/L mg/L mg/L mg/L
	Outlet	SFI-1	0.093	Pembrolizumab HCPs DNA Endotoxins Lipids Cell Debris	6.98 529 670 $9.70x10^{-3}$ 465 0.62	g/L mg/L mg/L mg/L mg/L mg/L
Sterile Filtration	Inlet	SFI-1	0.093	Pembrolizumab HCPs DNA Endotoxins Lipids Cell Debris	6.98 529 670 $9.70x10^{-3}$ 465 0.62	g/L mg/L mg/L mg/L mg/L mg/L
	Outlet	PALI-1	0.093	Pembrolizumab HCPs DNA Endotoxins Lipids Cell Debris	6.91 397 603 $9.70x10^{-3}$ 349 $6.2x10^{-5}$	g/L mg/L mg/L mg/L mg/L mg/L
Protein A	Product Inlet	PALI-1	0.093	Pembrolizumab HCPs DNA Endotoxins Lipids Cell Debris	6.91 397 603 $9.70x10^{-3}$ 349 $6.2x10^{-5}$	g/L mg/L mg/L mg/L mg/L mg/L
	Wash Inlet	PALI-2	0.093	Bis Tris HCl	4.19 2.62	g/L g/L
	Waste Outlet	PALO-1	0.093	Bis Tris HCl Phosphoric Acid Pembrolizumab HCPs DNA Endotoxins Lipids	8.38 5.24 1.96 $6.70x10^{-1}$ $3.97x10^{2}$ $6.03x10^{2}$ $9.70x10^{-3}$ $3.49x10^{2}$	g/L g/L g/L g/L mg/L mg/L mg/L mg/L

				Cell Debris	6.20×10^{-5}	mg/L
	Elution Inlet	PALI-3	0.093	Glycine HCl	7.50 7.29×10^{-1}	g/L g/L
	Product Outlet	VII-1	0.093	Pembrolizumab Glycine HCl HCPs DNA Endotoxins Lipids Cell Debris	6.24 7.50 7.29×10^{-1} 5.96×10^{-2} 9.05×10^{-2} 1.94×10^{-6} 1.75×10^{-2} 1.24×10^{-8}	g/L g/L g/L mg/L mg/L mg/L mg/L mg/L
	Regen. Inlet	PALI-4	0.093	Phosphoric Acid	1.96	g/L
	Equil. Inlet	PALI-5	0.093	Bis Tris HCl	4.18 2.62	g/L g/L
Viral Inactivation	Inlet	VII-1	0.093	Pembrolizumab Glycine HCl HCPs DNA Endotoxins Lipids Cell Debris	6.24 7.50 7.29×10^{-1} 5.96×10^{-2} 9.05×10^{-2} 1.94×10^{-6} 1.75×10^{-2} 1.24×10^{-8}	g/L g/L g/L mg/L mg/L mg/L mg/L mg/L
	Outlet	DFAI-1	0.093	Pembrolizumab Glycine HCl HCPs DNA Endotoxins Lipids Cell Debris	6.24 7.50 7.29×10^{-1} 5.96×10^{-2} 9.05×10^{-2} 1.94×10^{-6} 1.75×10^{-2} 1.24×10^{-8}	g/L g/L g/L mg/L mg/L mg/L mg/L mg/L
Diafiltration For AEX	Product Inlet	DFAI-1	0.093	Pembrolizumab Glycine HCl HCPs DNA Endotoxins Lipids Cell Debris	6.24 7.50 7.29×10^{-1} 5.96×10^{-2} 9.05×10^{-2} 1.94×10^{-6} 1.75×10^{-2} 1.24×10^{-8}	g/L g/L g/L mg/L mg/L mg/L mg/L mg/L
	Product Outlet	AXI-1	0.093	Pembrolizumab Glycine HCl Bis Tris HCPs DNA	6.20 0.018 1.82 4.18 5.96×10^{-2} 9.05×10^{-2}	g/L g/L mg/L g/L mg/L mg/L

				Endotoxins Lipids Cell Debris	$1.94x10^{-6}$ $1.75x10^{-2}$ $1.24x10^{-8}$	mg/L mg/L mg/L
	Buffer Inlet	WDFAI-1	0.65	20 mM bis Tris @ pH of 5.8	4.18	g/L
	Waste Outlet	WDFAO-1	0.65	Pembrolizumab Glycine HCl	0.005 7.48 0.727	g/L g/L g/L
Anion Exchange	Product Inlet	AXI-1	0.093	Pembrolizumab Glycine HCl Bis Tris HCPs DNA Endotoxins Lipids Cell Debris	6.20 0.018 1.82 4.18 $5.96x10^{-2}$ $9.05x10^{-2}$ $1.94x10^{-6}$ $1.75x10^{-2}$ $1.24x10^{-8}$	g/L g/L mg/L g/L mg/L mg/L mg/L mg/L mg/L
	Wash Inlet	AXI-2	0.093	20 mM bis Tris @ pH of 5.8	4.18	g/L
	Product Outlet	DFCI-1	0.093	Pembrolizumab Glycine HCl Bis Tris HCPs DNA Endotoxins Lipids Cell Debris	5.81 8 1.00 4.18 1.19 1.81 $3.88x10^{-2}$ 8.75 $0.62x10^{-2}$	g/L mg/L mg/L g/L µg/L µg/L µg/L µg/L µg/L
	Clean Inlet	AXI-3	0.093	20 mM bis Tris NaCl	4.18 117	g/L g/L
	Regen. Inlet	AXI-4	0.093	20 mM bis Tris @ pH of 5.8	4.18	g/L
	Waste Outlet	AXO-1	0.093	Pembrolizumab Glycine HCl Bis Tris NaCl HCPs DNA Endotoxins Lipids Cell Debris	1.02 0.01 0.82 4.18 117 $5.90x10^{-2}$ $8.87x10^{-2}$ 1.90 8.75 $0.62x10^{-2}$	g/L g/L mg/L g/L g/L mg/L mg/L µg/L µg/L µg/L
Diafiltration For CEX	Product Inlet	DFCI-1	0.093	Pembrolizumab Glycine	5.81 8	g/L mg/L

				HCl Bis Tris HCPs DNA Endotoxins Lipids Cell Debris	1.00 4.18 1.19 1.81 $3.88x10^{-2}$ 8.75 $0.62x10^{-2}$	mg/L g/L µg/L µg/L µg/L µg/L µg/L
	Product Outlet	CXI-1	0.093	Pembrolizumab Glycine HCl Bis Tris Na_2HPO_4-$7H_2O$ $NaH_2PO_4H_2O$ HCPs DNA Endotoxins Lipids Cell Debris	5.78 0.02 2.5 0.01 1.10 3.58 1.19 1.81 $3.88x10^{-2}$ 8.75 $0.62x10^{-2}$	g/L mg/L µg/L g/L g/L g/L µg/L µg/L µg/L µg/L µg/L
	Buffer Inlet	WDFCI-1	0.65	Na_2HPO_4-7H2O $NaH_2PO_4H_2O$	1.10 3.57	g/L g/L
	Waste Outlet	WDFCO-1	0.65	Pembrolizumab Glycine HCl Bis Tris	0.04 7.98 0.998 4.17	g/L mg/L mg/L g/L
Cation Exchange	Product Inlet	CXI-1	0.093	Pembrolizumab Glycine HCl Bis Tris Na_2HPO_4-$7H_2O$ $NaH_2PO_4H_2O$ HCPs DNA Endotoxins Lipids Cell Debris	5.78 0.02 2.5 0.01 1.10 3.57 1.19 1.81 $3.88x10^{-2}$ 8.75 $0.62x10^{-2}$	g/L mg/L µg/L g/L g/L g/L µg/L µg/L µg/L µg/L µg/L
	Wash Inlet	CXI-2	0.189	Na_2HPO_4-$7H_2O$ $NaH_2PO_4H_2O$	1.10 3.57	g/L g/L
	Wash Outlet	CXO-1	0.189	Pembrolizumab Glycine HCl Bis tris Na_2HPO_4-$7H_2O$ $NaH_2PO_4H_2O$ HCPs	0.40 0.02 2.5 0.01 1.10 3.57 1.19	g/L mg/L µg/L mg/L g/L g/L µg/L

