Design of a Pembrolizumab Manufacturing Plant Utilizing a Perfusion Bioreactor and Precipitation Chromatography

A Technical Report submitted to the Department of Chemical Engineering

Presented to the Faculty of the School of Engineering and Applied Science
University of Virginia • Charlottesville, Virginia

In Partial Fulfillment of the Requirements for the Degree
Bachelor of Science, School of Engineering

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Spring, 2023

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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

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2. Summary

The purpose of this capstone project was to design a continuous manufacturing facility for pembrolizumab. Pembrolizumab is a monoclonal antibody (mAb) that is used to treat lung, bladder, stomach, colon, and cervical cancers (Keown, 2019; Merck & Co., 2019). Pembrolizmab is currently produced by Merck under the brand name Keytruda. However, Merck's patent is expiring in 2028 (Hagen, 2021). The FDA has recently approved pembrolizumab as the first-line treatment for colorectal cancer, making it the first FDA approved mAb alternative to chemotherapy (Voelker, 2020).

To account for the increase in demand for pembrolizumab due to the patent expiring and the recent FDA approval, this manufacturing facility will use a continuous bioprocess to increase production of pembrolizumab. The facility will also use single use equipment throughout the process to decrease the amounts of harmful caustic chemicals needed for cleaning. This facility will also utilize precipitation chromatography, a more cost effective alternative to protein A chromatography, which is traditionally used in mAb purification. Precipitation chromatography is less expensive than protein A, but has similar yields and purity (Großhans et al., 2018).

The proposed design of this facility will produce 1400 kilograms of Keytruda per year, enough for 7 million doses per year. This accounts for 20% of the 2024 projected pembrolizumab demand (Liu, 2022). The total capital investment in the facility is $63.6 million. The plant will undergo construction and then operate at full capacity without selling the product while undergoing FDA validation, for a total startup time of 1.5 years between construction and selling product. From the economic analysis, the facility has a net present value of $61.4 million and an internal rate of return of 1132% for 15 years of operation. The analysis of this facility suggests this is an economically feasible design.

3. Introduction

3.1 Motivation and Background

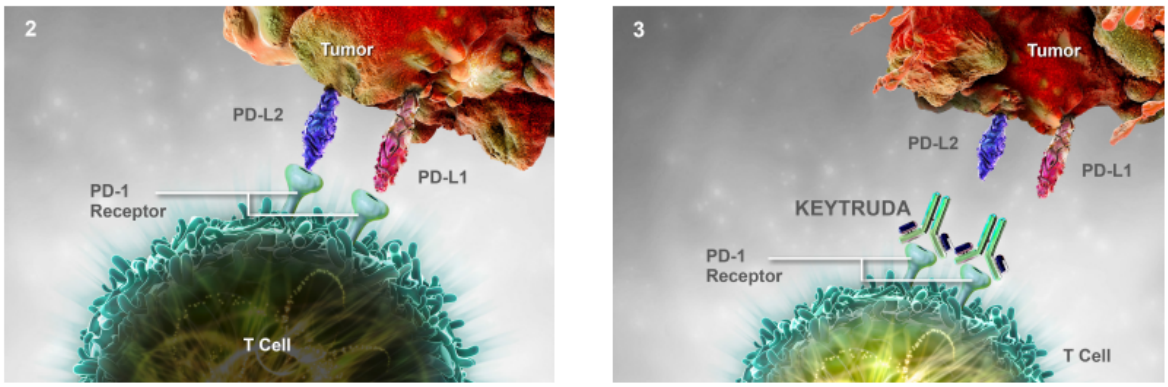
As of 2021, cancer is one of the leading causes of death in the United States (CDC, 2021). Pembrolizumab (Keytruda), is a monoclonal antibody manufactured by Merck & Co that serves as a blocking agent to prevent the cancer cells from hiding and spreading (Merck & Co., 2019). In 2014, pembrolizumab was initially FDA approved for treatment of advanced melanoma (Keown, 2019). Today, pembrolizumab is approved for treatment of various conditions including lung, bladder, stomach, colon, and cervical cancers (Keown, 2019; Merck & Co., 2019). Pembrolizumab averaged a 38% reduction in risk of death due to cancer versus chemotherapy alone. In 2020, it drew 14.4 billion dollars in sales, the second highest selling monoclonal antibody on the market during this time (Carta, 2022; Merck, 2020). In June 2020, the U.S. FDA approved a new indication for pembrolizumab as the first-line treatment for people with unresectable or metastatic microsatellite instability-high (MSI H) or mismatch repair deficient (dMMR) colorectal cancer; this marks the first immunotherapy approved for that population in the US as a first-line treatment and which is administered to people without also giving chemotherapy (Voelker, 2020).

Pembrolizumab is insufficiently accessible in low to middle income countries (LMICs) due to three core reasons: inharmonicity among global regulatory agencies, a lack of effort and awareness put towards registering novel mAbs in LMICs from the manufacturers and governments of the countries in need, and a lack of healthcare infrastructure in LMICs capable of undertaking the expenses and bureaucratic complexities of mAb drug products (Reck et al., 2016; Wellcome, 2020). The high cost of mAbs has rendered these barriers frequently as insurmountable both in LMICs and in underprivileged regions of high-income countries (Wellcome, 2020). With patent protection over the pembrolizumab originator due to expire in

2028, it is an opportune time to develop a cheaper alternative process to production (Hagen, 2021).

We designed a more efficient pembrolizumab manufacturing plant. Operating with perfusion or continuous bioreactors instead of batch bioreactors allows for increased product quality and productivity (Yang et al., 2019). Currently, the most expensive part of the process is the chromatography used to separate and purify the final protein product; many chromatography methods have been explored to optimize chromatography cost, including continuous antibody precipitation (Burgstaller et al., 2019). We utilized Chinese Hamster Ovary (CHO) cells to express pembrolizumab in a perfusion reactor and precipitation chromatography supplemented by other continuous filtration methods for product purification.

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).

T cells are released from the immune system to fight against infections and diseases, such as cancer; however, the PD-1 (programmed death receptor-1) pathway is used by cancer cells to hide from T cells (Merck & Co., 2019). Pembrolizumab is a monoclonal antibody manufactured by Merck & Co that serves as a programmed cell death inhibitor, or a blocking agent to prevent the cancer cells from hiding along the PD-1 pathway (Merck & Co., 2019).

3.3 Treatment and Dosage

Keytruda is used to treat lung, bladder, stomach, colon, and cervical cancers (Keown, 2019; Merck & Co., 2019). It is the current market lead in treating lung, gastric, and kidney cancers with the potential for use in early-stage treatment around surgery (Dunleavy, 2022).Pembrolizumab averaged a 38% reduction in risk of death due to cancer versus chemotherapy alone. One dose is administered to patients every three weeks. The final Keytruda drug can either be lyophilized and combined with sterile water for injection (WFI) before it is injected, or it can be sold as a solution already in the WFI. The final product in this project is sold in solution form, with one dose containing the active ingredient pembrolizumab, sucrose, L-Histidine, and polysorbate 80. The product must exceed 99% purity to be safely injected (Kelley, 2009).

3.4 Plant Capacity

Keytruda is one of the fastest growing oncology drugs currently on the market and also a very consistent performer in sales (Dunleavy, 2022). In 2021, Keytruda was the world's best-selling cancer drug and the 4th highest grossing drug by sales showing 19.5% year-over-year growth (Dunleavy, 2022). Continued growth for Keytruda is expected due to the success and hoped success of present and future clinical trials. Keytruda has recently gained approval in recent phase 3 trials in treating adjuvant non-small cell lung cancer (NSCLC) and is hopeful for approval as both a neoadjuvant and adjuvant treatment for stages 2 through 2B NSCLC (Liu, 2022). Merck & Co. continues to run additional phase 3 trials specifically for Keytruda targeting early-stage disease numbering 14 total between 2022 and 2025 opening the possibility for additional users and demand. Due to the expectation that users are expected to double, the decision was made to capture at least 1/5th of the expected demand due to the large growth rate of

Keytruda. In 2018, Keytruda's explosive growth saved a Merck Ireland manufacturing facility to keep up with pembrolizumab manufacturing needs (Palmer, 2018).

Based upon these data we designed this new more efficient manufacturing facility to produce 1400 kg of pembrolizumab annually to provide approximately 7 million doses, accounting for 20% of the 2024 projected demand, as users of pembrolizumab are projected to double from 1 million to 2 million (Liu, 2022). This number was determined by assuming 2 million users used Keytruda every 3 weeks for a year. The calculations were completed with the doses currently accepted by the FDA for treatment of NSCLC and head and neck squamous cell carcinomas (HNSCC), 200 mg every 3 weeks for 200,000 individuals.

4 Discussion

4.1 Upstream Process

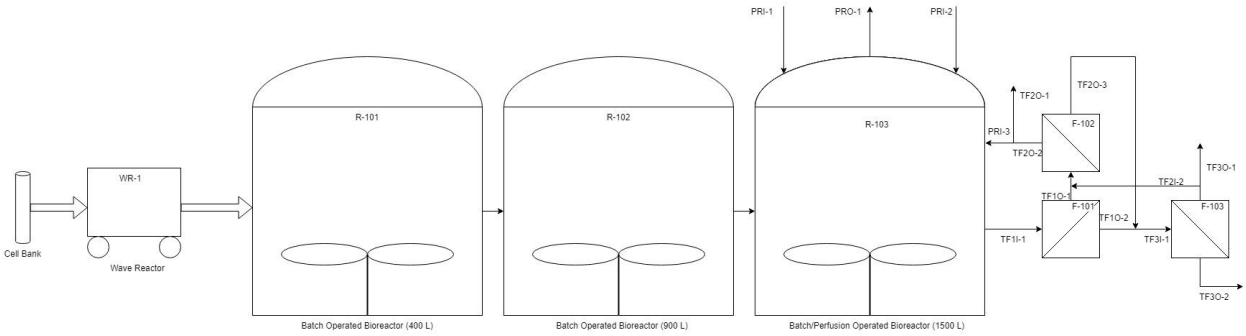


Figure 4.1.1. Upstream Process Flow Diagram.

4.1.1 Cell Line Acquisition and Storage

Over two thirds of recombinant therapeutic proteins on the market are produced from Chinese hamster ovarian (CHO) cells (Li et al., 2010). CHO cells make advantageous hosts due to their consistent and similar protein glycosylation profiles to humans and efficiency in undergoing posttranslational modifications (Orellana et al., 2015). Through heavy focus on their development in research and industry, they also have been engineered to produce high titers of monoclonal antibodies (Wurm, 2004). We will develop pembrolizumab using recombinant CHO cells acquired from Merck & Co.'s master cell bank in West Point, PA. Doing so will enable us to bypass the regulatory requirements of utilizing a novel cell bank, such as pursuing FDA approval and clinical trials. To avoid deviations in the quality of the cells, strict control and documentation of the cell transportation conditions will be necessary to ship the cells from West Point, Pennsylvania to our plant in Norristown, PA**.** Specifically, we will store the cells at -86 ºC in a VIP ECO Model MDF-DU702VH-PA Freezer throughout the shipping process and upon arrival at the production facility. This freezer model has an operating range of -40°C to -86°C (PHC Corporation, 2021). Two freezers will be used for storage in accordance with GMP guidelines to reduce cell loss related to freezer malfunctions or a disaster (FDA, 2006).

We will assume a high cell density of 50 x 10^6 cell/mL in each 4.5mL vial of the CHO cell line acquired from Merck & Co. This high density cell banking is proven to significantly reduce scale-up time (Tao et al., 2011). For use, the working cell bank (WCB) will be thawed in a 2 liter Thermo Fisher Precision GP 02 Water Bath operated at 37 ^{O}C (Thermo Fisher Scientific, 2022).

4.1.2 Inoculum Train

Before reaching the continuous perfusion stage operations, the cell volume must be scaled from the 4.5 mL cell inoculum to a working volume of 1500 L. The Monod-type kinetic equations, as modeled by Kornecki and Strube, model the growth of the cells, the consumption of glucose and glutamine as substrates, the production of lactate and ammonium as metabolites, and the monoclonal antibody production as a function of time (Equations 4.1.2). The modeling performed is of an immunoglobulin of type IgG1 produced by CHO cells with a mass of 150 kDa, similar to the mass of pembrolizumab, which is 149 kDa. Due to the small difference in mass, the kinetic equations and parameters were not altered when applied to pembrolizumab.

$$\frac{dX_v}{dt} = (\mu - \mu_d) \times X_v$$

$$\mu = \mu_{max} \times \frac{[GLC]}{K_{glc} + [GLC]} \times \frac{[GLN]}{K_{gln} + [GLN]} \times \frac{K_{\mathrm{Ilac}}}{K_{\mathrm{Ilac}} + [LAC]} \times \frac{K_{Iamm}}{K_{Iamm} + [AMM]}$$

$$\mu_d = k_d \times \frac{[LAC]}{K_{Dlac} + [LAC]} \times \frac{[AMM]}{K_{Damm} + [AMM]}$$

$$\frac{d[GLC]}{dt} = -\left(\frac{\mu - \mu_d}{Y_{X_V/glc}} + m_{glc}\right) \times X_V$$

$$\frac{d[LAC]}{dt} = Y_{lac/glc} \times \left(\frac{\mu - \mu_d}{Y_{X_V/glc}}\right) \times X_V$$

$$\frac{d[GLN]}{dt} = -\left(\frac{\mu - \mu_d}{Y_{X_v/gln}} + m_{gln}\right) \times X_V$$

$$m_{gln} = \frac{a_1 \times [GLN]}{a_2 + [GLN]}$$

$$\frac{d[AMM]}{dt} = Y_{amm/gln} \times \left(\frac{\mu - \mu_d}{Y_{X_V/gln}}\right) \times X_V - r_{amm} \times X_V$$

$$\frac{d[mAb]}{dt} = Q_{mAb} \times X_V$$

Equations 4.1.2. The Monod-type kinetics equations for monoclonal antibody production by CHO cells.

Table 4.1.2. Constants for the Monod-type kinetics equations.

Parameter	**Description**	**Value**	**Unit**
μ_{max}	*Maximum growth rate*	0.029	h^{-1}
k_d	*Maximum death rate*	0.0066	h^{-1}
$Y_{X/glc}$	*Yield coefficient cell conc./glucose*	0.413	e9 cells $mmol^{-1}$
$Y_{X/gln}$	*Yield coefficient cell conc./glutamine*	0.573	e9 cells $mmol^{-1}$
$Y_{lac/glc}$	*Yield coefficient lactate/glucose*	1.391	mmol $mmol^{-1}$
$Y_{amm/gln}$	*Yield coefficient ammonium/glutamine*	0.739	mmol $mmol^{-1}$
Q_{mAb}	*Specific production rate*	2.25	e-12 g $cells^{-1}$ h^{-1}
r_{amm}	*Ammonium removal rate*	6.3	e-12 mmol $cells^{-1}$ h^{-1}
m_{glc}	*Glucose maintenance coefficient*	69.2	e-12 mmol $cells^{-1}$ h^{-1}
a_1	*Coefficient for m_{gln}*	3.2	e-12 mmol $cells^{-1}$ h^{-1}
a_2	*Coefficient for m_{gln}*	2.1	mM
K_{glc}	*Monod constant glucose*	0.15	mM
K_{gln}	*Monod constant glutamine*	0.04	mM
K_{Ilac}	*Monod constant lactate for inhibition*	45.0	mM
K_{Iamm}	*Monod constant ammonium for inhibition*	9.5	mM
K_{Dlac}	*Monod constant lactate for death*	40.0	mM
K_{Damm}	*Monod constant ammonium for death*	4.0	mM

Initiating the upstream process with a high-density working cell bank fosters a more efficient inoculum train in terms of time and equipment (Repligen, 2022). The inoculum train we will use to bolster the number of cells to process-scale is illustrated in Figure 4.1.2 below.

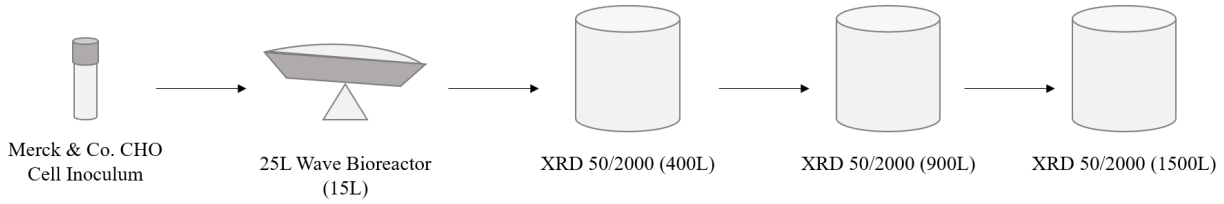


Figure 4.1.2. Fed-batch inoculum train equipment and working volumes from 4.5 mL vial to 1500 L perfusion reactor.

As shown, the ReadyToProcess WAVE 25L Rocker from Cytiva will constitute the first step in our scale-up process. To allow for headspace, we will operate it at a final working volume of only 15 L. Each step in our inoculum train prior to perfusion will be done in fed-batch mode, including the wave rocker, which will be operated at a temperature of 37 ^{O}C, a pH of 7.1, and a rocking speed of 20-29 RPM. This particular model has built-in temperature and dissolved oxygen sensors and will adjust the heat and rocking speed appropriately to achieve our set parameters. As recommended by the manufacturer, the wave rocker will run with a dissolved oxygen level of 40% (Cytiva, 2017).

From dilution of the working cell bank with 1 liter of WFI, the initial cell count in the wave rocker will be $2.25 * 10^8$ cells. Media will be fed into the wave rocker at a flow rate of 0.000667 L/min. This includes a glucose mass flow of 0.0120 g/min and glutamine mass flow of 0.00175 g/min. Operation for 350 hours will bring the total cell count up to $1.26 * 10^{10}$ and 15 L working volume, at which point the slurry will be transferred to a 400 L bag in the first XDR bioreactor.

In the XDR bioreactor, the 15 L working volume slurry will be diluted to 400 L with WFI, then commence the first round of fed batch operation, increasing the working volume to

900 L over the course of 100 hours. The feed rate in this stage is 0.0833 L/min, and the mass flow rates of glucose and glutamine are 0.499 g/min and 0.0731 g/min, respectively. The second fed batch stage in the bioreactor brings the working volume from 900 L to 1500 L over 500 hours. The flow rate is 0.0200 L/min with a glucose mass flow rate of 0.479 g/min, and a glutamine mass flow rate of 0.0526 g/min.

Using the Monod-type kinetic equations modified for fed batch operations, the concentration of cells and pembrolizumab product were calculated for the duration of the 950 hour inoculation train, as seen in Figure 4.1.2.1. The final concentration of cells in the bioreactor is 17.35 g/L, and a total of 25.40 kg of pembrolizumab is produced in one bioreactor during the seed train. In order to meet production demands, two bioreactors are needed, and both will have identical seed trains and perfusion operating conditions.

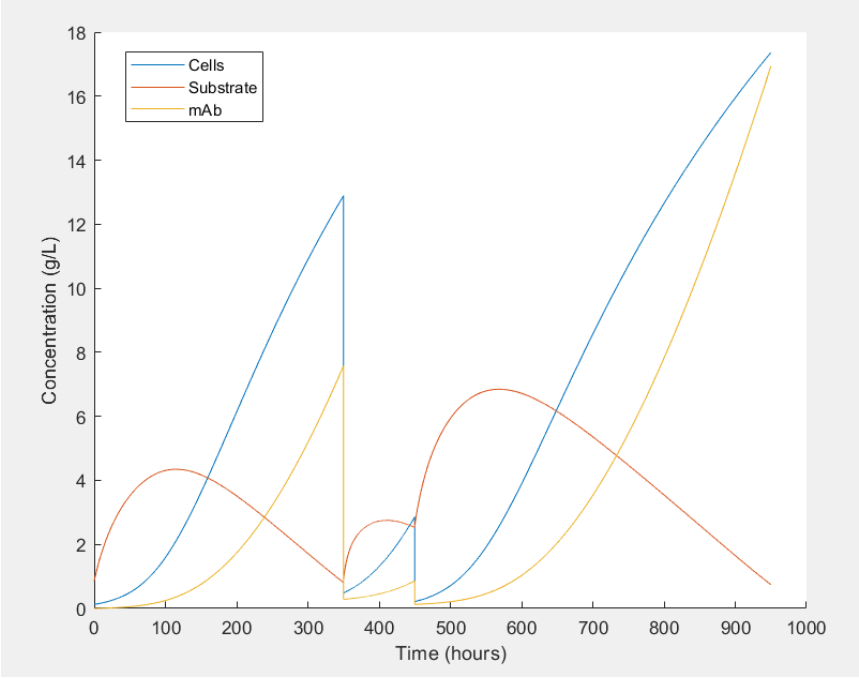


Figure 4.1.2.1. Concentration of cells, substrates, and pembrolizumab throughout the inoculation train.

4.1.3 Perfusion Reactor

We chose a Cytiva Xceller XDR 2000 Pro Single-Use Stirred Tank reactor for our perfusion reactor. We decided to use a perfusion reactor over a traditional batch or fed-batch bioreactor because cells stay in the exponential growth phase longer in perfusion, allowing for higher cell densities (Cytiva, 2020). We picked the Cytiva reactor because it has disposable bags and impellers, allowing for easier clean in place and sterilization of the fermentor. It also has a flexible range of working volumes, from 400 L to 1500 L, so most of the seed train can be grown in place in the reactors before perfusion begins (Cytiva, 2020b). These bioreactors are commonly used in industry. We will have five bioreactors, with two used to grow the seed train and two operated in perfusion mode over the course of the 25 day campaign. The fifth bioreactor will be a spare in case one breaks. Perfusion bioreactors can operate for 2-3 months at a time while maintaining a constant cell density in perfusion mode, so this is easily feasible (Burns et al., 2021).

We will operate at steady state while in perfusion mode, maintaining a constant working volume of 1500 L (Table 4.1.3.1). Several studies have examined the recommended perfusion bioreactor media exchange rate, which is expressed in vessel volumes per bioreactor volume per day (vvd^{-1}) to be between 1-2 vvd^{-1}, which would correspond to 1.04-2.08 L/min per bioreactor (Bielser et al., 2018). The flow rate into the bioreactor is determined by the dilution rate, as explained in section 4.1.4, and will be 1.55 L/min per bioreactor, which is within this range. During perfusion operations, the concentration of cells will be $3.10 * 10^{10}$ cells/L, and the resulting consumption of substrate requires a feed rate of 0.186 g/min and 0.0755 g/min for glucose and glutamine, respectively.

The bioreactor will operate at a temperature of 37℃ and 1.01 bar. We will maintain a constant pH of 7.2 in order to optimize cell viability (Ghafuri-Esfahani et al., 2020). Bioreactor dimensions are given in Figure 4.1.3.1.

Table 4.1.3.1. Xceller XDR Pro 2000 Tank Dimensions (Cytiva, 2020b).

Parameter	Value	Unit
V	2000	L
D_t	123	cm
H_t	185	cm
D_i	42	cm
H_i	42	cm
H_L	126	cm

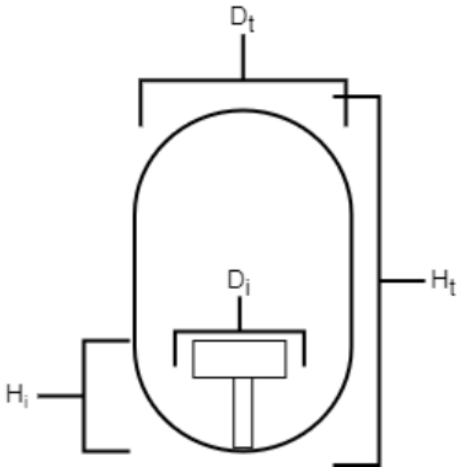


Figure 4.1.3.1. Perfusion Bioreactor Dimensions.

The Xceller XDR Pro also comes with a 40° pitched blade four blade impeller that is built into the bags for easy maintenance and cleaning, as well as sensors for dissolved oxygen concentration and pH monitoring that will be autoclaved between campaigns to prevent contamination.

Dissolved oxygen will be supplied to the system by adding compressed air to the bioreactor, which can be mathematically modeled using Equation 4.1.3.1.

$$\frac{dC_{O2}}{dt} = [k_L a(C^*_{O2} - C_{O2})] - [\frac{1}{Y_{X/O2}}\mu X]$$

Equation 4.1.3.1. Change in Oxygen Concentration Over Time.

To determine the oxygen demand and mass transfer rate of the system (k_La), we assumed the rate of oxygen transferred to the cells was equal to the rate of oxygen consumed by the cells. This assumption is true under steady state conditions, which occur when the bioreactor is operated in perfusion mode (Davis & Davis, 2003). Using the steady state assumption,the target k_La can be determined using the following equation.

$$target\ k_L a = \frac{Q_{O2}X}{C^*_{O2} - C_{O2}}$$

Equation 4.1.3.2. Target k_La with Steady State Assumptions.

In Equation 4.1.3.2, Q_{O2} is the cell oxygen consumption rate, X is the concentration of cells, C^*_{O2} is the solubility of oxygen at 37℃ and 1.01 bar, and C_{O2} is the minimum concentration of oxygen. Values under these conditions are given in Table 4.1.3.2.

Table 4.1.3.2. Theoretical Aeration Parameters for Xceller XDR Pro 2000 Bioreactor.

Parameter	**Value**	**Unit**
Q_{O2}	0.4107	mmol/g-h
X	17.35	g/L
C^*_{O2}	6.73	mg/L
C_{O2}	1.35	mg/L
K_La	42.40	h^{-1}

Q_{O2} was determined based on literature for CHO cells under steady state conditions (Goudar et al., 2011). X was found using the mass of a CHO cell (Abt et al., 2020) and the concentration of CHO cells in the media, which we determined was 3.1e10 cells/L media. C^*_{O2} was found using solubility tables for dissolved oxygen in water at 37℃ and 1.01 bar (Xylem,

2019). We approximated C_{O2} was 20% of C^*_{O2} under these conditions (Prpich, 2020). We calculated the target k_La as 42.4 h^{-1}.

We also determined the mixing and aeration conditions for the bioreactor, as well as the power requirements using the following equations.

$$Re = \frac{ND_i^2\rho}{\mu}$$

Equation 4.1.3.3. Reynold's Number for Stirred Tank Bioreactor.

$$P = N_P\rho N^3 D_t^5$$

Equation 4.1.3.4. Power Requirement Equation for Stirred Tank Bioreactor.

$$N_a = \frac{Q_g}{ND_i^3}$$

Equation 4.1.3.5. Aeration Number Equation for Stirred Tank Bioreactor.

$$k_La = \frac{0.0333}{D_t^4}\left(\frac{P_g}{V}\right)^{0.541} Q_g^{\frac{0.541}{\sqrt{D_t}}}$$

Equation 4.1.3.6. Predictive Model for k_La.

In Equation 4.1.3.3, Re is the Reynold's number, N is the impeller speed, ρ is the density and μ is the viscosity. We assumed the density was 997 kg/m^3 and the viscosity was 0.001 kg/m-s based on previous work (Burns et al., 2021). We chose an impeller speed of 68 rpm. In mammalian cell cultures, it is very important to maintain a low shear rate, as mammalian cells are shear sensitive and can be easily damaged at high impeller speeds. Most bioreactors for mammalian cell cultures operate at a maximum shear rate of 1.5 m/s as a result (Isailovic et al., 2015). Keeping this in mind, we chose an impeller speed of 68 rpm, which corresponds to a shear rate of 1.5 m/s. This gave us a Reynolds number of 2.00e5.

In Equation 4.1.3.4, N_P is the power number and P is the power requirement. Based on a correlation between the power number and the Reynolds number, we found an N_P of 0.35 for the system, which led to a power requirement of 1.44 kW (Prpich, 2020). In Equation 4.1.3.5, Q_g is the aeration rate and N_a is the aeration number. For this system, we found an aeration rate of 0.042 vvm. This gave the system an aeration number of 0.0125. Using a correlation between the aeration number and P_g, the power input for a gassed system, we determined P_g was 0.51 kW (Prpich, 2020). Using Equation 4.1.3.6, this gave us a predictive k_La of 43.0 h^{-1}.

For a good bioreactor design, the predictive k_La should be within 10% of the theoretical k_La (Prpich, 2020). Our theoretical k_La for the system is 42.4 h^{-1}, so this is well within these requirements. We also checked our bioreactor design against some good rules of thumb given in Table 4.1.3.3. In this table, v_s represents the superficial velocity. Our design satisfies all of these guidelines except for sufficient shear; based on the sensitivity of the CHO cells, we are choosing to prioritize their stability over the rules of thumb.

Table 4.1.3.3. General Rules of Thumb for Bioreactor Design (Bloom et al., 2022).

Rule	Requirement	Design Value	Agreement
Avoid Slugging	$v_s = \frac{Q_g}{A_t} < 125\ m/h$	3.18 m/h	Yes
Avoid Flooding	$Q_g \leq 0.6(\frac{D_i^5 N^2}{D_t^{1.5}})$	0.00105 m^3/s < 0.00743 m^3/s	Yes
Number of Impellers	$\frac{H_L - D_i}{D_i} \geq n_i \geq \frac{H_L - 2D_i}{D_i}$	$2 \geq 1 \geq 1$	Yes
Sufficient Shear	$\pi N D_i > 2.5\ m/s$	1.5 m/s < 2.5 m/s	No
Energy Input	$\frac{P_g}{V} < 15,000\ W/m^3$	337 W/m^3 < 15,000 W/m^3	Yes

4.1.4 Tangential Flow Filtration

Tangential Flow Filtration (TFF) is the method used to separate the desired pembrolizumab and other small components from the larger cells. For modeling purposes, the flow rates calculated in this section are from both of the bioreactors, that is double the flow rate from an individual bioreactor. The TFF and Recycling scheme is illustrated in Figure 4.1.4.

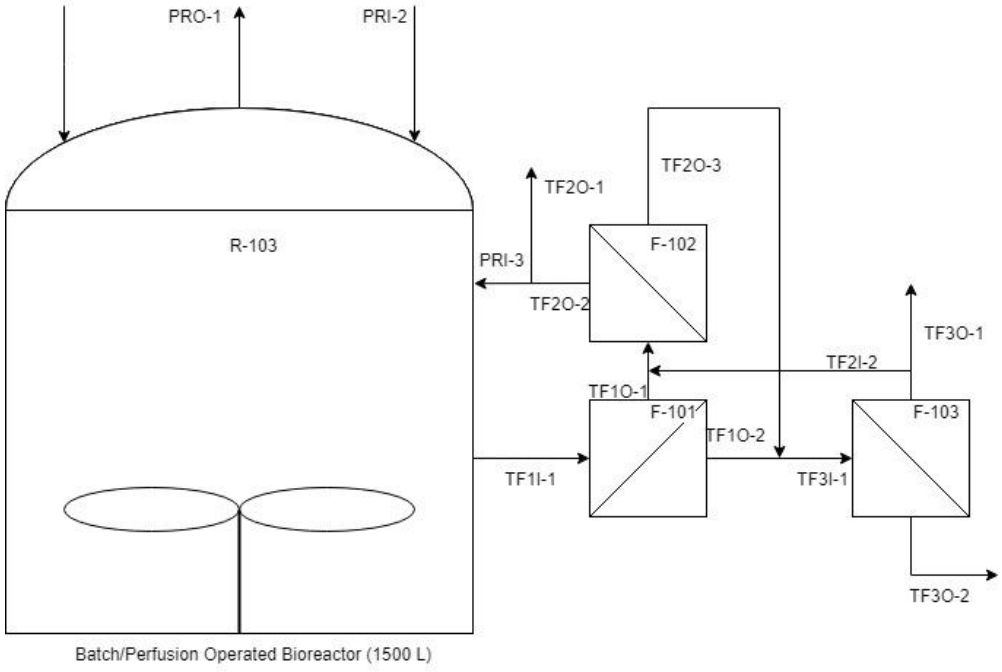


Figure 4.1.4. Tangential Flow Filtration and Recycling during perfusion operations.

When evaluating the kinetics of the bioreactor, it is important to consider the dilution rate D, which has an effect on the consumption of substrate and production of cells. The dilution rate can have an impact on productivity, with too high of a dilution rate preventing cells from consuming substrate since the flow in and out of the bioreactor is at the washout stage, or $D_{washout}$. The optimum dilution rate or D_{opt} can be determined by solving the equations below using the Monod kinetics parameters. While our system has two substrates, both produce the same values since they have different constants.

$$D = \frac{\mu_{max} S}{(K_s + S)\big(1 - a(b-1)\big)}$$

$$S = \frac{D K_s \big(1 - a(b-1)\big)}{\mu_{max} - D\big(1 - a(b-1)\big)}$$

$$b = \frac{X_r}{X_1} \quad a = \frac{F_r}{F_0}$$

Equations 4.1.4.1. Design equations for chemostat bioreactor with recycle stream for dilution rate.

$$D_{washout} = \frac{\mu_{max} S_0}{(K_s + S_0)\big(1 - a(b-1)\big)}$$

Equation 4.1.4.2. Dilution washout rate.

Based on the equations above, washout occurs when $D_{washout}$ = 0.0567 hr^{-1}, and our process is run at D = 0.0475 hr^{-1}.

In our system, three filters are used in the TFF and recycling process in order to capture more product. When the stream leaves the perfusion bioreactor at a flow rate of 4.55 L/min, it is passed through a filter (F-101), where 82% of the liquid and pembrolizumab pass through to stream TF1O-2 towards F-103. Due to the filter size, it is assumed that only 0.1% of cells pass through this filter, while the rest get sent to F-102. F-102 acts as a secondary harvesting filter for the pembrolizumab, collecting 82% of the remaining pembrolizumab for a total harvesting of 96.5% of the pembrolizumab produced. Based on thermodynamics, there is nothing 'pulling' the pembrolizumab across the filters, and therefore the streams entering and exiting both F-101 and F-102 will have identical concentrations of pembrolizumab.

F-102 has two streams feeding into it, one from F-101 and one from F-103. The purpose of stream TF2I-2 is to increase the flow rate across the filter such that the concentration of cells leaving F-102 is feasible. Stream TF2O-2 represents the concentrated cells leaving F-102, which acted as a 'dewatering' step, which was necessary to balance the dilution rate equations. This

stream splits into the recycling stream (PRI-3) and one of the purge streams (TF2O-1) to balance the flow rates around the bioreactor, and to return some of the cells. The cells leaving in TF2O-1 are equivalent to the cells produced in the bioreactor such that the concentration of cells within the bioreactor remains constant during perfusion operations, and is known as the bleed cells.

The third filter, F-103, acts as a dewatering step for the collected pembrolizumab and other smaller proteins and debris for further downstream processing. This filter concentrates the protein from the previous two filters, then uses the resulting water for the dilution around F-102.