				DNA Endotoxins Lipids Cell Debris	1.00 $3.61x10^{-2}$ 8.75 $0.62x10^{-2}$	µg/L µg/L µg/L µg/L
	Elution Inlet	CXI-3	0.093	Na_2HPO_4-7H2O $NaH_2PO_4H_2O$	5.71 1.20	g/L g/L
	Product Outlet	VFI-1	0.093	Pembrolizumab Glycine HCl Bis Tris Na_2HPO_4-$7H_2O$ $NaH_2PO_4H_2O$ HCPs DNA Endotoxins Lipids Cell Debris	5.38 0 0 0 5.71 1.20 0 0.81 $2.7x10^{-3}$ 0 0	g/L mg/L µg/L g/L g/L g/L µg/L µg/L µg/L µg/L µg/L
	Regen. Inlet	CXI-4	0.093	Na_2HPO_4-7H2O $NaH_2PO_4H_2O$ NaCl	1.10 3.57 117	g/L g/L g/L
	Regen. Outlet	CXO-2	0.093	Na_2HPO_4-7H2O $NaH_2PO_4H_2O$ NaCl	1.10 3.57 117	g/L g/L g/L
	Equil. Inlet	CXI-5	0.112	Na_2HPO_4-7H2O $NaH_2PO_4H_2O$	1.10 3.57	g/L g/L
	Equil. Outlet	CXO-3	0.112	Na_2HPO_4-7H2O $NaH_2PO_4H_2O$	1.10 3.57	g/L g/L
Viral Filtration	Inlet	VFI-1	0.093	Pembrolizumab Na_2HPO_4-$7H_2O$ $NaH_2PO_4H_2O$ DNA Endotoxins	5.38 5.71 1.20 0.81 $2.7x10^{-3}$	g/L g/L g/L µg/L µg/L
	Outlet	UFI-1	0.093	Pembrolizumab Na_2HPO_4-$7H_2O$ $NaH_2PO_4H_2O$ DNA Endotoxins	5.38 5.71 1.20 0 0	g/L g/L g/L µg/L µg/L
Ultrafiltration	Product Inlet	UFI-1	0.093	Pembrolizumab Na_2HPO_4-$7H_2O$ $NaH_2PO_4H_2O$	5.37 5.71 1.20	g/L g/L g/L

	Product Outlet	DFI-1	0.019	Pembrolizumab Na_2HPO_4-$7H_2O$ $NaH_2PO_4H_2O$	25.7 5.71 1.20	g/L g/L g/L
	Waste Outlet	UFO-1	0.074	Pembrolizumab Na_2HPO_4-$7H_2O$ $NaH_2PO_4H_2O$	0.026 5.71 1.20	g/L g/L g/L
Final DF	Product Inlet	DFI-1	0.019	Pembrolizumab Na_2HPO_4-$7H_2O$ $NaH_2PO_4H_2O$	25.7 5.71 1.20	g/L g/L g/L
	Product Outlet	FFI-1	0.019	Pembrolizumab Na_2HPO_4-$7H_2O$ $NaH_2PO_4H_2O$	25.0047 0.006 0.001	g/L g/L g/L
	Water Inlet	DFI-2	0.136	Water	--	--
	Waste Outlet	DFO-1	0.136	Pembrolizumab Na_2HPO_4-$7H_2O$ $NaH_2PO_4H_2O$	0.095 5.70 1.20	g/L g/L g/L

5.7 Plant Location

The manufacturing facility will be located in Carlow, Ireland, approximately 84 km outside of Dublin. The facility and surrounding area will cover an area of 33 acres, and this will provide capacity for 350 new jobs in the area (Palmer, 2018). In the surrounding area, there exists a variety of universities that offer degrees in chemical engineering and biotechnology, such as the University College Cork and the University College Dublin. As a result, there will be local talent available to maintain the facility. Additionally, as the 13th largest city in Ireland, Carlow is an ideal location due to the availability of resources. The moderate climate of Carlow will additionally reduce the need for excessive energy costs. Merck has also gained the support of Ireland's federal government due to the facility's economic benefits.

5.8 Process Economics

5.8.1 Plant Capital Costing

The capital costs of the plant are all the one-time, fixed expenses. According to Peters and Timmerhaus, the fixed-capital investments include expenses of purchased equipment, equipment installation, instrumentation and controls, piping, electrical, buildings, yard improvements, service facilities, land, engineering and supervision, construction expense, contractor's fee, and contingency. The estimated percentage range for these expenses can be seen below in Table 5.10.

Table 5.10. Typical percentages of fixed-capital investment values for direct and indirect cost segments for multipurpose plants or large additions to existing facilities (Peters and Timmerhaus, 1980)

Component	Range (%)
Direct Costs	
Purchased Equipment	15-40
Purchased Equipment Installation	6-14
Instrumentation and Controls (installed)	2-8
Piping (installed)	3-20
Electrical (installed)	2-10
Buildings (including services)	3-18
Yard Improvements	2-5
Service Facilities (installed)	8-20
Land	1-2
Total Direct Costs	
Indirect Costs	
Engineering and Supervision	4-21
Construction Expense	4-16
Contractor's fee	2-6
Contingency	5-15
Total Fixed-Capital Investment	

The selected percentages of fixed capital investment values are shown below in table 5.11. The numbers were selected based on the typical needs of pharmaceutical facilities. For example, the upper percentage for building cost was selected because no pharmaceutical processes occur inside, and the buildings must comply with stricter regulations. The lower percentage of piping was selected because pharmaceutical processes focus more on disposable

tubing rather than permanent structural pipes. The selection of the other percentages followed a similar logic.

Table 5.11. Percentages and costs of fixed-capital investment values

Components	% of Fixed Capital	Cost
Purchased equipment	15	$2,315,203
Installation	6	$926,081
Instrumentation	6	$926,081
Piping	4	$617,387
Electrical	4	$617,387
Buildings	18	$2,778,244
Yard improvements	1	$154,347
Service facilities	11	$1,697,815
Land	1	$154,347
Engineering and supervision	12	$1,852,162
Construction expense	10	$1,543,469
Contractor's fee	2	$308,694
Contingency	10	$1,543,469
	Total	$15,434,686
	Total with validation	**$16,434,686**

In order to obtain the total capital cost, we first determined the cost of all the purchased equipment. The prices for the purchased equipment is shown below in Table 5.12 and 5.13. The main equipment is any equipment directly represented by a block in the overall process flow diagram, seen in Figure 5.1. The ancillary equipment is any equipment that is necessary to support the entire process but is not directly involved in the pharmaceutical process, such as freezers, pumps, and storage tanks. All equipment costing was found by obtaining direct price quotes from the manufacturers, or from prices listed by the manufacturer online. The starred unit operations were obtained via other means. Since the plug flow reactor for viral inactivation is a

custom-made spiral tube made of stainless steel, the pricing was determined by calculating the cost of the steel required to construct the reactor, then multiplying this price conservatively by 5 to account for the cost of construction. The WFI system and holding tank prices were obtained from a capstone project produced last year since these costs were not able to be obtained with the available correlations. The total purchased equipment costing is $2.32 million. This number was then used to obtain the remaining capital costs based on fixed capital percentages. As later discussed, the initial FDA validation is estimated to cost $1 million, resulting in a total fixed capital cost of $16.3 million.