Pembrolizumab leaves the bioreactor at an average concentration of 1.08 g/L with a flow rate of 4.55 L/min for a mass flow of 4.90 g/min and is concentrated to 20.6 g/L at a flow rate of 0.229 L/min for a mass flow rate of 4.73 g/min, for a total pembrolizumab recovery of 96.5%. The remaining stream balances can be found in Section 5.6.1.

4.1.5 Media Selection and Campaign Requirements

Throughout a campaign, 24.3 kg of glucose and 4.77 kg of L-glutamine are required for the seed train and perfusion operations. Many commercially available media for CHO cells do not contain L-glutamine by default, and recommend adding it. Since our process is either fed-batch or a continuous perfusion operation, we will use Thermo Scientific's High-Intensity Perfusion CHO Medium to provide the D-glucose and other necessary nutrients for cell growth (Thermo Scientific, 2023). L-glutamine is not stable in solution, but will be added shortly before the feed is added to the fermentation broth. The supplement will also be provided by Thermo Fisher.

4.2 Downstream Process

Figure 4.2.1 summarizes the downstream processing of pembrolizumab. Our proposed downstream processing utilizes precipitation chromatography as the primary capture step, anion and cation exchange chromatography as the polishing steps, and the necessary viral inactivation stages, all of which will be conducted at room temperature.

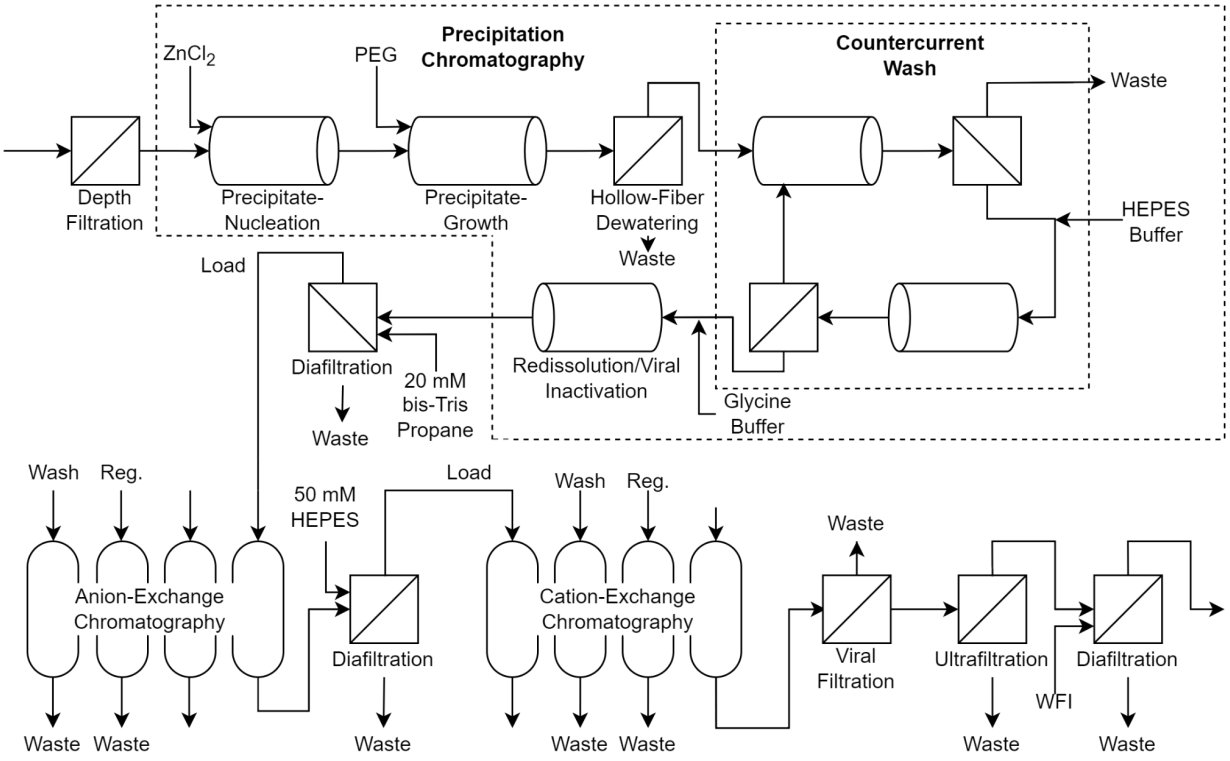


Figure 4.2.1. Overall Downstream Process Flow Diagram.

4.2.1 Depth Filtration

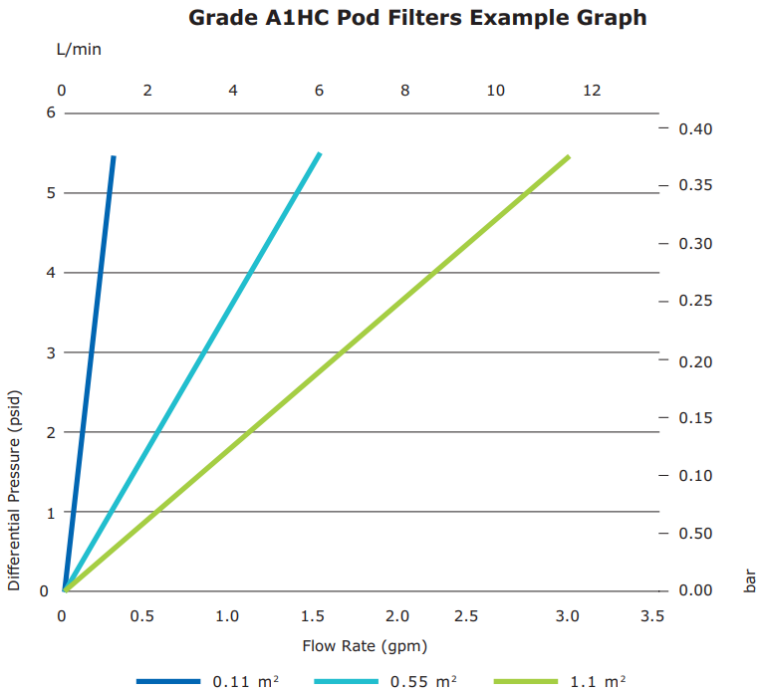


Figure 4.2.2. Differential Pressure in comparison to flow rate of the depth filter system based on experimental data (Merck KGaA, 2022).

Following TFF, depth filtration will be used to remove remaining cells and cell debris. Depth filtration utilizes a porous medium to capture large impurities based on particle size (Yigzaw et al., 2006). A 0.11 m^2 Millistak+ A1HC Pod Depth Filter with a length of 62 cm will be utilized in this process (Merck KGaA, 2022). This particular filter was chosen due to its greater surface area, multiple graded-density layers, and low hold-up volume resulting in greater product yield. The adsorptive, positively charged filter media aids in the reduction of smaller impurities such as DNA, endotoxins, host cell proteins (HCPs), and lipids; however, these impurities are neglected in the material balance due to their relatively small presence in comparison to the desired protein product. The mAb protein, which is much smaller than the pores of the depth filter, will not be collected through the filter and a 99% recovery will be assumed. The flow rate entering and exiting the depth filter will remain constant at 0.229 L/min. Based on Figure 4.2.2, this flow rate would result in a differential pressure of approximately 1.5

psi (10 kPa), well below the maximum operating pressure of the system: 50 psi (345 kPa) at 25℃ (Merck KGaA, 2022). These filters are 100% disposable and will be replaced during the shutdown period after each 25 day campaign.

4.2.2 Precipitation Chromatography

Following depth filtration, precipitation chromatography will be used as the primary capture method of pembrolizumab. Traditionally, protein A chromatography has been used in industrial manufacturing of monoclonal antibodies, including Merck's production of pembrolizumab. Protein A affinity chromatography uses a proteic ligand from *Staphyloccocus aureus* which binds to the Fc region of immunoglobulin (Ramos-de-la-Peña et al.). It is a commonly used technique due to its high binding affinity and therefore high purity levels with reduction of host cell proteins (HCP) to below 100 ppm (Bracewell et al.). However, the process, particularly the resins used, is economically costly at $8,000 - $ 15,000 per L with resin costs for a single column exceeding $1 million (Ramos-de-la-Peña et al.; Bracewell et al.). Furthermore, there is often resin fouling, and the aging of the resin can result in variations in purity or elution rate of the product, and cleaning and reuse of the resin is often a large problem, despite the resins being recycled up to 100 times (Wang et al.).

Upstream processes such as perfusion reactors have increased cell titers over a continuous yield to greater than 5 g/L therefore continuous purification is required to keep up with the yield. Precipitation chromatography relies on a series of selective precipitation steps which results in an innately continuous process allowing for cost reduction at the pilot and industrial scale, with multiple studies reporting an overall cost reduction of greater than 40% for monoclonal antibodies, incorporating equipment and carbon footprint costs (Hammerschmidt et al.; Pons Royo et al.). Precipitation chromatography can be broken down into two steps–the precipitation stage and the washing stage as shown in Figure 4.2.3.

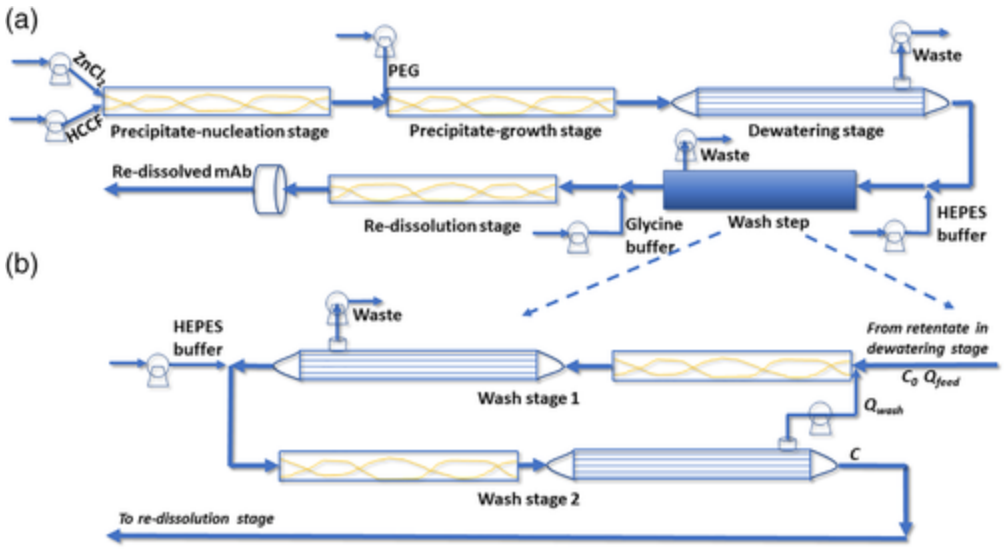


Figure 4.2.3. a) Continuous precipitation chromatography scheme, b) Detailed imagery of counter current washing scheme (Li et al.).

4.2.2.1 Precipitation Stage

During the precipitation stage, zinc chloride ($ZnCl_2$) and polyethylene glycol (PEG) are added sequentially to the cell culture feed from the depth filtration. $ZnCl_2$ acts as a reversible cross-linking agent for proteins since the metal ion binds to histidine and cysteine residues, forming a metal-protein complex (Dutra et al.). Immunoglobulins have a higher precipitation rate compared to other proteins due exposed histidines favoring the cross-linking, and the success of $ZnCl_2$ as a cross-linking agent is independent of a monoclonal antibody's pI since it is a highly favorable process (Dutra et al.). PEG is added after the metal-protein complexes have had time to form and acts as a volume-exclusion agent. The volume exclusion, or hard interactions, is the PEG removing space for the protein to remain dissolved, forcing the large metal-protein complexes to fall out of solution, or incur an entropic penalty (Miklos et al.). In the continuous precipitation chromatography of pembrolizumab, the addition of a large quantity of PEG will increase the viscosity of the solution resulting in lower flux through filtering membranes and increased system pressure (Dutra et al.). Additionally, in continuous processes, there is significantly lower yield with $ZnCl_2$ as the sole agent, therefore our process will include both to

minimize the risk of PEG increasing the viscosity and maximizing the yield in the precipitation stage (Dutra et al.).

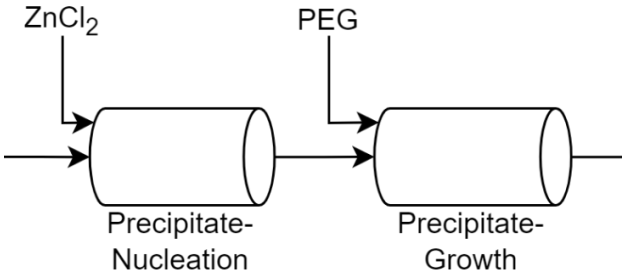


Figure 4.2.4. The precipitation chromatography stage steps showing sequential addition of reagents.

The precipitation nucleation step is the mixing of $ZnCl_2$ with the harvested cell culture fluid (HCCF). Since the HCCF leaves depth filtration at a volumetric flow rate of 0.229 L/min, 0.1 M $ZnCl_2$ will be pumped in at a flow rate of 0.0458 L/min, based on the work of Li et al., resulting in a flow rate of 0.275 L/min. The concentration of pembrolizumab is 17.2 g/L and $ZnCl_2$ is 0.0167 M. A residence time of 30 seconds is required to create the metal-protein complexes and the solution also needs to be well mixed. Static mixers will be used during precipitation steps in order to not break the coordinate covalent bonds of the metal-protein complexes and to ensure sufficient mixing. For the addition of $ZnCl_2$, this will be accomplished with the StaMixCo static mixer model HT-50-1.50-6-C, which has an inner diameter of 3.48 cm and a length of 35.6 cm. Assuming a working inner area of 7.13 cm^2, this mixer allows for a residence time of approximately 55 seconds.

The PEG is added at a concentration of 17.5 wt % through a pump at a flow rate of 0.183 L/min, or 80% of the initial HCCF flow rate, based on the work of (Li et al.). The resulting flow rate is 0.458 L/min with pembrolizumab concentration of 10.2 g/L, $ZnCl_2$ concentration of 0.01 M, and a PEG concentration of 7 wt %. Based on previous work with Gu et al., the expected

solubility of pembrolizumab under these conditions is 0.08 g/L (Figure 4.2.5), allowing for a precipitation recovery rate of 99.2% from the concentration of 10.2 g/L. The concentration of 7 wt% PEG is ideal for precipitation of monoclonal antibodies while limiting the precipitation of HCP (Großhans et al.). The solution is also mixed with a static mixer to ensure the lowest solubility rate, giving a residence time of approximately 45 seconds.

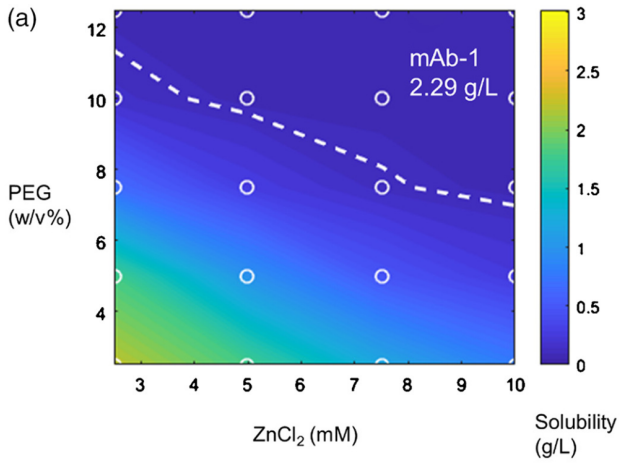


Figure 4.2.5. Solubility of the monoclonal antibody as a function of $ZnCl_2$ and PEG concentration.

The last step of the precipitation stage before the washing stage is the dewatering step in order to concentrate the precipitates to approximately 40% of the current volume. The dewatering stage will use a MiniKros hollow fiber membrane module with 0.2 µm hydrophilized PES hollow fiber membranes with a surface area of 5,100 cm^2 which was chosen due to the manufacturers recommendation of operation between 5 and 50 L/hr, matching the current flow rate of 27.5 L/hr. Based on the work of Li et al., this chosen filter will correspond with a permeate flow rate of 0.275 L/min, and a 2.5 fold increase in pembrolizumab concentration in the retenate with a flow rate of 0.183 L/min, as well as the removal of 60% of HCPs and DNA. A single waste stream is present in the precipitation stage, which is located as the permeate flow in the dewatering stage.

4.2.2.2 Washing stage

Following the precipitation stage, soluble impurities such as HCP, DNA, excess $ZnCl_2$ and PEG, are removed by washing the precipitates using 50 mM (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer at a pH of 7.0 to ensure the precipitates remain undissolved. The HEPES buffer has minimal interactions with the zinc cations and is used without the addition of any PEG or $ZnCl_2$ in order to limit the attraction of the precipitates to the buffer (Ferreira et al.).

The washing stage of the precipitation chromatography is a two stage countercurrent washing step, as based on the work of Li et al., with fresh HEPES buffer being added to the second stage of washing, the precipitated pembrolizumab being diluted with the buffer, then reconcentrated through filters as shown in Figure 4.2.6. The countercurrent system is used to reduce the quantity of buffer added, thus reducing the amount of pembrolizumab that can re-solubilize, while also allowing for sufficient HCP removal. Furthermore, the countercurrent washing scheme has a low pressure drop further reducing the operating cost of this stage (Dutta et al.; Nambiar et al.).