Table 5.12. Main equipment costs

Main Equipment				
Unit operation	**Product**	**Price**	**Quantity**	**Total**
Inoculation	GE wave 25	$75,000	1	$75,000
Bioreactor	GE XDR 2000	$450,000	2	$900,000
TFF	Repligen KrosFlo	$25,000	2	$50,000
Depth	Pod Holder	$15,080	2	$30,160
Protein A	GE column holder	$105	5	$525
	GE column holder rod	$770	5	$3,850
PFR*	Custom made	$2,736	2	$5,472
AEX DF	Pall cadence inline diafiltration module	$5,641	2	$11,282
AEX	GE column holder	$105	3	$315
	GE column holder rod	$770	3	$2,310
CEX DF	Pall cadence inline diafiltration module	$5,641	2	$11,282
CEX	GE column holder	$105	4	$420
	GE column holder rod	$770	4	$3,080
UF	GE AKTA flux tangential flow system	$35,000	2	$70,000
Final DF	Pall cadence inline diafiltration module	$4,721	2	$9,442
			Total =	**$1,173,138**

Table 5.13. Ancillary equipment costs

Ancillary Equipment				
Unit Operation	**Product**	**Price**	**Quantity**	**Total**
Peristaltic pumps	Welch peristaltic pump	$1,330	66	$87,780
WFI system*	MECO Vapor Compression Distillation Module	$350,000	1	$350,000
Holding tanks*	5000 L HyPerforma single-use mixer	$20,000	8	$160,000
	2000 L HyPerforma single-use mixer	$20,000	3	$60,000
	1000 L HyPerforma single-use mixer	$20,000	5	$100,000
	500 L HyPerforma single-use mixer	$20,000	6	$120,000
	200 L HyPerforma single-use mixer	$20,000	2	$40,000
	100 L HyPerforma single-use mixer	$20,000	3	$60,000
	50 L HyPerforma single-use mixer	$20,000	6	$120,000
Freezers	Panasonic Healthcare VIP series freezer	$22,143	2	$44,285
			Total =	**$1,142,065**

5.8.2 FDA Approval and Validation Costs

Keytruda has already been approved by both the FDA and European Commission in The yearly upkeep was determined in the operating cost section to be $532,000 for yearly fees to upkeep the approval of the drug. Because the drug has already been validated we have estimated that validation of the plant and the process should take about a year. The facility will complete construction in 1.5 years, immediately after which the 1-year FDA validation and production at half capacity will begin simultaneously. This timeline is accounted for in the cash flow analysis, detailed later. The validation cost for the plant was estimated to be about $1 million dollars which includes all necessary legal paperwork and governmental tests of the plant.

The plant will also undergo two weeks of yearly maintenance in between the 5th and the 6th campaign of the plant yearly to ensure that the whole system is working correctly,

governmental maintenance checks can be conducted, and an overview of the facility will take place.

5.8.3 Operating Expenses

The operating expenses include any expenses that must be made continuously throughout the lifeline of the plant, such as labor, waste management, utilities, and raw materials. The Table for the annual operating costs can be seen below in Table 5.14. The fixed capital investment (*FCI*), raw material, waste treatment, utilities, and operating labor must be first determined in order to calculate the remaining capital costs. Additionally, some expenses such as patents and royalties can only be calculated from the total cost of manufacture (*COM*). Therefore, the values based on the *COM* could only be calculated once all other values were filled in. The total cost of manufacture was then determined as seen in the last 2 rows of Table 5.14.

Table 5.14. Annual operating costs

Direct Costs	**Nomenclature**	**Cost**
Raw materials	C_RM	$49,794,935
Waste treatment	C_WT	$2,678,015
Utilities	C_UT	$139,570
Operating labor	C_OL	$3,546,230
Direct and supervisory and clerical labor	0.18*C_OL	$638,321
Maintenance and repairs	0.06*FCI	$986,081
Operating supplies	0.009*FCI	$147,912
Laboratory charges	0.15*C_OL	$531,935
Patents and Royalties	0.03*COM	$2,389,272
Fixed Costs		
Depreciation	0.1*FCI	$1,643,469
Local taxes and insurance	0.032*FCI	$525,910
Plant overhead costs	0.708*C_OL + 0.036*FCI	$3,102,380
General costs		

Administration	0.177*C_OL+0.009*FCI	$775,595
Distribution and selling costs	0.11*COM	$8,760,665
Research and development	0.05*COM	$3,982,121
	Cost - 0.19COM =	$64,510,352
	Total operating cost	**$79,642,410**

The raw material cost includes buffers, media, water, filters, single-use bags, filters, resins and any other materials that must be regularly supplied to the facility. We first calculated the cost of any constant flows, such as media and buffers. We then calculated the cost of solids that must be frequently replaced, such as filters and chromatography resins. All costs were obtained from the respective manufacturer websites. However, the single-use bag prices were obtained from the previous year's capstone due to current unavailability. The cost of process water was determined based on the EPA estimation for process water costs since we were unable to find the cost of process water in Ireland. It is important to note that the majority of the raw material costs can be attributed to the media, since we are not preparing it in the facility. Additionally, since we are creating the WFI in-facility, we are only calculating the cost of process water, also known as tap water, below, then separately calculating the cost of the power required to purify the water in the utilities section. These costs can be seen in Tables 5.15 and 5.16.

Table 5.15. Constant flow costs

Flows				
Unit Op	**Material**	**Quantity per Campaign (kg or L)**	**Price/Unit**	**Cost per Campaign**
Fermentor	Process water	39800	$0.000528	$21
	Media	2022	$2,095	$4,236,446
	L-glutamine	49.3	$566	$27,926

Protein A	Process water	14100	$0.000528	$7
	Bis Tris	28.1	$766	$21,525
	Glycine	30.2	$91	$2,760
	HCl	2.94	$1,670	$4,902
	Phosphoric acid	6.58	$1,100	$7,233
AEX + DF	Process water	33440	$0.000528	$18
	Bis Tris	18.5	$766	$14,171
	HCl	13.89	$1,670	$23,191
	NaCl	102	$3	$318
	NaOH	0.16	$944	$151
CEX + DF	Process water	38950	$0.000528	$21
	NaH2PO4 H2O	33.6	$165	$5,555
	Na2HPO4 7H2O	22	$130	$2,869
	NaOH	0.786	$944	$742
Final DF	Process water	5870	$0.000528	$3
Formulation	Sucrose	58.8	$49.80	$2,928
	L-histidine	1.302	$641.00	$835
	Polysorbate 80	0.168	$113.00	$19
			Total =	**$4,351,641**