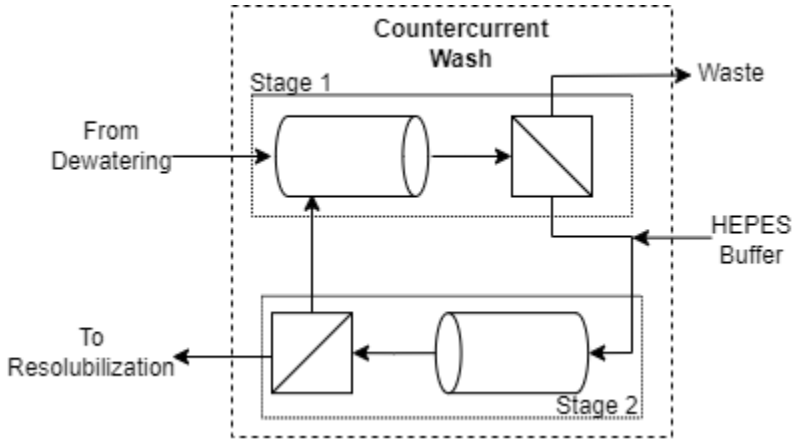


Figure 4.2.6. Countercurrent washing scheme demonstrating locations of the addition of HEPES buffer and output of waste stream.

The dewatered concentrated precipitated protein is fed to the first washing stage, where it is mixed through a short static mixer with the permeate from the second stage. The permeate is assumed to have a relatively low concentration of HCP and DNA. This mixture is then concentrated through tangential flow filtration back to a flow rate of 0.275 L/min, resulting in an approximately 60% reduction of HCP and DNA. Fresh HEPES buffer is then added to the retenate containing the precipitated protein, mixed with a static mixer and filtered again, with the retenate moving on to the re-dissolution stage and the permeate acting as buffer to the first washing stage.

While the inlet and outlet flow rate of the precipitated pembrolizumab for the washing stage is 0.183 L/min, the flow rates between the washing stages and the flow rate of fresh HEPES buffer depend upon the recycling rate within the countercurrent scheme. After a comparable Protein A chromatography stage, the expected contamination of HCP in solution is approximately 5,000 ppm, from a starting point of 120,000 ppm from the HCCF (Zhang et al.). Given a 60% reduction during the dewatering stage, it is assumed the HCP contamination is 48,000 ppm, requiring a 9.6-fold reduction. As demonstrated by Nambiar et al., the degree of impurity removal can be solved by mass balances resulting in Equation 4.2.1 and Equation 4.2.2.

$$R = \frac{\alpha^{N+1}-1}{\alpha-1}$$

Equation 4.2.1. Degree of reduction of HCP and DNA contamination, where R is reduction, N is the number of stages, and α is the flow removal factor.

$$\alpha = \frac{q_{DF}S}{q_F}$$

Equation 4.2.2. Flow removal factor α, where q_{DF} is the flow of the diafiltration buffer, q_F is the feed flow rate, and S is the solute sieving coefficient.

A solute that is not retained, such as the precipitated monoclonal antibody, will have a value of S = 1. While these equations neglect impurity binding to the membrane and assume

perfect mixing, the work of Nambiar et al. and Shao and Zydney suggest this is negligible. For a 9.6-fold reduction, the flow removal factor, α, is required to be 2.47, for a buffer flow rate of 0.452 L/min.

Given the total flow rate within the washing stage is 0.635 L/min, two static mixers from StaMixCo with a model number of HT-50-1.50-6-C will be used. These mixers have a working area of approximately 7.13 cm^2 and a length of 35.6 cm, allowing for a residence time of approximately 24 seconds, consistent with the suggested 20 seconds as used by Li et al. Additionally, the MiniKros hollow fiber membrane module with 0.2 μm hydrophilized PES hollow fiber membrane will be used to concentrate the precipitated protein between wash stages, resulting in a permeate waste flow rate equivalent of the HEPES buffer inlet at 0.452 L/min. Assuming minimal protein lost to binding with the filters, the concentration of pembrolizumab leaving the countercurrent washing stage is 22.6 g/L, for 88.3% yield during the washing stage. The loss of protein is expected to occur upon the removal of the cross-linking $ZnCl_2$ with the addition of the HEPES buffer–Li et al. found the concentration of $ZnCl_2$ to be below 0.0009 M and the PEG concentration to be below 0.6 wt%. Based on their previous studies, these concentrations would correspond to a monoclonal antibody solubility of greater than 3.0 g/L. The concentration of HCP and DNA contamination is expected to decrease from 48,000 ppm from the dewatering stage to approximately 5,000 ppm.

The washing stage has one waste stream flowing from the permeate of the filter in the first stage of the countercurrent washing stage. There are two pumps required, one to add the HEPES buffer, and one to pump the permeate from the second stage back to mix with the retentate from the dewatering stage in the first stage.

The addition of $ZnCl_2$ as a cross-linking agent requires a physiological pH for the protein to interact with the Zn^{2+} cation in place of other hydrogens in solution. However, with the

decrease in pH through addition of a buffer, the cross-linking will be disrupted, and the pembrolizumab will be able to redissolve. This process will use a 2M glycine buffer at pH 3.2. When the pH of the solution is below 5, the precipitation yield is greater than 90%. At a volumetric ratio of 0.5 glycine to 1 precipitates, a final pH of 3.5 is achieved with a 93% recovery. Thus, the inlet flow rate of glycine buffer will be 0.0917 L/min at a concentration of 2M, and the final concentration of pembrolizumab for the precipitation chromatography stage will be 14.0 g/L at a flow rate of 0.275 g/L. The mixing will be done with a static mixer provided by StaMixCo, model HT-50-1.00-6-C, which has an internal diameter of 2.21 cm and a length of 27.9 cm, allowing for a residence time of 20 seconds. The solution will then pass through a double-layer Sartoclear depth filter with filtration size of 8um and 0.8um provided by Sartorius Corporation in order to remove any insoluble material.

4.2.3 Viral Inactivation

In standard monoclonal antibody manufacturing process, viral inactivation occurs during a separate process unit in which the product stream is subjected to low pH conditions to inactivate enveloped viruses (David et al., 2020). However, in this process, a separate unit will not be needed to lower the pH of the solution following precipitation chromatography. Continuous viral inactivation will take place in-line directly after the capture step in a low pH (~3.5) glycine solution. As the solution exiting the precipitation chromatography stage will already be at a pH of 3.5, additional acid will not be needed to lower the pH. In traditional processes, viral inactivation occurs in a holding tank with a minimum hold time of 30 minutes; however, studies have shown that complete viral inactivation can occur with even shorter hold times (Gillespie et al., 2018). A continuous stainless steel plug flow reactor with a coiled flow inverter will be used to hold the solution at the low pH for one hour.

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

Equation 4.2.3. RTD function for a laminar flow reactor (Fogler, 2008).

Equation 4.2.3, shows that the residence-time distribution (RTD) is equal to 0 at any time less than half of the average residence time. Therefore, a reactor design with a residence time of 60 minutes ensures that the solution remains in the PFR for a minimum of 30 minutes, enough time to safely confirm complete viral inactivation. However, damage to the solution can occur if held at a low pH for too long; a coiled flow inverter can be used to produce a tight residence time distribution by decreasing axial dispersion and increasing radial mixing (David et al., 2020).

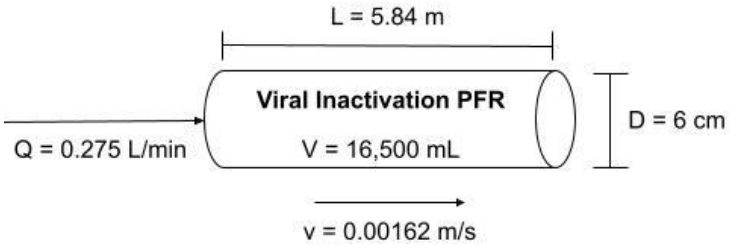


Figure 4.2.7. Viral Inactivation Plug Flow Reactor design.

As shown in Figure 4.2.7, the stainless steel plug flow reactor will have an inner diameter of 6 cm and a length of 5.84 m. The flow rate entering the PFR will remain constant at 0.275 L/min. Based on the calculated Reynolds number of 97, the flow will be laminar. Equation 4.2.4 displays the equation used to calculate the pressure drop which will be needed for pump design; this was found to be 1.2E-5 psi (8.4E-5 kPa).

$$\Delta p = \frac{64}{Re} * \frac{L}{D} * \frac{\rho}{2} * v^2$$

Equation 4.2.4. Pressure drop across a circular pipe in laminar flow.

4.2.4 Diafiltration for Anion Exchange Chromatography

For buffer exchanges throughout our process, we will utilize continuous constant volume diafiltration. Diafiltration is a membrane-based separation technique in which the feed buffer is passed through a porous membrane, while the pembrolizumab is retained. Concurrently, a fresh buffer of the appropriate pH and composition is fed through the filter to gradually replace the old buffer. Constant volume diafiltration specifically entails an equal feed flow rate and retentate flow rate - this is the current standard for continuous downstream processing (Millipore Sigma, 2023).

As seen in Figure 4.2.8, continuous volume diafiltration requires fewer diavolumes of fresh buffer than batch DF to achieve the same percentage of contaminant removal. Our goal is to achieve over 99.9% buffer replacement within each diafiltration step. According to the data provided by Millipore, we must run our diafilters with seven diavolumes of fresh buffer to do so, where a diavolume is defined as the volume of product feed entering the filter per unit time (Millipore, 2003).

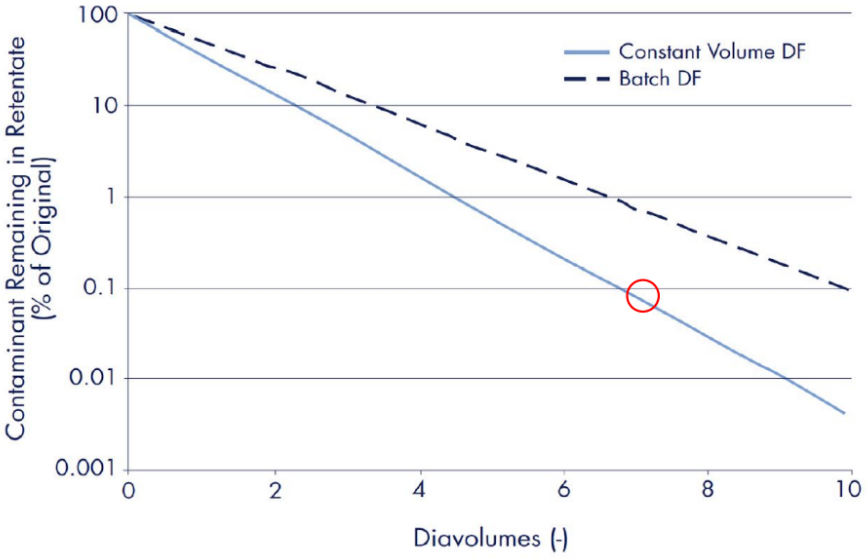


Figure 4.2.8. Comparison of contaminant removal per diavolume of fresh buffer in constant volume versus batch diafiltration (Millipore, 2003).

Our diafiltration steps will operate with an inline Pall Cadence module and 0.11m^2 cellulose membrane with a cutoff size of 30 kDa. We calculated this filter area in the ultrafiltration section below (section 4.2.9). The 30 kDa cutoff size is standard for mAb purification (Pall, 2023). Pembrolizumab has a molecular weight of 149 kDa, so we assumed 0.995 rejection coefficient of the protein to account for concentration polarization, caking, and flux decay within the filter in each diafiltration and ultrafiltration step throughout the downstream process (Food and Drug Administration, 2016; Pall, 2023). For our assumptions to remain valid, we will clean diafiltration membranes each campaign and replace them each year. According to Pall (2023), the filters foster minimal fouling and can fully regain permeability from cleaning with 0.1M NaOH.

Each of our downstream ultrafiltration and diafiltration modules will operate as single-pass tangential flow filtration (SPTFF) systems. SPTFF systems are designed to accommodate continuous operation and only require a single pump by avoiding any form of recirculation (Figure 4.2.9). Additionally, SPTFF is advantageous compared to recirculation for the following reasons: its smaller required hold-up volume allows for a higher product yield, it imposes less shear on the proteins, and it is capable of attaining a larger concentration factor (Pall, 2023). Because protein interacts less with any one membrane, however, SPTFF requires multiple sections of cassettes to attain more efficient buffer exchange for diafiltration or higher concentration factors for ultrafiltration. Our process will utilize 3-section cassettes for each UF and DF unit due to the large quantity of commercial data available for this configuration (MilliporeSigma, 2020).

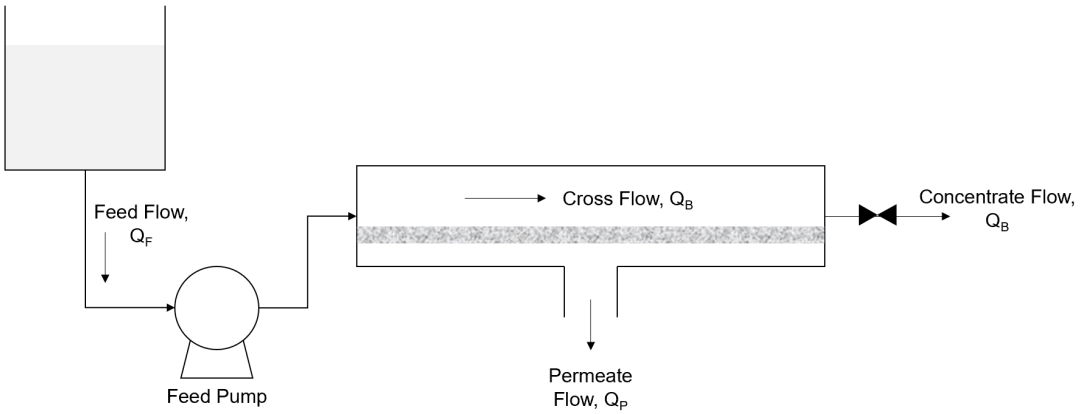
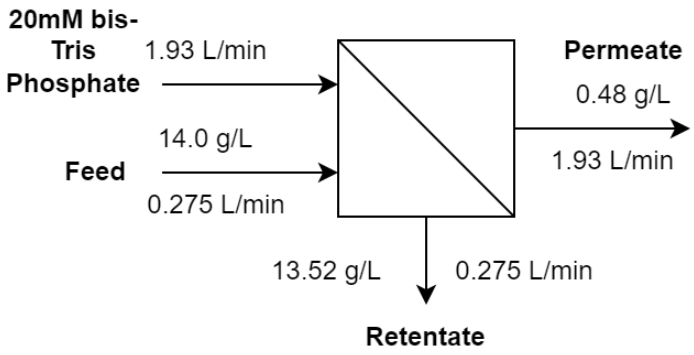


Figure 4.2.9. Simplified single-pass tangential flow filtration schematic.

Our downstream process will operate with a total of three diafiltration modules: The first two will serve to raise the pH of the product buffer to the appropriate values for anion-exchange chromatography and cation-exchange chromatography. We will exchange the product buffer with WFI with the final DF unit as preparation for fill and formulation.

In preparation for anion-exchange chromatography, we will use diafiltration to suspend the pembrolizumab in a buffer of 6.6 pH. To do so, we will replace the glycine with 20mM bis-Tris propane fed at 1.93 L/min (Lebendiker, 2002). The pembrolizumab solution will be fed into this step at a flow rate of 0.275 L/min and a concentration of 14.0 g/L. The majority of it will exit in the retentate, which has the same flow rate as the feed and a pembrolizumab concentration of 13.52 g/L. The waste permeate will have a flow rate of 1.93 L/min and a pembrolizumab concentration of 0.48 g/L. These values were calculated using the material balances in Figure 4.2.10, which apply to each of the following diafiltration units.



$$Q_R + Q_P = Q_F + Q_W$$

$$7Q_F = Q_W$$

$$\%Product\ Loss\ =\ 100\ *\ (1\ -\ exp[(\sigma\ -\ 1)\ *\ N])$$

$$\sigma\ =\ 0.995$$

Figure 4.2.10. Flows, material balances, and concentration calculation for diafiltration prior to anion-exchange chromatography, where N is the number of diavolumes and σ is the rejection coefficient.

4.2.5 Anion Exchange Chromatography

Anion Exchange Chromatography (AEX), a form of ion exchange chromatography, takes advantage of charge differences between a protein and impurities using a positively charged resin in flow-through mode to provide viral, host-cell protein (HCP), and DNA clearance while the mAb product flows through (Ichihara, 2018). The AEX columns used in this design will be used for polishing of the mAb product. The ability to separate the mAb from these acidic impurities depends on the isoelectric point (pI) of the mAb and the pH of the buffer solution in the column. If the pH of the column is above the pI of the mAb it binds in an AEX, therefore we will operate the column at 6.6 pH, in this condition the mAb will be positively charged as Pembrolizumab has a pI of 7.6 (Jungbauer, 2009). The pH of 6.6 was chosen to operate at 1 pH unit away from the protein of interest, while the impurities attracted to the resin have pIs in the range of 2-5. The buffers used for this step are listed in section 4.2.4.

This AEX step will operate using Cytiva Capto Q Impact resin as it is suitable for large scale protein processing, has a high dynamic binding capacity (DBC), and recovers protein at a

desired rate with the chosen operating conditions. Using Cytiva data from Figure 4.2.11, a DBC value of 130 mg/mL at a residence time of 2.1 minutes was determined (Cytiva, 2020c). The Capto Q Impact Resin has a particle size of 90 micrometers, max linear flow of 300 cm/hr, and functions at pH ranges from 2-12 (Cytiva, 2020c).