Table 5.16. Solids costs

Solids					
Unit Op	**Material**	**Product**	**Quantity per Campaign**	**Price/Unit**	**Cost per Campaign**
Wave Reactor	single-use bags	n/a	1	$333	$333
Fermentor	single-use bags	n/a	3	$500	$1,500
TFF	Filter	MidiKros 20cm 0.2um PES 1 mm FLLxFLL sterile	1	$237	$237
Depth	Filter	Millistak+ HC pod depth filter, C0HC media series, 1.1 m2	1	$680	$680
Sterile	Holder	ULTA prime GF normal flow filtration capsule	1	$212	$212
	Filter	MilliporeSigma Millipak	1	$61	$61

		disposable filter units			
Protein A	Resin	POROS MabCapture A Select Resin	2.8	$7,240	$20,272
	Column	GE 50/160 XK columns	5	$312	$1,560
AEX DF	Filter	Pall 30kD T02 Centramate	12	$434	$5,204
AEX	Resin	Capto Q resin	1.59	$2,102	$3,343
	Column	GE XK 26/20 columns	3	$743	$2,229
CEX DF	Filter	Pall 30kD T02 Centramate	12	$434	$5,204
CEX	Resin	Capto S ImpAct resin	7.86	$3,860	$30,356
	Column	GE 50/20 XK columns	4	$994	$3,976
VF	Filter	Sartorius Virosart HF	90	$650	$58,500
UF	Filter	Millipore Sigma Ultracel Ultrafiltration Disc	1	$59	$59
Final DF	Filter	Pall Delta 30kD T01 Centramate	12	$350	$4,202
Air filtration	Filter	Sartorius Sartpore Capsule	1	$343	$343
Pumps	Tubing	Pharmed tubing	3000	$7	$20,400
Storage tanks	single-use bags	n/a	33	$500	$16,500
				Total =	**$175,171**

Since this is a pharmaceutical facility, all waste will be deemed hazardous in order to allow the appropriate waste treatment protocols are carried out. Based on Turton, the maximum for hazardous waste management costs $2 per pound of hazardous waste, which we used to be conservative in our economic estimations. As previously determined in the waste management section, our facility will be producing approximately 121,160 L of liquid waste and 570 kg of solid waste per campaign. Assuming a liquid waste density of 1 kg/L, this results in a total of 121,730 kg of waste produced per campaign, which has a waste management cost of $243,000 per campaign, or $2.68 million per year.

The power requirement of the facility was organized by unit operation, as can be seen below in Table 5.17. The pump power requirement was previously determined in the pump section. The power requirement of all other unit operations was determined based on the respective data sheets associated with the unit operation. Although there was no datasheet for the vapor compression distillation water purification system, these operations typically require 8 kWh per metric tonne of water (Lokiec & Ophir, 2007). The most recent cost of electricity in Ireland was found to be $0.27 per kWh (Statista, 2020), resulting in $12,700 of utilities per campaign, or $140,000 per year. It is important to note that this utility cost does not include the cost of utilities for general lighting, service facilities, etc., as these correlations were not available to us.

Table 5.17. Power requirements of process equipment (GE Healthcare, 2020a; *Xcellerex™ XDR Cell Culture Bioreactor Systems*, 2020; *WAVE Bioreactor Systems Cell culture procedures*, 2019; Lokiec & Ophir, 2007; Repligen, 2018; ThermoFischer, 2020)

Unit Operation	**Power Requirement (kW)**	**Cost per campaign**
Inoculation	1.5	$292
Bioreactor	0.432	$84
TFF	0.096	$19
UF	0.3	$58
Peristaltic pumps	0.81	$158
WFI system	1.47	$286
Holding tanks	59.4	$11,547
Freezers	1.26	$245
Totals =	**65.3**	**$12,688**

Assumptions regarding worker salary, vacation times, shift length, etc. were obtained from Turton. A single operator will work 5 8-hour shifts per week for 49 weeks out of the year,

leaving 3 weeks for vacation and sick leave. This gives each operator 245 shifts per year. Since a chemical plant must operate 24 hours a day, this requires 1095 operating shifts per year. This requires 4.5 operators to be hired for each operator needed in the plant at any time. Each operator will be paid $66,910. The operating labor was obtained using Equation 5.1 below, where N_{OL} is the number of operators per shift, P is the number of processing steps involving the handling of particulate solids, and N_{np} is the number of non-particulate processing steps.

$$N_{OL} = (6.29 + 31.7P^2 + 0.23N_{np})^{0.5}$$

Equation 5.1. Equation to find the number of operators per shift

We determined that there are 2 steps involving the handling of particulate solids; 1 for the inoculation steps, and 1 encompassing the addition to powdered media and buffers to the mixing tanks. We determined there to be 23 non-particulate processing steps, one for each unit operation, in which each individual chromatography column counts as a unit operation. This results in 11.8 operators per shift. Since 4.5 operators are needed per operator in the plant at any time, (11.8)*(4.5) gives 53 operators needed to run our pharmaceutical facility, resulting in $3.55 million on annual operator labor costs.

5.8.4 Economic Analysis using Discounted Cash Flow

In order to perform the discounted cash flow analysis, we must first outline the general timeline of the plant. The plant will undergo 18 months of construction. Once construction is complete, the plant will operate at half capacity despite requiring full operating costs. The product produced during this time will not be sold. The 1-year validation process will also occur simultaneously. Assuming that the validation occurs without problems, the plant will be in full

production 2.5 years after construction begins, or halfway through year 2, and will continue production for another 18.5 years into year 20, for a full lifetime of 21 years.

Although many of the economic values have already been calculated, it is important to isolate a few values for the discounted cash flow analysis. The initial capital cost is $15.4 million and the validation cost is $1 million. The annual operating cost is $79.6 million. The revenue is $11.7 billion based on our annual production rate of 226 kg and a raw cost of $51.79 per mg, giving an annual profit of $11.6 billion. The Ireland corporate tax rate is a flat 12.5% (PWC, 2020), and we will be using a discount rate of 8%, which is a low estimation of pharmaceutical discount rates (Avance, 2020). These values are summarized in Table 5.18 below.

Table 5.18. Summary of important values for discounted cash flow analysis

Initial Capital Cost =	$15,434,686
Validation Cost =	$1,000,000
Discount Rate =	0.08
Tax Rate =	0.125
Annual Operating Cost =	$79,642,410
Annual Revenue =	$11,704,540,000
Annual Profit	$11,624,897,590

The discounted cash flow analysis Table and figures can be seen below in Table 5.19 and Figures 5.3-5.4. The cash flow was determined based on the storyline outlined previously. The taxed cash flow was calculated by applying the 12.5% corporate tax rate in Ireland after the plant begins making money. The discounted cash flow was determined using Equation 5.2 below, where TCF_t is the taxed cash flow at year *t*, DCF_t is the discounted cash flow at year *t*, and *i* is the discount rate. The cumulative cash flow was calculated simply by adding the discounted cash flows from all previous years. It is important to note that both the initial construction costs and

depreciation are not visible on the figures due to relative size, however these values can be seen in the table below.

$$TCF_t = DCF_t(1+i)^t$$

Equation 5.2. Used to calculate discounted cash flows.

Table 5.19. Cash flow analysis.

Year	Cash flow	After-tax cash flow	Discounted cash flow	Cumulative cash flow
0	-$10,289,791	-$10,289,791	-$10,289,791	-$10,289,791
1	-$45,466,101	-$45,466,101	-$42,098,241	-$52,388,032
2	$5,811,948,795	$5,085,557,912	$4,360,046,221	$4,307,658,189
3	$11,624,897,590	$10,171,990,825	$8,074,854,272	$12,382,512,461
4	$11,624,897,590	$10,171,990,825	$7,476,716,918	$19,859,229,379
5	$11,624,897,590	$10,171,990,825	$6,922,886,036	$26,782,115,415
6	$11,624,897,590	$10,171,990,825	$6,410,079,663	$33,192,195,077
7	$11,624,897,590	$10,171,990,825	$5,935,258,947	$39,127,454,024
8	$11,624,897,590	$10,171,990,825	$5,495,610,136	$44,623,064,160
9	$11,624,897,590	$10,171,990,825	$5,088,527,904	$49,711,592,064
10	$11,624,897,590	$10,171,990,825	$4,711,599,911	$54,423,191,974
11	$11,624,897,590	$10,171,990,825	$4,362,592,510	$58,785,784,484
12	$11,624,897,590	$10,171,888,108	$4,039,396,719	$62,825,181,203
13	$11,624,897,590	$10,171,785,391	$3,740,144,378	$66,565,325,582
14	$11,624,897,590	$10,171,785,391	$3,463,096,647	$70,028,422,228
15	$11,624,897,590	$10,171,785,391	$3,206,570,969	$73,234,993,198
16	$11,624,897,590	$10,171,785,391	$2,969,047,194	$76,204,040,391
17	$11,624,897,590	$10,171,785,391	$2,749,117,772	$78,953,158,163
18	$11,624,897,590	$10,171,785,391	$2,545,479,418	$81,498,637,582
19	$11,624,897,590	$10,171,785,391	$2,356,925,387	$83,855,562,969
20	$11,624,897,590	$10,171,785,391	$2,182,338,322	$86,037,901,291