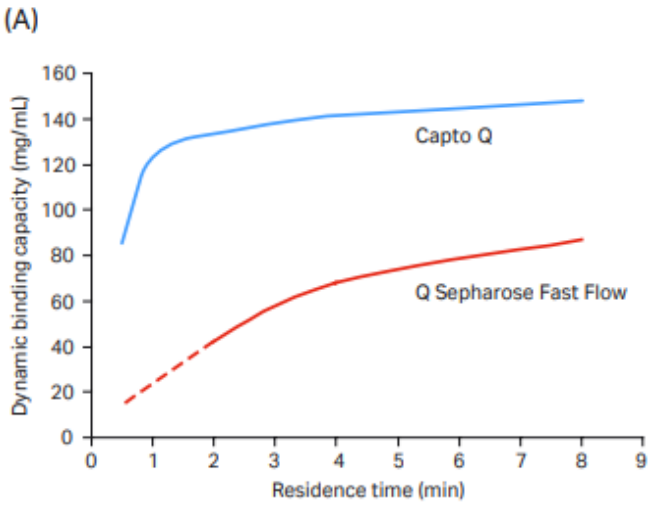


Figure 4.2.11. DBC vs residence time for Capto Q Impact Resin.

We chose the ReadyToProcess Capto Q 1 L column by Cytiva with an inner diameter of 8 centimeters for consistency in resin and column brand (Cytiva, 2023). The column diameter was chosen due to the incoming flow of 0.275 L/min which will split into two columns for flows of 0.1375 L/min due to the operating requirements of the resin. This flow with the residence time and cross sectional area specified was used to calculate a linear flow velocity of 164.2 cm/hr which is far below the operating limits of the resin. A bed height of 5.74 cm was then calculated giving a total column volume of 288.8 cm^3. The pressure drop of the column was calculated using the Carman-Kozeny equation (Equation 4.2.5) to be 0.766 kPa.

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

Equation 4.2.5. Carman-Kozeny calculation of IEX chromatography column pressure drop, where ε is the porosity of the bed, η is the liquid viscosity, L is the column length, u is the superficial velocity, and d_p is the particle diameter.

5 total AEX columns will be in use with 2 being actively used for purification, 2 being recharged, and 1 as backup. The schedule can be seen below in Figure 4.2.12.

Column 1	10CV					2CV	2CV	2CV	2CV	2CV	10CV					2CV	2CV	2CV	2CV	2CV
	0.1375 L/min					0.1375 L/min					0.1375 L/min					0.1375 L/min				
	Load/Collect					Wash	Strip	CIP	Regen.	Re-eq.	Load/Collect					Wash	Strip	CIP	Regen.	Re-eq.
Column 2	2CV	2CV	2CV	2CV	2CV	10CV					2CV	2CV	2CV	2CV	2CV	10CV				
	0.1375 L/min					0.1375 L/min					0.1375 L/min					0.1375 L/min				
	Wash	Strip	CIP	Regen.	Re-eq.	Load/Collect					Wash	Strip	CIP	Regen.	Re-eq.	Load/Collect				
Column 3	10CV					2CV	2CV	2CV	2CV	2CV	10CV					2CV	2CV	2CV	2CV	2CV
	0.1375 L/min					0.1375 L/min					0.1375 L/min					0.1375 L/min				
	Load/Collect					Wash	Strip	CIP	Regen.	Re-eq.	Load/Collect					Wash	Strip	CIP	Regen.	Re-eq.
Column 4	2CV	2CV	2CV	2CV	2CV	10CV					2CV	2CV	2CV	2CV	2CV	10CV				
	0.1375 L/min					0.1375 L/min					0.1375 L/min					0.1375 L/min				
	Wash	Strip	CIP	Regen.	Re-eq.	Load/Collect					Wash	Strip	CIP	Regen.	Re-eq.	Load/Collect				

Figure 4.2.12. Proposed anion-exchange chromatography run schedule.

The time breakdown of our proposed AEX run schedule for a given column is 21.02 minutes of loading time followed by 4.20 minutes of washing. Then, the column will be stripped, cleaned in place (CIP), regenerated, and re-equilibrated for 4.20 minutes. Each column cycle will total to 42.04 minutes.

The number of column volumes to be input during loading was calculated using Equation 4.2.6 from our DBC at 10% breakthrough and our column volume and feed concentration (C_F) of pembrolizumab. For both anion-exchange chromatography and cation-exchange chromatography, the load volume (V_{load}) was ten times the column volume (V_{col}) when rounded up to an integer value.

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

Equation 4.2.6. Calculation of the sample loading volume into the IEX columns from the DBC at 10% breakthrough, the column volume, and the feed concentration.

We then found the approximate number of column volumes for washing, stripping, cleaning in place (CIP), regeneration, and re-equilibration from experimental data provided by Cytiva. This study used the same resins and chromatography type (flow-through) as our project (Cytiva, 2020).

4.2.6 Diafiltration for Cation Exchange Chromatography

In preparation for cation-exchange chromatography, we will use diafiltration to suspend the pembrolizumab in a buffer of 8.1 pH. To do so, we will replace the 20mM bis-Tris propane buffer with 50mM HEPES buffer fed at 1.93 L/min (Lebendiker, 2002). The anion-exchange chromatography outlet will be fed into the diafilter at 0.275 L/min with a pembrolizumab concentration of 12.90 g/L, the DF product outlet will run at 0.275 L/min and a concentration of 12.46 g/L of pembrolizumab, and the waste permeate will have a flow rate of 1.93 L/min and a pembrolizumab concentration of 0.44 g/L.

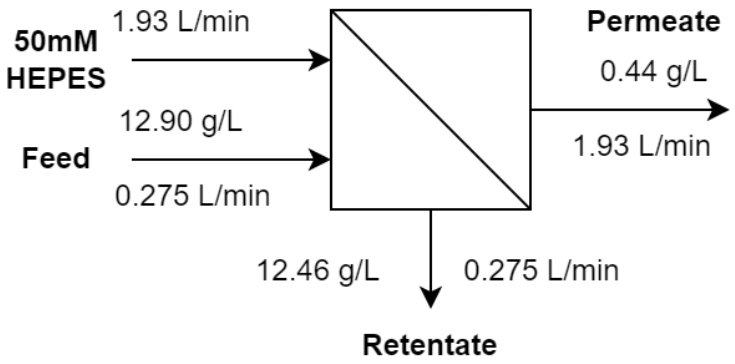


Figure 4.2.13. Pembrolizumab stream flow rates and concentrations around diafiltration prior to cation-exchange chromatography.

4.2.7 Cation Exchange Chromatography

Cation Exchange Chromatography (CEX), another form of ion exchange chromatography, takes advantage of charge differences between a protein and impurities using a negatively charged resin to provide further polishing of charge variants and aggregates by binding positively charged impurities and allowing the mAb to flow through (Ichihara, 2018). The ability to separate the mAb from these basic impurities relies on the same principle as AEX but in opposite charges, the isoelectric point (pI) of the mAb is the same but a different buffer solution in the column is used as described in the previous diafiltration step. If the pH of the solution in the column is below the pI of the mAb it binds in an CEX, therefore we will operate the column at 8.1 pH, in this condition the mAb will be negatively charged due to the Pembrolizumab pI of 7.6 (Jungbauer, 2009). The pH of 8.1 was chosen to operate at 0.5 pH units away from the protein of interest. The buffers used for this step are listed in section 4.2.6.

This CEX step will operate using Cytiva Capto S Impact resin as it is suitable for large scale protein processing, has a high dynamic binding capacity (DBC), and recovers protein at a desired rate with the chosen operating conditions. Using Cytiva data from Figure 4.2.14, a DBC value of 130 mg/mL at a residence time of 5.4 minutes was determined (Cytiva, 2020c). The Capto S Impact Resin has a particle size of 50 micrometers, max linear flow of 220 cm/hr, and functions at pH ranges from 4-12 (Cytiva, 2020d).

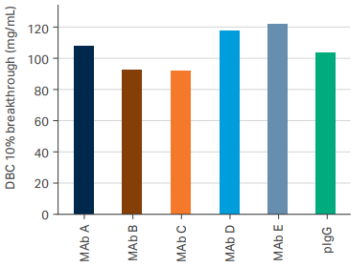


Fig 4. DBC of Capto S ImpAct for five MAbs and one pIgG. The samples, approximately 10 mg/mL medium of each five MAbs and pIgG were run in a Tricorn 5/50 column at a flow corresponding to a residence time of 5.4 min. The pH values of the start buffers were 5.5 for MAbs A, B, D, and pIgG, and 5.25 for MAbs C and E. The start buffer for MAb A also contained 50 mM NaCl.

Figure 4.2.14. DBC of Capto S ImpAct for five MAbs and one pigG.

We chose the ReadyToProcess Capto S 1 L column by Cytiva with an inner diameter of 8 centimeters for consistency in resin and column brand with CEX, but the specified residence time led to the difference in diameter specification (Cytiva, 2023). The incoming flow of 0.275 L/min will again split into two columns for flows of 0.1375 L/min due to the operating requirements of the resin. This flow with the residence time and cross sectional area specified was used to calculate a linear flow velocity of 164.2 cm/hr which is below the operating limits of the resin. A bed height of 14.8 cm was then calculated giving a total column volume of 742.5 cm^3. The pressure drop of the column was calculated using the Carman-Kozeny equation (Equation 4.2.5) to be 1.72 kPa. 5 total CEX columns will be in use with 2 being actively used for purification, 2 being recharged, and 1 as backup, the same configuration as AEX. The schedule for CEX can be seen below in Figure 4.2.15.

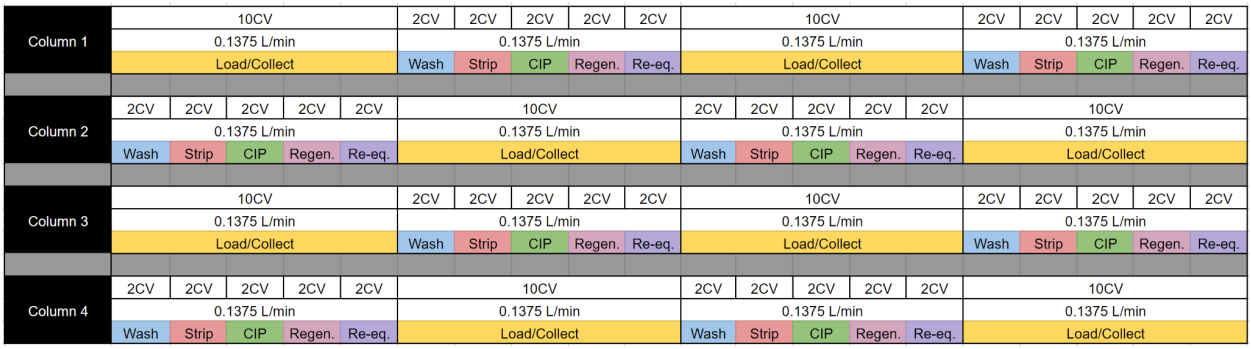


Figure 4.2.15. Proposed cation-exchange chromatography run schedule.

The time breakdown of our proposed CEX run schedule for a given column is 54.0 minutes of loading time followed by 10.8 minutes of washing. Then, the column will be stripped, cleaned in place (CIP), regenerated, and re-equilibrated for 10.8 minutes each. Each column cycle will total to 108.0 minutes.

4.2.8 Viral Filtration

Viral filtration is the last viral clearance step required by the FDA to ensure patient safety. It is designed to clear any viruses that may have been introduced to the product in the downstream purification process by using a size exclusion filter (Liu et al., 2010). Due to the small size of the viruses in the solution, we can assume that the flux of the solution through the viral filtration membrane will be approximately constant. We can also assume the concentration of pembrolizumab through the filter will be unchanged, which is true as long as there is no fouling on the filter. Due to the high purity of the stream entering the viral filter, this assumption holds as long as the filter is changed frequently (Burns et al., 2021). We will use a Virosart HF Mid-Scale Module sterile filter with an area of 200 cm^2. The inlet flow rate will be 0.275 L/min, and the concentration of pembrolizumab will be constant at 16.136 g/L (Figure 4.2.16).

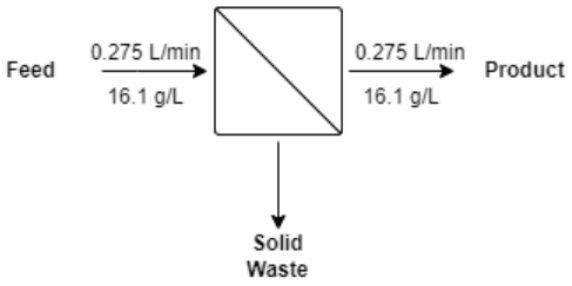


Figure 4.2.16. Mass flow rates around viral filter.

Based on the inlet flow rate and product specifications provided by the manufacturer in Figure 4.2.17, the filter will operate at a pressure of 65 psi (448 kPa) under these conditions.

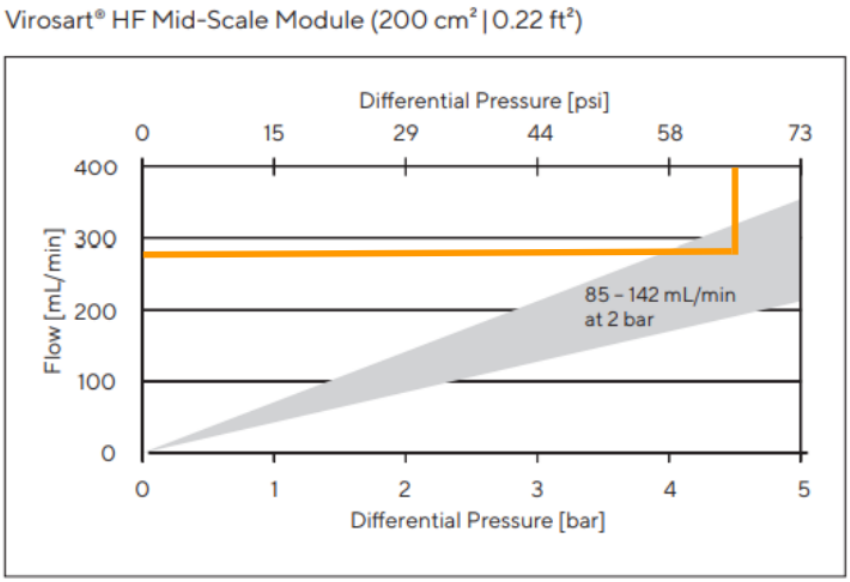


Figure 4.2.17. Characteristic flow rates for the Virosart HF Mid-Scale Module filter (Sartorius, 2020).

4.2.9 Final Ultrafiltration and Diafiltration

We will use ultrafiltration (UF) to concentrate the pembrolizumab in its buffer prior to the final buffer exchange. Similarly to our diafiltration modules, the buffer solution will pass through the membrane while the pembrolizumab is retained; however, no fresh buffer will be added simultaneously. Our ultrafiltration unit will operate as a 3-section SPTFF system illustrated in Figure 4.2.18. SPTFF UF enables continuous concentration of the pembrolizumab with a series of easily removable and cleanable cassettes (MilliporeSigma, 2018).

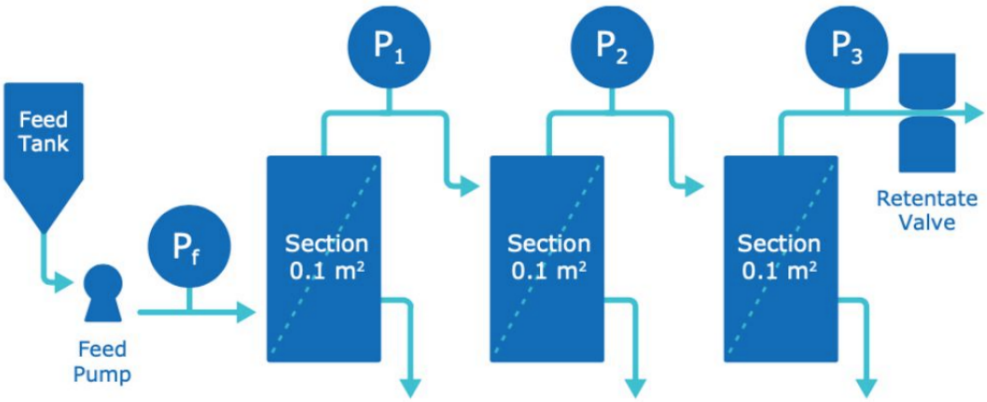


Figure 4.2.18. Schematic representation of SPTFF ultrafiltration sections (MilliporeSigma, 2020).

Using the following purified mAb data from Arunkamar et al. (2017), we found that the optimal transmembrane pressure and permeate flux at our desired feed flux was 8 psi (55.16 kPa) and 63 LMH (Figure 4.2.19). This applies to each ultrafiltration and diafiltration unit in the downstream process except for the last diafiltration unit. The final diafilter will maintain the $0.11 m^2$ membrane area; however its feed flow flux will inherently be lower as a result of the ultrafiltration step prior to it. To accommodate this difference, we will run the final diafiltration step at a lower operating pressure of 4 psi (27.58 kPa).

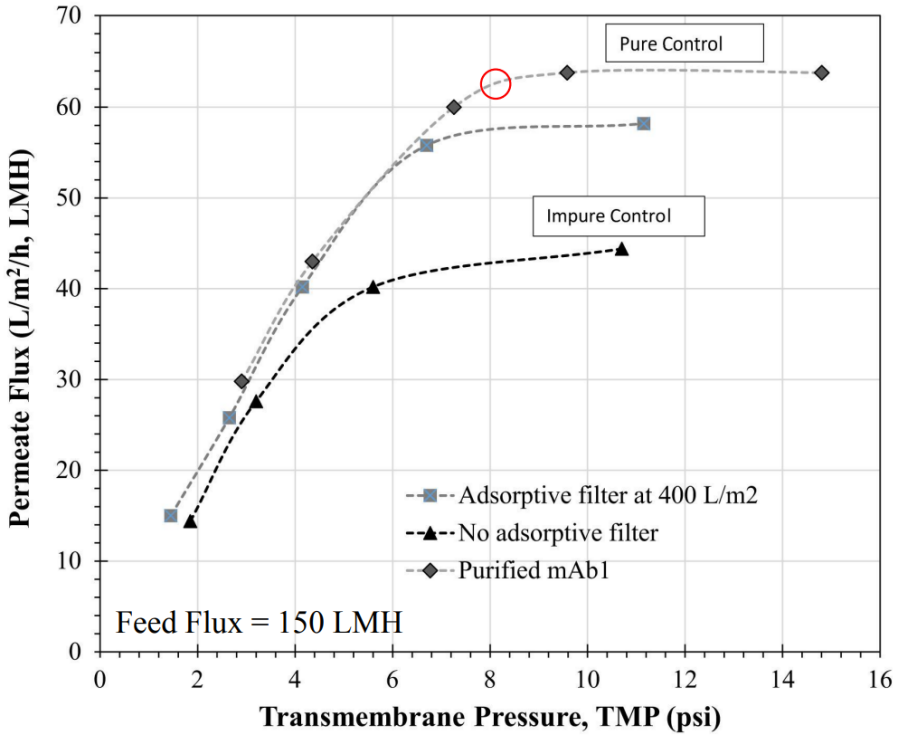


Figure 4.2.19. Permeate flux and transmembrane pressure optimization data at a feed flux of 150 LMH (Arunkumar et al., 2017).

These optimal values reside at the "elbow" of the flux versus TMP plot, and insomuch, the flux is not too large as to induce significant concentration polarization or too small as to require a large membrane area (MilliporeSigma, 2023). In dividing the selected permeate flux by

the permeate flow rate calculated in the final diafiltration section, we deduced a membrane area of 0.11 m^2, which is a standard commercial size of the Pall cellulose membranes selected for diafiltration.

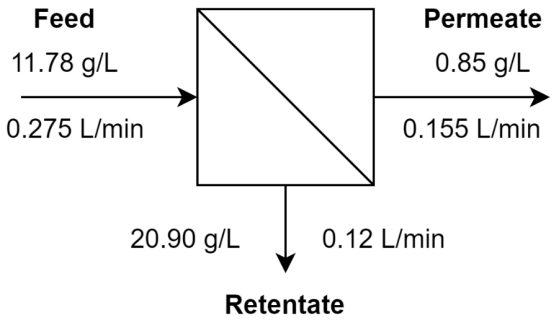
The resulting ultrafiltration feed flow rate is 0.275 L/min. This was obtained by multiplying the membrane area (A) by the feed flux (150 LMH) used by Arunkamar et al. (2017) to generate the permeate flux versus TMP data. To calculate the cross-membrane flow, we assumed this optimal permeate flux value to be the average across the membrane ($u_{p,avg}$). In doing so, we found the cross-membrane flow of each filter to be 0.160 L/min using Equation 4.2.7.

$$\frac{A}{Q_F} = \frac{1 - Q_B / Q_F}{u_{p,avg}}$$

Equation 4.2.7. Calculation of cross-membrane flow from feed flow, average permeate flux, and membrane area for a single-pass tangential flow filter (Carta, 2022).

We are employing the Millipore Pellicon 3 Cassettes with 30 kDa cutoff Ultracel membrane for ultrafiltration. As with the diafiltration modules, we will operate UF with 0.11 m^2 area filters, which is also a standard size of the Ultracel membranes (MilliporeSigma 2018). Our ultrafiltration unit will have an operating pressure of 8 psi (55.16 kPa) and permeate flux of 63 LMH.

As shown in Figure 4.2.20, the feed concentration of pembrolizumab will be 11.78 g/L. The retentate flow will be 0.12 L/min with a pembrolizumab concentration of 20.90 g/L, and the permeate flow will be 0.16 L/min with a pembrolizumab concentration of 0.85 g/L.



$$Q_F = Q_P + Q_B$$

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

$$Y = 1 - \frac{Q_B}{Q_F}$$

$$VRF = \frac{1}{(1-Y)}$$

$$C_{ret} = C_{feed} * VRF^{\sigma}$$

$$\sigma = 0.995$$

Figure 4.2.20. Ultrafiltration flows, material balances, and concentration calculations.

The final diafiltration unit will exchange HEPES buffer fed at 0.12 L/min with WFI fed at 0.84 L/min. Pembrolizumab will enter at a concentration of 25.90 g/L The product outlet will move to fill and finish at a flow rate of 0.12 L/min and a concentration of 25.0047 g/L. The waste will have a flow rate of 0.84 L/min and a pembrolizumab concentration of 0.025 g/L. With a transmembrane pressure of 4 psi (27.58 kPa), the cross-membrane flow calculated using Equation 4.2.7 for this final unit is 0.047 L/min.

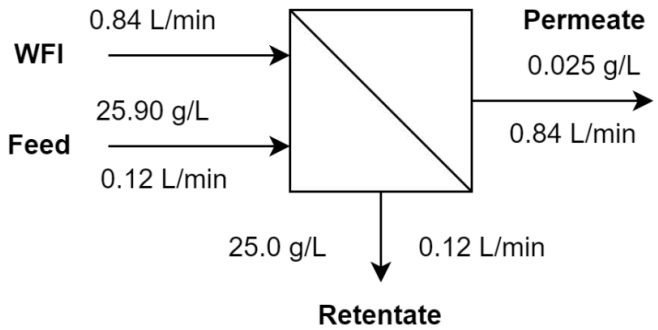


Figure 4.2.21. Final diafiltration flows and pembrolizumab concentrations.

In total, our downstream process will require 9 Pall cellulose membranes and 3 Ultracel membranes per year.

4.2.10 Formulation and Filling

Following the downstream purification the API drug substance is suitable for final formulation and filling to become semi-finished product prior to packaging. This involves the addition of excipients such as L-histidine, polysorbate-80, and sucrose which makes up the 4 mL and 25 mg/mL vial of drug product. Additionally there is approximately 0.30 mL of overfill in each vial to meet US pharmacopeia requirements due to syringes not being able to remove all liquid from the vial prior to injection. Each vial contains 6.2 mg of L-histidine, 0.8 mg of polysorbate, and 280 mg of sucrose in addition to the 200 grams of active pembrolizumab.

To achieve this final solution from the downstream operations the pembrolizumab will flow from final diafiltration into a 50 L Sartorius Palletank at a rate of 0.12 L/min taking approximately 7 hours to fill. Once filled the Palletank will be transferred to an aseptic parenteral environment for the following formulation and filling steps. The contents of the Palletank will be drained into a 200 L Pall Allegro Single-Use Mixer through a Pall Fluorodyne EX EDF - Kleenpak Capsule which contains a 0.2 micron filter to achieve bioburden reduction. The mixing tank will be filled at a rate of 0.25 L/min which will take approximately 3.3 hours for excipients and pembrolizumab. Mixing will then take place for 50 minutes at 150 RPM to achieve homogenization (de Boulard, 2022). The mixing unit will then be drained at a rate of 0.25 L/min which will again take approximately 3.3 hours to the Cytica SA25 Aseptic Filling workcell which has a 20,000 vial batch throughput, each 50 L of solution will provide 12,500 vials of pembrolizumab so this unit is sufficient for the chosen volumes (SA25 aseptic filling workcell, n.d.). Each campaign will fill 540,000 vials so 54,000 will be ordered in excess per campaign in case of breakage. Due to the overfill requirements 4.05 kg of excess active ingredient is required

per campaign but this was not factored into the final design and production capacity. There will be 3 formulation lines each with a palletank and mixing tank totalling six of each unit with a spare of each in the event of malfunction only one filling unit is at the facility.

4.3 Ancillary Equipment

4.3.1 Pump Design

The various flows described in the sections above will be controlled by in-system pumps or by Masterflex peristaltic pumps. Peristaltic pumps have been implemented in pharmaceutical industries for a variety of reasons including use of single-use tubing to prevent cross-contamination, enable quicker validation, and reduce cleaning needs due to no contact between fluids and the pump itself, only sterile tubing (Lambert, 2008). Additionally, peristaltic pumps provide ease in adjusting flow speed to prevent foaming and splashing and maximize throughput while also reducing shear due to the low pressure and low mechanical stress vital to shear sensitive pharmaceutical products (Lambert, 2008). 25 peristaltic pumps will be required with each specified in Table 4.3.1.1 below and each with a spare for 50 total pumps. The pumps will be either Masterflex Ismatec or I/P series for the range in flow rates required.

Table 4.3.1.1. Pump Design.

	From	To	Flow Rate (L/min)	Quantity	Pump
#	Upstream				
1	Media Tank	Wave rocker	0.000667	1	ismatec
2	Slurry	XDR reactor	Unknown	1	Ismatec
3	Media tank	XDR reactor	0.02 - 1.55	1	I/P
4	XDR Reactor	TFF	4.55	1	I/P
5	TFF	Purge	Unknown	1	I/P
6	TFF	Recycle	Unknown	1	I/P
#	Downstream				
7	TFF	Depth Filtration	0.229	1	Ismatec
8	ZnCl2	Precip Nucleation	0.0458	1	Ismatec
9	PEG	Precip Growth	0.183	1	Ismatec
10	Wash 1 TFF	Wash 2	0.275	1	ismatec
11	Dewatering	Waste	Unknown	1	I/P
12	HEPES	Wash	0.452	1	I/P
13	Wash 2	Wash 1	Unknown	1	I/P
14	Wash	Waste	Unknown	1	I/P
15	Glycine	Redissolution	0.0917	1	Ismatec
16	Precip chrom	Viral filtration	0.275	1	Ismatec
17	bisTrisProp	Diafiltration	1.93	1	I/P
18	Diafiltration	AEX	0.1375	2	Ismatec
19	HEPES	Diafiltration	1.93	1	I/P
20	CEX	VF/UF	0.275	1	Ismatec
21	UF	Waste	0.16	1	Ismatec
22	UF	Palletank	0.12	1	Ismatec
23	HEPES	Diafiltration	0.12	1	Ismatec
24	Palletank	Mixing	0.25	3	I/P
25	Mixing	Filling	X	1	I/P

4.3.2 Tank Design

Holding tanks will be used throughout the process to store and mix buffers and media, as well as for emergency draining of the bioreactors, and for storing waste. The holding tanks will be designed to store 25% of the volume of buffer needed for one 25 day campaign. Each buffer and media holding tank will be connected to a CSTR that will mix solid additive and WFI to create more buffer. Each CSTR will be designed to store 10% of the volume of the associated holding tank. Five 5000 L waste tanks will be used to collect and denature the waste created throughout the process. ThermoFisher HyPerforma Single-Use Mixers will be used throughout the process. These are stainless steel tanks with single use mixing bags (ThermoFisher, 2020). A comprehensive list of all tanks and sizes is found in Table 4.3.3.1.

Table 4.3.3.1. Tank specifications for holding, mixing, and waste storage.

Tank Identifier	Volume (L)	Type	Contents	Purpose
Upstream				
TH-1	20,000	Holding	Media	Holding bioreactor feed
TH-2	2000	Holding	Media	Holding bioreactor feed
TM-1	2000	Mixing	Media	Mixing bioreactor feed
TM-2	200	Mixing	Media	Mixing bioreactor feed
TH-3	200	Holding	WFI	Holding WFI for bioreactor dilution
TE-1	2000	Holding	Bioreactor Contents	Emergency holding for the bioreactor
TE-2	2000	Holding	Bioreactor Contents	Emergency holding for the bioreactor
TE-3	2000	Holding	Bioreactor Contents	Emergency holding for the bioreactor
TE-4	2000	Holding	Bioreactor Contents	Emergency holding for the bioreactor
Downstream				
TH-4	500	Holding	0.1 M $ZnCl_2$	Holding buffer for precipitation chromatography
TM-3	50	Mixing	0.1 M $ZnCl_2$	Mixing buffer for precipitation chromatography
TH-5	2000	Holding	PEG	Holding buffer for precipitation chromatography
TM-4	200	Mixing	PEG	Mixing buffer for precipitation chromatography
TH-6	5000	Holding	50 mM HEPES	Holding buffer for precipitation chromatography, CEX chromatography and DF before CEX
TH-7	2000	Holding	50 mM HEPES	Holding buffer for precipitation chromatography, CEX chromatography and DF before CEX

TM-5	500	Mixing	50 mM HEPES	Mixing buffer for precipitation chromatography, CEX chromatography and DF before CEX
TM-6	200	Mixing	50 mM HEPES	Mixing buffer for precipitation chromatography, CEX chromatography and DF before CEX
TH-8	1000	Holding	2 M Glycine buffer	Holding buffer for precipitation chromatography
TM-7	100	Mixing	2 M Glycine buffer	Mixing buffer for precipitation chromatography
TH-9	20,000	Holding	20 mM bis-Tris Propane	Holding buffer for AEX chromatography and DF before AEX
TH-10	2000	Holding	20 mM bis-Tris Propane	Holding buffer for AEX chromatography and DF before AEX
TM-8	2000	Mixing	20 mM bis-Tris Propane	Mixing buffer for AEX chromatography and DF before AEX
TM-9	200	Mixing	20 mM bis-Tris Propane	Mixing buffer for AEX chromatography and DF before AEX
TH-11	1000	Holding	50mM Sodium acetate buffer	AEX and CEX chromatography
TM-10	100	Mixing	50mM Sodium acetate buffer	AEX and CEX chromatography
TH-12	5000	Holding	WFI	Holding WFI for Final DF
TH-13	5000	Holding	WFI	Holding WFI for Final DF
TH-14	2000	Holding	0.1 M NaOH	Cleaning chromatography columns
TM-11	200	Mixing	0.1 M NaOH	Cleaning chromatography columns
Waste Storage				
TW-1	5000	Holding	Waste	General Waste Tank
TW-2	5000	Holding	Waste	General Waste Backup

TW-3	5000	Holding	Waste	General Waste Backup
TW-4	5000	Holding	Waste	General Waste Backup
TW-5	5000	Holding	Waste	General Waste Backup

4.3.3 Heat Exchanger

A heat exchanger will be required for temperature regulation between the upstream and downstream process. The upstream process is conducted at a temperature of 37°C following the CHO cell kinetic information obtained from literature. Aggregation is affected by factors such as pH, temperature, and salt concentration. A study found that the formation of aggregates is greater at low pH conditions and high temperatures (Singla et al., 2016). Since necessary steps in the downstream process such as viral inactivation require subjecting our product to low pH conditions, we will conduct the downstream process at room temperature to minimize aggregation. We will use a stainless-steel counter current double-pipe heat exchanger.

$$\Delta T_{lm} = \frac{(T_{H,out} - T_{C,in}) - (T_{H,in} - T_{C,out})}{ln(\frac{T_{H,out} - T_{C,in}}{T_{H,in} - T_{C,out}})}$$

Equation 4.3.4.1. LMTD for a Counter Current Double-pipe Heat Exchanger.

The stream exiting tangential flow filtration will enter through the inner pipe of the heat exchanger at a flow rate of 0.229 L/min. Ethylene Glycol-water mixtures are commonly used in heat transfer applications to prevent the freezing of pipe fluids (CORECHEM, 2022). Therefore, the surrounding outer pipe will be 50 wt% ethylene glycol and 50 wt% water. Based on equation 4.3.4.1, the cooling stream will enter at 5°C and exit at 11°C to produce a log mean temperature difference (LMTD), ΔT_{lm}, of 19.998. Using Equation 4.3.4.2, the heat released by the inner pipe to effectively cool the stream from 37°C to 20°C was calculated to be 272 J/s.

$$Q = m \times C_p \times \Delta T$$

Equation 4.3.4.2. Heat Transfer Requirement for a Change in Stream Temperature.

$$A = \frac{Q}{U_0 \Delta T}$$

Equation 4.3.4.3. Required Heat Transfer Area.

Equation 4.3.4.3 was used to determine the required heat transfer area. Using an overall heat transfer coefficient, U_o, of 285 W/m^2•K for stainless steel, the area was calculated to be 32.89 cm^2. Resolving Equation 4.3.4.2 using the obtained heat transfer, Q, temperature change of the cooling stream, and heat capacity of the ethylene-glycol mixture, the required mass flow rate of the cooling stream was found to be 0.742 L/min.

4.4 Water for Injection (WFI) System Design

Because our process necessitates a 99% purity, sterile product to be suitable for direct intravenous injection, we must use water for injection (WFI) in each operation requiring water. Using tap or other non-sterilized water sources would compromise the process by introducing an unquantifiable amount of minerals or biological matter. According to continuous Good Manufacturing Practices (cGMP) enforced by the FDA, unknowns of this sort are unacceptable in production processes for injectables (Hunter, 2022). WFI on the other hand is attained through either membrane purification or distillation and is both sterile and nonpyrogenic. With no added salts and a pH of approximately 5.5, it can act as a diluent to buffers used within the process and is key to dilute the product for the final formulation. When integrated with the appropriate additives, it will be safe to inject as a part of our final pembrolizumab product (Baxter Corporation, 2014). Based on the need of each unit operation listed below in table 4.4.1, we will need to supply 9.60 L/min of WFI to the process, or a total of 345,000 L per 25-day campaign.