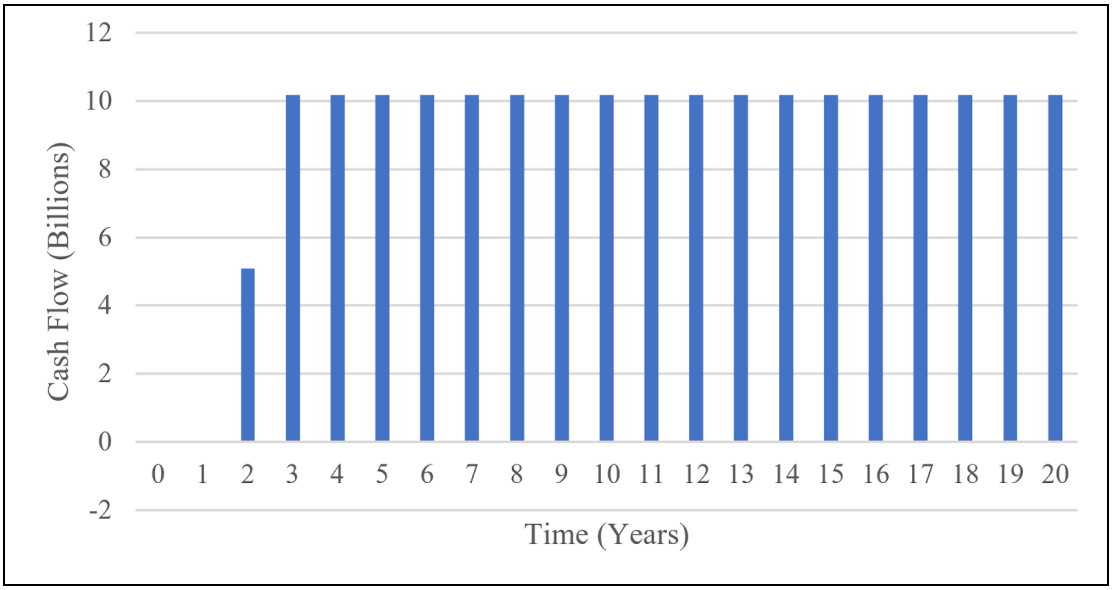


Figure 5.3. Taxed cash flow.

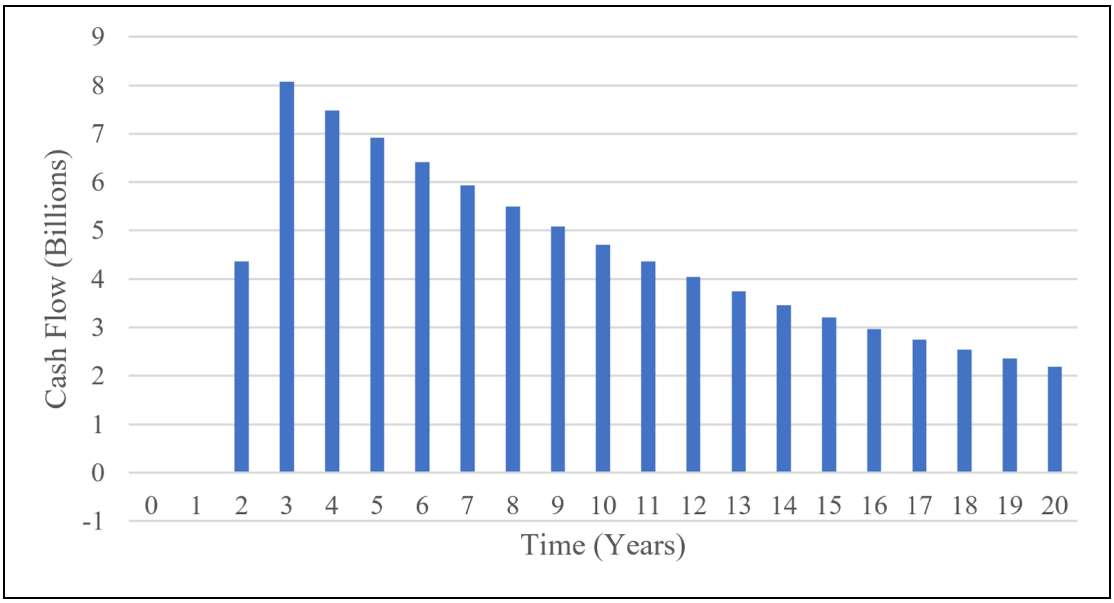


Figure 5.4. Discounted cash flow.

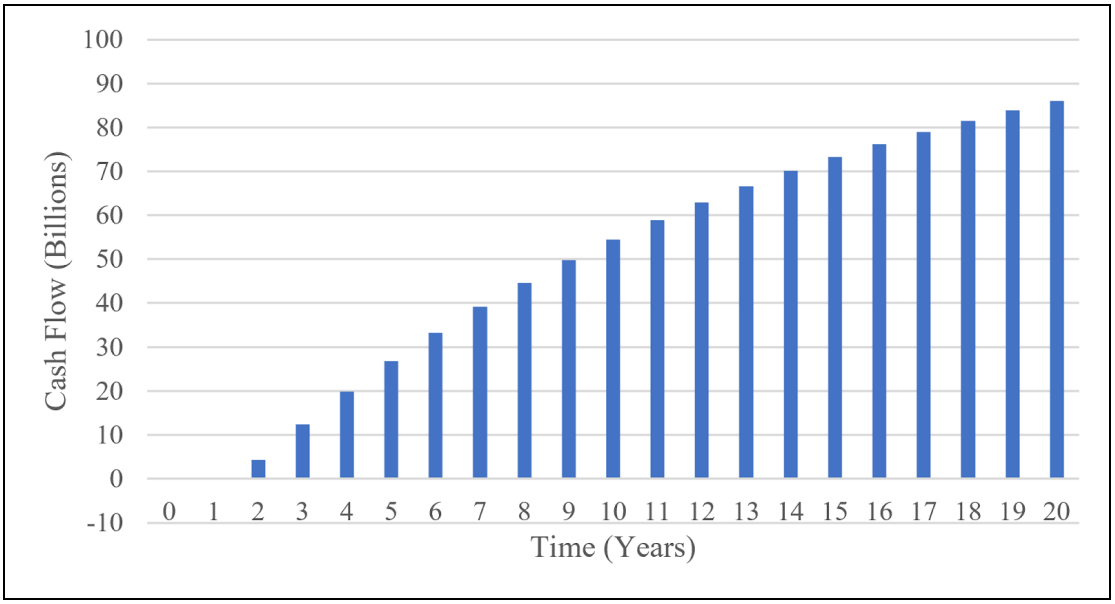


Figure 5.5. Cumulative cash flow.

The net present value of the plant at the end of year 20 is $401 billion, and the internal rate of return is 69.7%. These values were determined using equations 5.3 and 5.4 below, where NPV_{20} is the net present value at the end of 20 years, CCF_{20} is the cumulative cash flow at 20 years, i is the discount rate, P is the initial capital investment, and R is the internal rate of return. Based on the high net present value and internal rate of return, we can determine that this is a profitable project.

$$NPV_{20} = CCF_{20}(1+i)^{20}$$

$$P(1+R)^{20} - P(1+i)^{20} = NPV_{20}$$

Equations 5.3-4. Used to find the net present value and internal rate of return.

5.8.5 Risk Analysis

Though pharmaceutical drugs are typical inelastic markets, the quantity demanded is not a strong function of price, the name brand drug Keytruda does vulnerability to biosimilar drugs. These biosimilar drugs are generic versions of the pembrolizumab monoclonal antibody and require less validation, reducing the cost of research and development significantly. In the case of Infliximab, before biosimilars, the price rose 6% annually for 6 straight years followed by a 1.3% drop for 3 years. Once biosimilars entered the market in 2017, pricing dropped 13.6% annually (Center for Biosimilars Staff, 2019b) Another monoclonal antibody Humira once accounted for 62.2% of Abbvie's revenue but biosimilar competition forced revenue down 33.5% (Center for Biosimilars Staff, 2019a). Forecasting similar revenue drops for Keytruda, a revenue of still remains at 7,780,500,000 which far outweighs operating costs with research and development costs already being recouped pre patent expiration. This is due to the exceptional performance of the drug, the widespread nature of its current designated ailments, and the potential for additional designations.

In order to foresee the economic impacts of undesired changes in the sales of Keytruda, we have examined two dramatic cases. For case 1, we have assumed that the revenue is slashed in half. This dramatically reduces the profit, which may reflect several undesirable events such as decreased sales, decreased production rate, workers demanding more pay, greater manufacturing costs, etc. Despite this dramatic decrease, the net present value and internal rate of return at the end of production is still very profitable at $199 billion and 63.9%, respectively. Not only does this indicate that this project will remain profitable despite drastic market changes, but this also indicates that it is extremely viable and ethically favorable to decrease the price of Keytruda. By

doing so, we can allow this otherwise expensive cancer treatment to be significantly more affordable, and therefore be more accessible to a greater population. In case 2, we have assumed that production is delayed for 3 years. Not only that, but we have assumed that this has occurred due to validation delays, which additionally incur greater validation costs. This may also reflect other scenarios such as delayed construction, prolonged start-ups, unexpected shutdowns, and early plant shutdown. Despite this change, the net present value and internal rate of return at the end of the production lifetime was still very high at $191 billion and 67.1%, respectively. The cumulative cash flows for both cases can be seen in Figures 5.6 and 5.7 below.

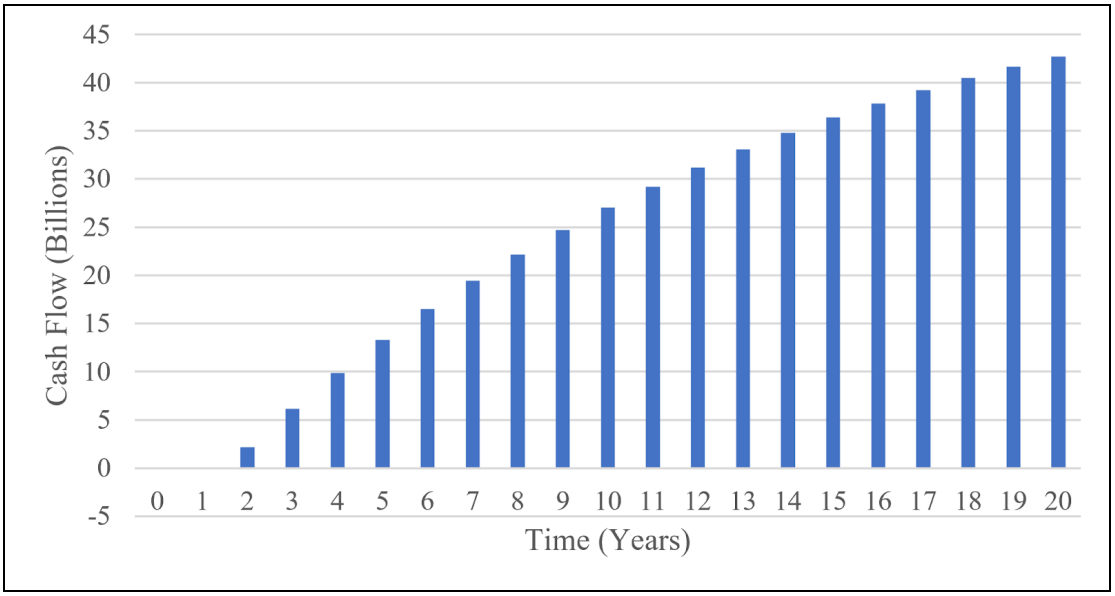


Figure 5.6. Cumulative cash flow for case 1.

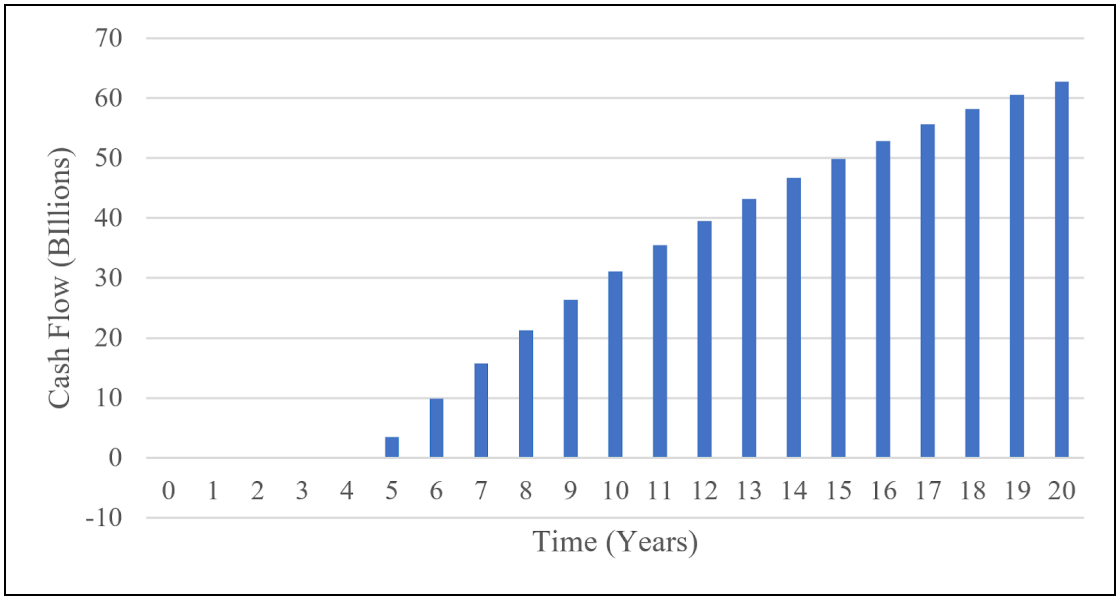


Figure 5.7 Cumulative cash flow for case 2.

5.9 Quality Control

The facility will be equipped with an on-site quality control lab for interval testing of final and intermediate products. The lab will be equipped with high-pressure liquid chromatography (HPLC), mass spectrometers, ion-exchange HPLC, biological cell density measuring equipment including fluorescent dyes, and size exclusion HPLC. These tests will allow the lab to test for purity, molecular weight, chemical identity, cell proliferation, and aggregation respectively. Each test needs to ensure that the product is within acceptable ranges for the location and step in the biomanufacturing process from which it is drawn.