Table 4.4.1. WFI requirements per campaign.

WFI Requirements	
Module	**L Water**
Fermentation	111,600

Precipitation	27,810
AEX DF	69,300
AEX	18,470
CEX DF	69,300
CEX	18,480
Final DF	30,240
Total	345,200

According to the International Society for Pharmaceutical Engineering (2022), membrane-based systems are the new state-of-the-art method for acquiring water for injection on an industrial scale. Unlike vapor compression distillation, which has a high footprint, high operating cost, and high capital cost due to the scale of the equipment, membrane-based WFI systems often come preassembled and run automatically (Wrampe, 2019). We will employ the MECO Masterpak™ Ultra system, which features integrated water pretreatment, reverse osmosis (RO), electrodeionization (EDI), ultrafiltration, and heat sanitization units (MECO, 2023). Pretreatment of the source water is necessary to prevent damage by chlorine or ammonia to the fragile polyamide thin-film membrane used in reverse osmosis. Tangential flow of the treated water across the RO membrane separates water with larger ion contents from that with lower quantities of ions. Because federal regulations demand a maximum WFI conductivity of less than 1.3 μS/cm, membrane-based WFI systems typically involve two passes through the RO membrane (Wrampe, 2019). This model has a backwash, rinse, and recirculation function that is controlled automatically via a PID system and a pump with variable drive frequency (VDF), which also enables a constant continuous flow of WFI to our process (MECO, 2023). Reverse osmosis will be followed by electrodeionization and ultrafiltration with a 6 kDa molecular weight cutoff to remove any endotoxin accumulated from a biofilm forming on the RO

membrane (MECO, 2023; Wrampe, 2019). The RO membrane also undergoes routine heat sanitization to mitigate biofilm formation as a part of MECO's clean-in-place system. Like for the water feed, PID is used to automatically control heat sanitization circuits, which are located throughout the system in addition to a stainless steel break tank with an integrated immersion heater used for the carbon pretreatment filter (MECO, 2023).

The MECO MP2Ultra model is specifically suitable for a 4-11 L/min flow rate of WFI (MECO, 2023). At flow rates of 25 L/min, MECO claims that a similar model consumes 9 kW per hour of runtime plus an additional 90 kW per heat sanitization cycle, which will be once per campaign in our case (Herold, 2021). Because our system will only run at a flow rate of 9.60 L/min, we assume that the running power consumption will only be 5 kW per hour. This assumption is based on the breakdown of power consumption per stage (RO, EDI, UF) and WFI distribution versus their individual feed rates located in a MECO cost analysis of the model one step up in capacity from ours (MECO, 2019).

4.5 Air Filtration Design

The air supply for the perfusion bioreactor must also be properly sterilized to maintain product quality. Therefore, a Sartofluor® Capsule 2 XLG Size 4 sterile grade air filter will be used to purify the inlet air stream. The filtration area is 0.015 m^2 and the maximum differential pressure is 4 bar (Sartorius, 2022). Based on Figure 4.5.1, with a required inlet air flow rate of 63 L/min which is equivalent to 3.78 m^3/h, the air filter will be operated at a pressure of 190 mbar, or 2.8 psi. The filter will be replaced after each 25-day campaign.

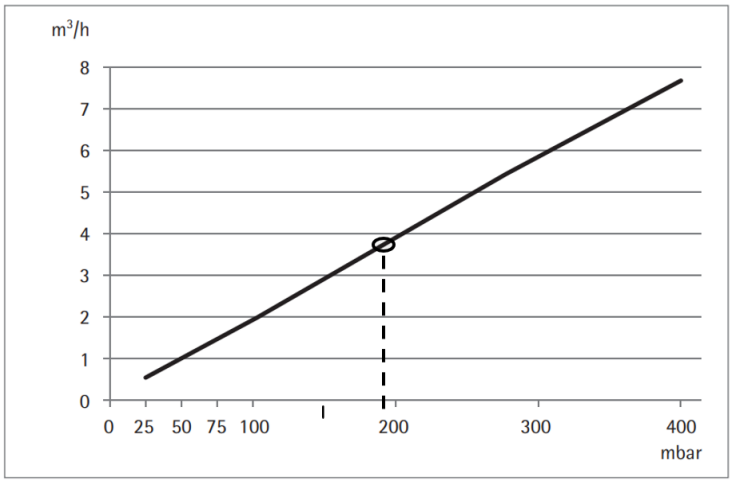


Figure 4.5.1. Sartopore flow rate capabilities (Sartorius Stedim Biotech, 2022).

4.6 Disposal

4.6.1 Liquid Waste

This process produces 314000 L of liquid waste per campaign, with waste sources including the perfusion bioreactor waste and purge streams, the waste streams of precipitation and ion exchange chromatographies, the ultrafiltration step, and the several diafiltration units built into the process. Waste streams from the perfusion bioreactor collected during upstream processing will be separated from the other waste collection in our waste collection tanks, which are designed to not be filled above 75%, in order to avoid overcapacity. Several waste tanks will be used as backup, in case of the need to purge an inoculation train or other plant related malfunctions. Based on our plant location, we will be contracting waste disposal with Eldredge Inc. in West Chester, PA, and their company headquarters and processing site is approximately 30 miles from our plant.

Unit Operation	Flow Rate (L/min)	L per Campaign
TFF	2.87	103000
Precipitation Chromatography	0.452	16300
Diafiltration for AEX	1.93	69500
Anion Exchange Chromatography	0.275	9900
Diafiltration for CEX	1.93	69500
Cation Exchange Chromatography	0.275	9900
Ultrafiltration	0.160	5760
Diafiltration	0.840	30200
Total	8.83	314000

4.6.2 Solid Waste

The solid waste in our process includes the depth filters, TFF filters, bioreactor bags and chromatography columns--our single-use equipment. This waste is a biohazardous material and will be disposed of by our contracted waste disposal company, Eldredge Inc.

4.7 Plant Scale Market Calculations

The plant scale was based upon the expected growth in demand by 2024 to 2 million users as discussed earlier. It was decided we would capture 20% of this demand to supply 400,000 users with their annual needs. This totaled 7 million doses produced annually and 1400 kg of pembrolizumab produced to meet the dosing requirement of every 3 weeks for these patients. The calculation can be seen in Table 4.7.1.

Table 4.7.1. Plant Scale Market Calculations.

Estimated Users for 2024: 2 million
Dose Size: Non-small cell lung cancer and head and neck squamous cell carcinomas: 200 mg
Administration: Every 3 weeks
Market Share: 20%
$kg\ of\ Product = 2,000,000 * 0.20 * 52\ weeks/3\ weeks * 0.0002\ kg\ /dose = 1384\ kg$
Rounding to 1400 kg for expected losses yields 7 million doses based upon the 200 mg dose size

5. Final Design

5.1 Upstream Process

5.1.1 Cell Line Acquisition and Storage

The CHO cell line will be obtained from Merck's master cell bank located in West Point, Pennsylvania. Each vial will be 4.5 mL with a high cell density of 50 x 10^6 cell/mL. The cells will be stored at -86℃ in a VIP ECO Model MDF-DU702VH-PA Freezer during shipment and at the production facility. At our facility, the cell vials will be equally distributed between two storage freezers to avoid cell loss as a result of freezer malfunctions. The cell bank will be thawed to 37℃ in a 2L Thermo Fisher Precision GP 02 Water Bath.

5.1.2 Inoculum Train

To begin our scale-up process, 15 L of WFI will be added to the 4.5 mL high-density CHO cell seed in the ReadyToProcess WAVE 25L Rocker from Cytiva. This unit will operate in fed-batch mode at a temperature of 37 ^{O}C, a pH of 7.1, and a rocking speed of 20-29 RPM depending on oxygenation levels. The wave rocker will run with a dissolved oxygen level of 40% (Cytiva, 2017). From dilution of the working cell bank with 1 liter of WFI, the initial cell count in the wave rocker will be 2.25 * 10^8 cells. Media will be fed into the wave rocker at a flow rate of 0.000667 L/min. This includes a glucose mass flow of 0.0120 g/min and glutamine mass flow of 0.00175 g/min. Operation for 350 hours will bring the total cell count up to 3.45 * 10^{11} and 15 L working volume, at which point the slurry will be transferred to a 400 L bag in the first XDR bioreactor.

Over 100 hours, the working volume will be increased to 900 L, where the cell count will change from 1.26 * 10^{10} to 2.65 * 10^{12}. The feed rate in this stage is 0.0833 L/min, and the mass flow rates of glucose and glutamine are 0.499 g/min and 0.0731 g/min, respectively. The second fed batch stage in the bioreactor brings the working volume from 900 L to 1500 L over 500

hours and the cell count from 2.65 * 10^{12} to 1.92 * 10^{13}. The flow rate is 0.0200 L/min with a glucose mass flow rate of 0.479 g/min, and a glutamine mass flow rate of 0.0526 g/min.

5.1.3 Perfusion Bioreactor

The bioreactor chosen for this design is Cytvia's Xceller XDR Pro 2000 L single use stirred tank perfusion reactor. It has a total volume of 2000 L, an inner diameter of 123 cm, and a height of 185 cm. It will be operated at 37℃ and 1.01 bar, and have a steady state working volume of 1500 L that will be constant throughout the 25 day campaign. The disposable four blade pitched impeller has a diameter of 42 cm, and a height of 42 cm from the bottom of the tank. It will be operated at a rotational speed of 68 rpm. Compressed air will be fed to the bioreactor at a rate of 0.042 vvm; under these conditions, the reactor will have a k_La of 43.0 h^{-1}. Two bioreactors will be operated in perfusion mode per 25 day campaign, while two others are used to grow the seed train in batch mode to maintain continuous operation. One back up reactor will also be purchased.

5.1.4 Tangential Flow Filtration

The reactor effluent will be sent through a series of three filters for tangential flow filtration. The first filter will receive a flow of 4.55 L/min and will allow 82% of the flow to pass through to the third filter. The remaining 18% will be diluted with waste water from the third filter to increase the flow from 0.819 L/min to 10.8 L/min. This flow will pass through a second filter, and 82% of this flow will be directed towards the flow entering the third filter, combining with the collection from the first. The second filter dewaters the cells and the permeate is then sent back to the reactor at a flow of 1.45 L/min and cell concentration of 39.9 g/L, with some of the second filters' permeate leaving the system as a cell bleed stream. The third filter will have an inlet flow concentration of 0.376 g/L pembrolizumab and a flow rate of 12.6 L/min. This third filter dewaters the protein to a concentration of 20.6 g/L and flow rate of

0.229 L/min with an overall yield of 96% through tangential flow filtration. All of the filters used in this process will be provided by Sartorius and will be the Sartocon Slice Disposable Hydrosart Cassette. The first two filters will be of pore size 0.2 um, and the third filter, which dewaters the protein will be of pore size 100 kDa to retain the protein.

5.2 Downstream

5.2.1 Depth Filtration

Following TFF, depth filtration will be used to capture large impurities. We will use a 0.11 m^2 Millistak+ A1HC Pod Depth filter with a length of 62 cm. A 99% recovery of the monoclonal antibody will be assumed since the mAb is relatively small compared to the pores of the filter. The flow rate entering and exiting the filter will remain constant at 0.229 L/min. This flow rate corresponds with an operating pressure of 1.5 psi (10 kPa) well below the maximum operating pressure of 50 psi (345 kPa). The filter will be replaced after each 25-day campaign.

5.2.2 Precipitation Chromatography

5.2.2.1 Precipitation Stage

The pembrolizumab is collected by precipitation chromatography as the primary capture step, with $ZnCl_2$ acting as a crosslinking agent, and PEG acting as a volume-exclusion agent. $ZnCl_2$ is added at a flow rate of 0.275 L/min to a harvested cell culture fluid (HCCF) with a flow rate of 0.229 L/min and allowed to mix for a pembrolizumab concentration of 17.2 g/L. PEG is then added at a flow rate of 0.183 L/min with a 17.5 wt% concentration for a resulting flow rate of 0.458 L/min and pembrolizumab concentration of 10.2 g/L. The protein-metal complex is then dewatered by filtration to protein flow rate of 0.183 L/min.

5.2.2.2 Washing Stage

The washing stage of precipitation chromatography removes impurities from the solution through a buffer, in this process HEPES was selected at a pH of 7.0 and a flow rate of 0.452

L/min was selected based on recycling flow rates in the countercurrent scheme to the protein flow rate of 0.183 L/min. The outlet of the washing has the same flow rate of 0.183 L/min, but the protein is re-solubilized using 2M glycine, which is added at a flow rate of 0.0917 L/min for a final pembrolizumab concentration of 14.0 g/L at a flow rate of 0.275 g/L.

5.2.3 Viral Inactivation

The product stream following precipitation chromatography must be subjected to low pH conditions for a minimum time of 30 minutes to inactivate enveloped viruses. As the product stream exiting the precipitation chromatography unit will already be at a low pH of 3.5, a continuous stainless-steel plug flow reactor with a coiled flow inverter will be used to hold the solution for one hour. With a minimum residence time of 30 minutes, this will ensure that the product is held at the low pH for at least 30 minutes to confirm complete inactivation. The total volume of the plug flow reactor will be 16,500 mL to accommodate the constant flow rate of 0.275 L/min. The plug flow reactor will have an inner diameter of 6 cm and a length of 5.84 m.

5.2.4 Diafiltration for Anion Exchange Chromatography

Our first diafiltration step will replace glycine from viral inactivation with bis-Tris propane with a pH of 6.6 in preparation for anion exchange chromatography. To do so, we will employ the inline Pall Cadence module (F-202) equipped with a 0.11m^2 cellulose membrane with a cutoff size of 30 kDa. The pressure drop across this filtration unit will be 8 psi (55.16 kPa). Each membrane will be cleaned after every 25-day campaign with 0.1 M NaOH and replaced each year. The inlet flow rate will be 0.275 L/min with a pembrolizumab concentration and mass flow of 14.0 g/L and 3.85 g/min, respectively. Buffer will flow in at a rate of 1.925 L/min at a concentration of 20.0 mM (10.87 g/min). Retentate will flow out at a rate of 0.275 L/min with a pembrolizumab concentration of 13.52 g/L and mass flow of 3.72 g/min. The retentate will also have 20 mM of bis-tris propane (1.55 g/min). Our waste permeate will flow at

a rate of 1.925 L/min with a pembrolizumab concentration of 0.07 g/L and mass flow of 0.13 g/min. This waste stream also contains zinc chloride, glycine, HEPES, PEG, and bis-tris propane.

5.2.5 Anion Exchange Chromatography

Anion Exchange Chromatography will be the first polishing step in the downstream purification process. The monoclonal antibody will be suspended in bis-tris propane according to the previous diafiltration step at a pH of 6.6. The column will utilize Cytiva Capto Q Impact resin in a Cytiva ReadyToProcess Capto Q 1 L column, a single use column which will be replaced every campaign. A residence time of 2.1 minutes was determined for a DBC value of 130 mg/mL. This column has a diameter of 8 centimeters and a bed height of 5.74 centimeters to give a total volume of 288.8 cm^3. The incoming flow to this column from diafiltration is 0.1375 L/min corresponding to a linear flow velocity of 164.2 cm/hr. The pressure drop in this column is calculated to be 0.766 kPa. 5 total AEX columns will be operated on a schedule according to Figure 4.2.12.

The time breakdown of our proposed AEX run schedule for a given column is 21.02 minutes of loading time followed by 6.31 minutes of washing. Then the column will be stripped for 4.2 minutes, cleaned in place (CIP) for 6.31 minutes, regenerated for 10.51 minutes, and re-equilibrated for 10.51 minutes. With a wait period of 4.20 minutes, each column cycle will total to 63.05 minutes.

5.2.6 Diafiltration for Cation Exchange Chromatography

Our second diafiltration step will replace bis-tris propane from AEX with 50 mM HEPES buffer at a pH of 8.1 in preparation for cation exchange chromatography. This buffer will be fed at a flow rate of 1.93 L/min into the unit. The inlet coming from AEX will flow at a rate of 0.275 L/min and contain 12.90 g/L of pembrolizumab at a mass flow of 3.55 g/min. The

retentate will exhibit a flow rate of 0.275 L/min with a pembrolizumab concentration of 12.46 g/L (mass flow of 3.43 g/L).Waste will flow out as the retentate at a rate of 1.93 L/min with a pembrolizumab concentration of 0.06 g/L and mass flow of 0.12 g/min. Like for the previous DF unit, we will employ the inline Pall Cadence module (F-203) equipped with a 0.11m^2 cellulose membrane having a cutoff size of 30 kDa. It will also be operated at a pressure of 8 psi (55.16 kPa), cleaned once per campaign with 0.1 M NaOH, and replaced once per year.

5.2.7 Cation Exchange Chromatography

Cation Exchange Chromatography will be the second polishing step in the downstream purification process. The monoclonal antibody will be suspended in HEPESe according to the previous diafiltration step at a pH of 8.1. The column will utilize Cytiva Capto S Impact resin in a Cytiva ReadyToProcess Capto Q 1 L column, a single use column which will be replaced every campaign. A residence time of 5.4 minutes was determined for a DBC value of 130 mg/mL. This column has a diameter of 8 centimeters and a bed height of 14.8 centimeters to give a total volume of 288.8 cm^3. The incoming flow to this column from diafiltration is 0.1375 L/min corresponding to a linear flow velocity of 164.2 cm/hr. The pressure drop in this column is calculated to be 1.72 kPa. 5 total CEX columns will be operated on a schedule according to Figure 4.2.15.