The testing strategy is key for this process as unlike with batch processing, a failed test can have undefined limits of product misspecifications due to the continuous nature. The testing will occur at fixed intervals of two hours at several locations. The locations of testing are seen in Figure 5.8 and are as follows: perfusion bioreactor, post sterile filtration, post chromatography columns (all), post-final UF/DF. A failure in a quality control test will trigger the disposal of a

"batch". For quality control purposes only, a batch constitutes a 2 hour period ahead of the failed test. Additionally, further testing of material before this 2 hours period is required along with a shorter 30-minute testing interval for a period of 2 hours immediately followed by a 1 hours testing interval for a period of 22 hours. A root cause study will take place and emergency holding tanks can be used to shut down the process if deemed necessary.

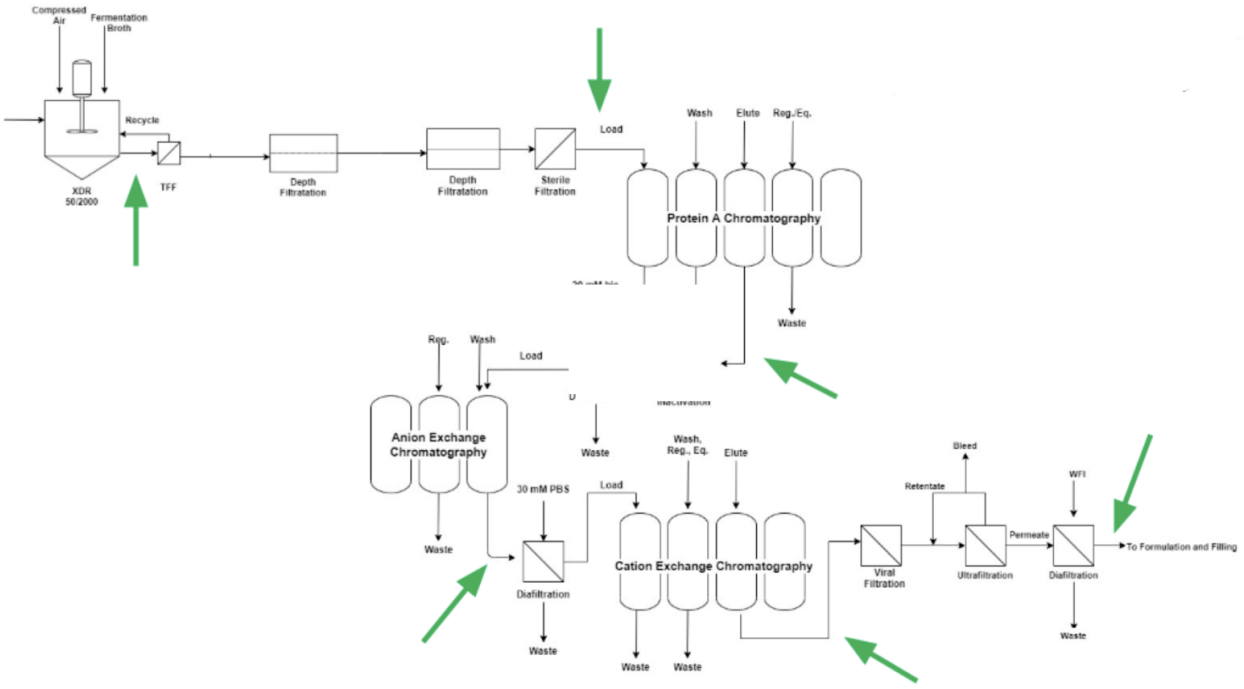


Figure 5.8. Location of Quality Control Testing.

6. Regulatory, Safety, Health, and Environmental Considerations

In order to maintain compliance with the FDA and EMA, this pembrolizumab facility will abide by the current Good Manufacturing Practices (GMP). These practices ensure public safety by maintaining safety, purity, efficacy, stability, and consistency. By following these procedures, we will ensure that the product leaving a pharmaceutical facility maintains quality at all moments along the supply chain. Additionally, these policies help ensure that any operators,

chemicals, or ancillary materials that come in contact with the equipment will not contaminate the product. All individuals working in the facility will follow these procedures. These protocols will additionally ensure that individuals maintain consistent documentation.

The safety hazards that arise from this process mostly arise from any caustic cleaning chemicals or biological hazards that may be utilized. In order to prevent these hazards, all individuals that work within the process must wear the appropriate personal protective equipment (PPE) as designated by the OSHA standards for PPE use. The specific caustic chemicals include exposure to NaOH, HCl, PBS, and bis Tris with the first two buffers being the most caustic. These caustic chemicals present a health hazard during the cleaning stages and buffer preparations for the ion-exchange chromatography steps, the protein affinity step, and the intermediate diafiltration steps. It is important to minimize exposure by mitigating the amount that comes into contact with individuals via contact with the skin, inhalation, or ingestion. The appropriate PPE, training, and GMP guidelines will all work to minimize safety hazards and prevent excessive exposure. The facility will be equipped with floor drains, eyewash stations, chemical showers, and non-slip flooring material to mitigate safety risks present in the facility.

The environmental impact of this facility arises primarily from the solid and liquid waste generated by the facility. This waste is generated in both upstream and downstream processing. Primarily, the use of single-use disposable bioreactor bags during the seed train scale-up will provide environmental waste. However, this will greatly minimize the use of caustic cleaning chemicals throughout this stage of the process, and this benefit will outweigh the waste due to disposed plastic. These bags will, nonetheless, have to be disposed of properly. Additionally, an environmental hazard arises from the NaOH and HCl cleaning buffers. As a result, all buffers

and waste that leave the system will be treated with 1.0 M NaOH to deactivate any active biologics, and subsequently neutralized with 1.0 M HCl. After the waste returns to a pH of 7.0, the waste can then be properly treated.

7. Social and Ethical Concerns

Social and ethical concerns are addressed in order to ensure the overall success of the facility and product, by ensuring little disruption caused to the environment in which the plant is being built as well as to its residents, and a healthy work atmosphere for employees working in the plant. If these concerns are addressed, it can then be ensured that the product of the utmost quality is made and that patients receiving treatment with the drug can benefit from its use. This will also ensure that this production facility and Merck as a company remain in good standing.

Anticipated impacts to the community surrounding the facility in Carlow, Ireland may include noise from construction of the plant and its related change in traffic flow, as well as a surge of new jobs to the area. We do not anticipate a great environmental impact to the area, as waste disposal will be conducted in a manner that minimizes environmental damage. Additionally, relying on a single-use system will reduce the environmental impact of the plant for an extended period of time. The continuous process will improve the efficiency of the plant while also decreasing the amount of natural resources that the plant demands. Employees will be paid competitive salaries, provided many benefits, and their safety will be prioritized at all times in order to ensure a strong workforce. All employees, including investigation authors, technical operators, quality control, and plant managers, will undergo safety and ethics training through a week-long orientation before they begin their official work in the plant.

A major reason why single-use and continuous bioprocessing technology is a priority in the plant design is to overall reduce costs in an effort to reduce the base price of the drug for patients. With the plant design aiming to reduce time and money spent in manufacturing, it would be feasible to price Keytruda at a lower base price that would further encourage patients to

seek treatment with this life-saving drug, as demonstrated by Case 1 in section 5.8.5. We aim to make the currently very expensive drug as affordable for patients as possible, while also making enough profit to go towards further research and maintenance of our drug. It is also worth mentioning that Keytruda is the leading PD-L1 inhibitor in the market, therefore market competition is not a large concern for this design.

8. Conclusions and Recommendations

The research conducted in this report on a pembrolizumab manufacturing facility utilizing single-use and continuous bioprocessing technology demonstrates that this facility will be financially viable, practical, and successful in filling the high demand there is for Keytruda.

Our economic analysis proves that the plant and its operation would be very profitable once product sales begin. Single-use technology and continuous bioprocessing will increase the profitability of the plant by reducing production time, costs, and personnel required. Additionally, single-use and continuous bioprocessing technology are becoming more relevant in the industry today, so this plant would serve as a source of information for other facilities attempting to change their operations to continuous or single-use technology-based. An added benefit of this design is that it would be able to overall reduce costs in an effort to reduce the base price of the drug, ensuring greater accessibility of the drug for consumers.

Aside from general risks or concerns of safety and product quality that are present with any plant design, there are no major risks or concerns to consider with this project. Keytruda is the leading PD-L1 inhibitor among competitors, and it is consistently high in demand. Concerns may include the long-term viability of keeping this process continuous or the ability to adhere to the product timeline, but this project's research has proven that this should be feasible. Overall, the project is capable of moving onto validation and construction of the plant.

9. Acknowledgments

Throughout the duration of this Capstone design project, a variety of sources and individuals have been crucial to its success. Primarily, the extensive resources offered by the University of Virginia were imperative in achieving the quality of design, and these resources allowed us to resolve any conflicts that arose quickly and efficiently. The team would specifically like to thank Professor Eric Anderson, Professor Giorgio Carta, Professor Mike King, and Professor George Prpich for their help throughout this year. Professor Anderson ensured that we kept to our proposed schedule and offered weekly guidance. Professors Carta, King, and Prpich provided expertise whenever it was asked of them. Finally, we would like to thank the University of Virginia Department of Engineering and Applied Science for all of the knowledge offered during our four years as students, without which this project would not have been possible.

10. Table of Nomenclature

Symbol	Definition	Unit
Q	Volumetric Flow Rate	L/min
d	Diameter	cm
v	Kinematic viscosity	cm^2/s
D_0	Diffusion coefficient	cm^2/s
Re	Reynolds Number	--
L	Length	cm
p	density	g/L
ΔP	Pressure Drop	psi
μ	Dynamic Viscosity	cP
ε	Extraparticular Porosity	--
u	Superficial Velocity	m/s
d_p	Particle Diameter	m
η	Dynamic Viscosity	Pa*s
L	Column Length	m
Ks	Half saturation constant	N/A
S_o	Initial Substrate concentration	g/L
μ_{max}	Maximum specific growth rate	hr^{-1}
F_o	Inlet feed flow rate	L/min
D_{opt}	Optimum Dilution Rate	hr^{-1}
a	Recycle flow rate divided by feed flow rate	N/A
b	Recycle stream cell concentration divided by reactor outlet cell concentration	N/A

N_{OL}	Number of operators per shift	N/A
N_{np}	Number of non-particulate processing steps	N/A
TCF	Taxed cash flow	$
DCF	Discounted cash flow	$
t	Time	Years
i	Discount rate	N/A
NPV	Net present value	$
CCF	Cumulative cash flow	$
P	Initial capital investment	$
R	Internal rate of return	N/A

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12. Appendix

Calculations for Viscosity of Pembrolizumab Solution

From Li et al 2014

#/mAb	pI	C20	η (cP)	C50	η (cP)	C75	η (cP)	C100	η (cP)	C125	η (cP)	C150	η (cP)	C175	η (cP)	C200	η (cP)
8/IgG4κ	7	17	1.2	43	1.5	91	3.3	114	5	137	8.3	160	14.5	--	--	189	33

Table I Concentration Dependent Viscosity Data on 11 mAbs Used in this Study*

mAb #, Isotype, pI[#]	C_{20}	η (cP)	C_{50}	η (cP)	C_{75}	η (cP)	C_{100}	η (cP)	C_{125}	η (cP)	C_{150}	η (cP)	C_{175}	η (cP)	C_{200}	η (cP)
1, IgG1κ, 8.3	19	1.1±0.0	49	1.5±0.0			98	2.6±0.0	126	3.7±0.0	153	5.4±0.1	172	8.0±0.1	204	13.5±0.2
2, IgG1κ, 8.7	18	1.2±0.0	52	1.6±0.0			97	2.7±0.0	127	3.7±0.1	149	5.4±0.1	171	8.1±0.2	195	13.3±0.7
3, IgG4κ, 7.5	23	1.2±0.0	46	1.5±0.0			95	2.9±0.1	126	4.0±0.0	148	6.2±0.1	171	11.1±0.6	198	19.1±3.7
4, IgG1λ, 8.7	19	1.1±0.0	45	1.4±0.0			99	2.7±0.0	126	4.1±0.1	147	6.6±0.1	176	12.2±0.6	196	20.4±0.9
5, IgG1κ, 8.1	21	1.2±0.0	48	1.7±0.0	74	2.3±0.0	98	3.3±0.0	129	5.3±0.0	150	8.4±0.1	175	14.1±0.2	203	28.9±0.8
6, IgG1λ, 8.2	25	1.3±0.0	49	1.7±0.0			105	3.9±0.0	127	6.9±0.0	153	12.9±0.1	183	27.6±0.2		
7, IgG2κ, 6.9	19	1.1±0.0	53	1.5±0.0			96	3.2±0.2	125	6.4±0.6	150	13.9±0.5	170	27.3±4.5		
8, IgG4κ, 7.0	17	1.2±0.0	43	1.5±0.0	91	3.3±0.0	114	5.0±0.1	137	8.3±0.1	160	14.5±1.1			189	33.0±2.7
9, IgG2κ, 7.4	25	1.3±0.0	50	1.7±0.0			107	4.2±0.1	134	7.6±0.1	155	15.6±0.6			191	44.9±1.8
10,IgG1λ, 7.2	19	1.2±0.0	52	2.5±0.0			103	15.4±0.2	131	43.6±0.6	149	94.7±0.6				
11,IgG1λ, 6.9	20	1.6±0.0	41	3.4±0.0	81	15.4±0.1	106	30.2±0.2	130	57.9±0.5	150	103.6±1.0				

* Measured concentration dependent solution viscosity data for 11 mAbs. Viscosities were measured at several targeted concentrations. For example, C_{20} means experimentally measured concentration at targeted concentration value of 20 mg/mL. The values shown in this table are for solution viscosity (η). These data were used to derive values for specific viscosity (η_{sp}) and relative viscosity (η_{rel}) of the mAbs plotted in Fig. 1. # pI values reported in this table were measured using cIEF method described in the text. The highly concentrated solutions of mAbs 10 and 11 show high viscosities.

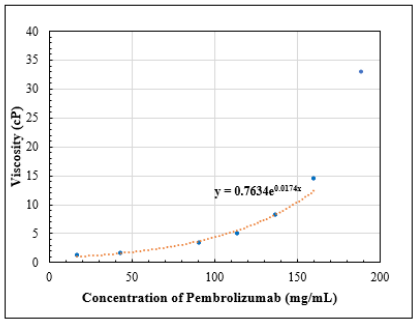

Figure A.1/Table A.1. Viscosity approximations for pembrolizumab using an IgG4 model with pI of 7.0.

Scale Calculations

Sales of Keytruda in 2019 = 10.8 *billion dollars*

Sales of Keytruda in 2025 = 22.5 *billion dollars*

Average Cost of Keytruda per mg = \$51.79/*mg*

Sales Increase from 2019 *to* 2025 = 11.7 *billion dollars*

$$\textit{Number of kg needed of Keytruda} = \frac{11.7\ \textit{billion dollars}}{\$51.79\ \textit{per mg}} = 226\ \textit{kg of Keytruda}$$