The time breakdown of our proposed CEX run schedule for a given column is 54.0 minutes of loading time followed by 16.2 minutes of washing. Then the column will be stripped for 10.8 minutes, cleaned in place (CIP) for 16.2 minutes, regenerated for 27.0 minutes, and re-equilibrated for 27.0 minutes. With a wait period of 10.8 minutes, each column cycle will total to 162.0 minutes.

5.2.8 Viral Filtration

Viral filtration will be performed using a Virosart HF Mid-Scale Module sterile filter with an area of 200 cm^2, operating at a pressure drop of 4.48 bar. The flow rate entering and exiting the filter is 0.275 L/min, and the initial and final pembrolizumab concentration is 16.136 g/L. The filter will be replaced every eight hours to prevent fouling.

5.2.9 Final Ultrafiltration and Diafiltration

Our downstream process will conclude with a final ultrafiltration and diafiltration step. Our ultrafiltration unit will consist of the Millipore Pellicon 3 Cassettes (F-205) with 30 kDa cutoff Ultracel membrane operated as a 3-section SPTFF system. The transmembrane pressure of this until will be 8 psi, and the total volumetric flow rate of the inlet will be 0.275 L/min. Pembrolizumab will flow in at a rate of 3.24 g/min with a concentration of 11.78 g/L. Pembrolizumab will exit the unit predominantly with the retentate at a mass flow of 3.11 g/min and a concentration of 25.90 g/L, while the permeate will contain 0.85 g/L of pembrolizumab flowing at a rate of 0.13 g/min. The volumetric flows of the retentate and permeate will be 0.120 L/min and 0.155 L/min, respectively. The cross-membrane flow of this filter will be 0.160 L/min, and each filter will be replaced once per campaign.

Our final diafiltration unit will replace the HEPES buffer from AEX with water for injection (WFI) in preparation for formulation and filling. The inlet from viral filtration will have a flow rate of 0.120 L/min, pembrolizumab mass flow rate of 3.11 g/min, and a pembrolizumab concentration of 25.90 g/L. WFI will flow into the unit at a rate of 0.840 L/min, and the retentate will flow out at a rate of 0.120 L/min. The pembrolizumab mass flow in the retentate will be 3.00 g/min with a concentration of 25.0047 g/L. The waste stream will have a volumetric flow rate of 0.840 L/min, a pembrolizumab mass flow rate of 0.11 g/min, and a pembrolizumab concentration of 0.13 g/L. This unit will be operated at 4 psi, and like for the previous units, we will use the

inline Pall Cadence module (F-206) equipped with a 0.11m^2 cellulose membrane having a cutoff size of 30 kDa. We will clean the filters once per campaign with 0.1 M NaOH and replace them once per year.

5.2.10 Formulation and Filling

Formulation and filling will be made up of three production lines with a palletank and mixing tank. A 50 L Sartorius Palletank will be filled at a rate of 0.12 L/min until full from the final diafiltration unit in downstream production. The palletank will be moved to a formulation area where it will be drained at a rate of 0.25 L/min into a 200 L Pall Allegro Single-Use Mixing Tank. Mixing will take place for 50 minutes at 150 rpm using the built in impeller for each disposable bag in the mixer. After the completion of mixing with pembrolizumab and excipients the unit will be brought to the SA25 Aseptic Filling Cell from Cytiva to fill approximately 12,500 vials per drained mixing unit. At the conclusion of filling one 50 L Palletank the next line's Palletank will begin to be filled, thus the three Palletanks will rotate in filling, draining, changeover procedures as seen in Figure 5.2.10.1.

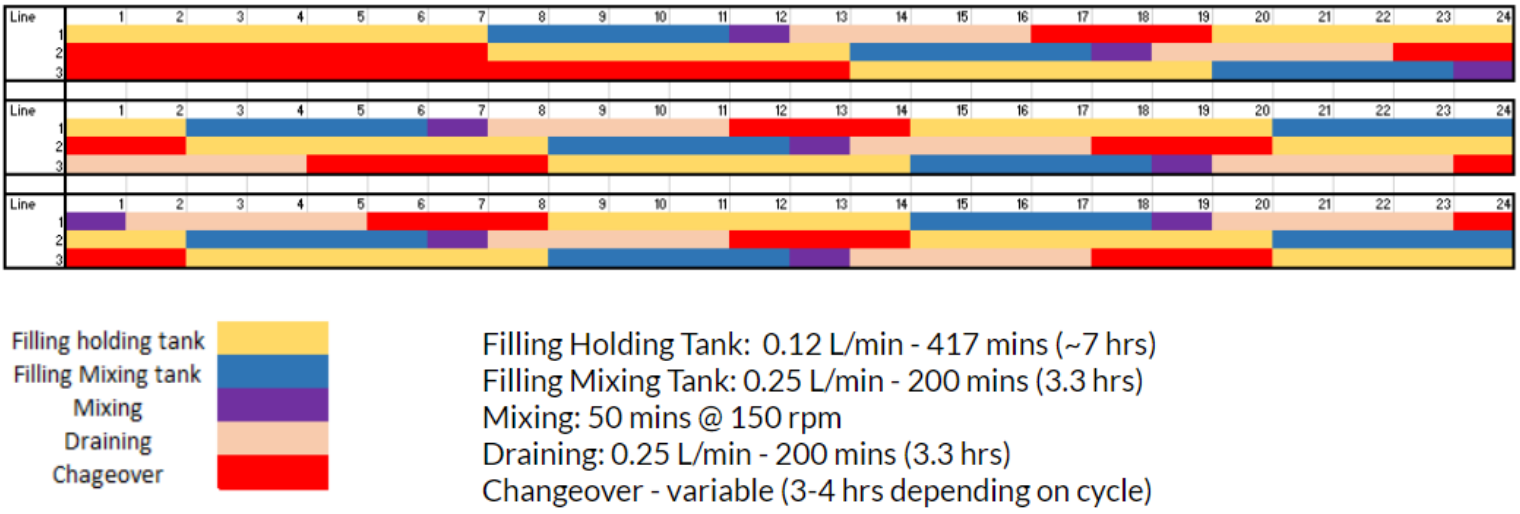


Figure 5.2.10.1 Sample 3 Day Schedule for the 3 Formulation/Fill Lines.

5.4 Production Schedule

As mentioned previously, the production schedule will consist of thirteen 25 day perfusion campaigns. The schedule for each campaign is shown in Table 5.4.1. There will be a three day upstream equipment cleaning period at the start of each campaign, with a one day offset between the start of the upstream and downstream production, to allow for cleaning and startup of the downstream process. Due to the length of the seed train, we will run two wave reactors in the seed train and two bioreactors in the seed train while perfusion occurs in the other two bioreactors. There will be a 40 day annual shutdown of the plant for cleaning and maintenance on the process. This shutdown time can also be used to accommodate any delays in the process to mitigate process losses. The annual campaign schedule is shown in Table 5.4.2. Due to the length of the seed train, the wave reactors will shut down at the end of the thirteenth campaign and undergo cleaning and maintenance then, and will start producing the seed train for the first campaign of the next year while the rest of the plant is still in shutdown. For all campaigns except the first campaign, the seed train will begin while the previous campaign is in operation.

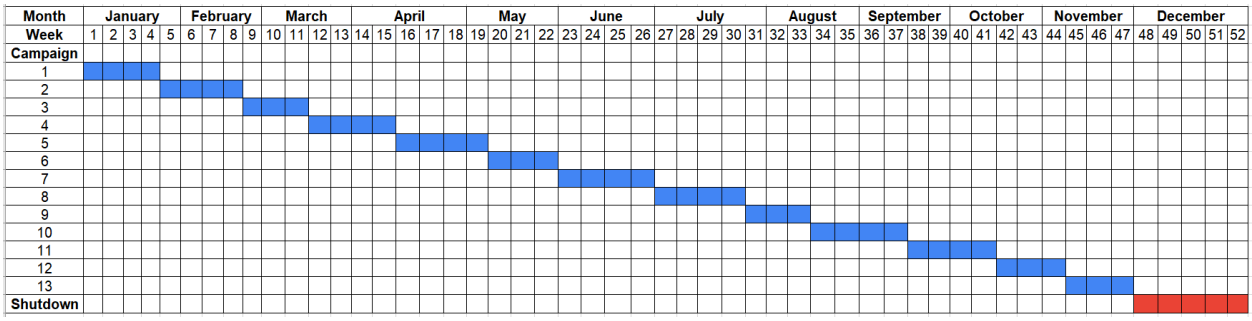


Table 5.4.2. Yearly production schedule for the pembrolizumab facility (13 campaigns).

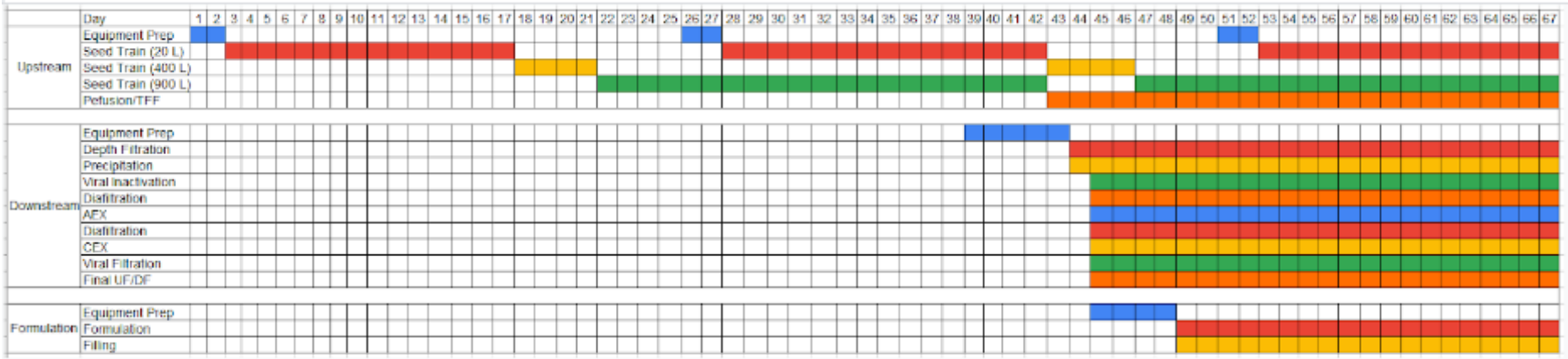


Table 5.4.1. Production schedule for a single campaign of the pembrolizumab facility, including start up time for the seed train..

5.5 Equipment Tables and Specifications

5.5.1 Upstream Equipment Table

Table 5.5.1. Upstream Equipment Table.

Operation	Unit	Unit No.	Quantity	Temperature (°C)	Pressure (psi)	Size
WCB Storage	VIP ECO Model MDF-DU7 02VH-PA Freezer	FR-101	2	-86	n/a	730 L
WCB Thawing	Thermo Fisher Precision GP 02 Water Bath	WB-101	2	37	n/a	2 L
Fermentation	WAVE 25 L Rocker Reactor	WR-1	2	37	n/a	25 L
	Xcellerex XDR 50/2000	R-101	2	37	n/a	2000 L
		R-102	2			
		R-103	2			
TFF	Repligen KrosFlo KR2i	F-101	2	37		A= L=
		F-102	2	37		A= L=
		F-103	2	37		A= L=

5.5.2 Downstream Equipment Table

Table 5.5.2. Downstream Equipment Table.

Operation	Unit	Unit No.	Quantity	Temperature (°C)	Pressure Drop (psi)	Size
Depth Filtration	Millistak+ A1HC Pod Depth Filter	F-201	2	RT	1.5	A = 0.11 m^2 L = 62 cm
Precipitation Chromatography		C-201		RT		D= L=
Viral Inactivation	Custom built Stainless Steel PFR with CFI	V-201	1	RT	n/a	D = 6 cm L = 584 cm
Diafiltration	Pall Delta Cadence Inline Diafiltration Module	F-202	2	RT	8	A = 0.11 m^2
Anion-Exchange Chromatography	ReadyToPro-cess Capto Q 1 L column by Cytiva	C-202	5	RT	0.11	ID = 8 cm h = 5.74 cm
Diafiltration	Pall Delta Cadence Inline Diafiltration Module	F-203	2	RT	8	A = 0.11m^2
Cation Exchange Chromatography	ReadyToPro-cess Capto S 1 L column by Cytiva	C-203	5	RT	0.25	ID = 8 cm h = 14.8 cm
Viral Filtration	Virosart HF Mid-Scale Module sterile filter	F-204	2	RT	65	A = 200 cm^2
Final Ultrafiltration	3-section SPTFF system with	F-205	2	RT	8	A = 0.1 m^2

	Pellicon® cassettes					
Final Diafiltration	Pall Delta Cadence Inline Diafiltration Module	F-206	2	RT	4	A = 0.11 m^2
Formulation and Fill	Weinas VD 160	FF-201	2	RT		

5.5.3 Miscellaneous Equipment Table

Table 5.5.3. Miscellaneous Equipment Table.

Operation	Unit	Unit No.	Quantity	Temperature (°C)	Pressure (psi)	Size
Autoclave	Custom Autoclave	A-1	1	-	-	-
Pump (See 4.3.1)						
Heat Exchanger (See 4.3.3)	Stainless Steel Counter Current Double-pipe Heat Exchanger	HE-201	1	n/a	n/a	Heat transfer area = 32.89 cm^2
WFI (See 4.4)	MECO Masterpak ™ Ultra system	WFI-1	1	Varies	n/a	n/a
Tanks (See 4.3.2)	Holding and Mixing Tanks	TH-1	1	RT	n/a	
		TM-1	1	RT	n/a	
		TE-1	1	RT	n/a	1500 L
		TH-2	1	RT	n/a	500 L
		TM-2	1	RT	n/a	50 L
		TH-3	1	RT	n/a	1700 L
		TM-3	1	RT	n/a	170 L
		TH-4	1	RT	n/a	22,000 L
		TM-4	1	RT	n/a	2200 L
		TH-5	1	RT	n/a	850 L
		TM-5	1	RT	n/a	85 L
		TH-6	1	RT	n/a	18,000 L
		TM-6	1	RT	n/a	1800 L

		TH-8	1	RT	n/a	5000 L
		TH-8	1	RT	n/a	3000 L
		TH-9	1	RT	n/a	
		TM-9	1	RT	n/a	
		TW-1	1	RT	n/a	5000 L
		TW-2	1	RT	n/a	5000 L
		TW-3	1	RT	n/a	5000 L
		TW-4	1	RT	n/a	5000 L
		TW-5	1	RT	n/a	5000 L

5.6 Material and Energy Balances

5.6.1 Upstream Material Balances

Table 5.6.1. Mass Balances for Upstream Fed-Batch Processes.

Description	Material	Initial Amount	Final Amount	Unit
25 L Wave Reactor (WR-1)	Pembrolizumab Cells	- $2.25 * 10^8$	45.23 $1.26 * 10^{10}$	g # of cells
400 L Reactor (R-101)	Pembrolizumab Cells	45.23 $1.26 * 10^{10}$	14310.00 $3.05 * 10^{10}$	g # of cells
900 L Reactor (R-102)	Pembrolizumab Cells	14310.00 $3.05 * 10^{10}$	41133.00 $3.68 * 10^{10}$	g # of cells
1500 L Reactor (R-103)	Pembrolizumab Cells	41133.00 $3.68 * 10^{10}$	- $3.68 * 10^{10}$	g # of cells

Table 5.6.2. Mass Balances for Upstream Continuous Processes.

From		R-103	F-101	F-101	F-102	F-102	F-102	F-102	F-102 F-101	F-103	F-103	F-103
To	R-103	F-101	F-102				R-103		F-103	F-102		
Stream	PRI-1	TF1I-1	TF1O-1	TF1O-2	TF2O-3	TF2O-2	PRI-3	TF2O-1	TF3I-1	TF2I-2	TF3O-1	TF3O-2
Cells (g/min)	0	79.0	78.9	0.0790	0.0789	78.12	57.8	20.3	0.282	0.000224	0	0.282
Pembrolizumab (g/min)	0	4.90	0.882	4.02	0.723	0.159	0.117	0.0413	4.74	0.00376	0.000979	4.73
Substrate (g/min)	36.0	0.625	0.113	0.513	0.0923	0.0203	0.0150	0.00527	3.36	2.66	0.632	0.0610
Total Flow Rate (L/min)	3.1	4.55	10.8	3.73	8.87	1.95	1.45	0.497	12.6	10	2.37	0.229
Concentration (g/L)												
Cells	0	17.4	7.29	0.0212	0.00890	40.1	39.9	40.9	0.0125	0	0	0.689
Pembrolizumab	0	1.08	0.0815	1.08	0.0815	0.0815	0.0810	0.0830	0.376	0.000376	0.000412	20.6
Substrate	11.6	0.137	0.0104	0.137	0.0104	0.0104	0.0103	0.0106	0.0480	0.0480	0.0480	0.0480

5.6.2 Downstream Material Balances

Table 5.6.1. Downstream Mass Balance Table

Mass Flows (g/min)														
Stream	DF1I-1	PCN-1	PCNI-2	PCGI-1	PCGI-2	PCHI-1	PCHO-1	PCWI-1	PCWI-2	PCWO-1	PCVI-1	PCVI-2	DF2I-1	DF2I-2
Pembrolizumab	4.73	4.73		4.73		4.69	0.33	4.36		0.51	3.85		3.85	
$ZnCl_2$			0.62	0.62		0.62	0.37	0.25		0.23	0.02		0.02	
PEG					34.87	34.87	20.72	14.15		12.99	1.16		1.16	
HEPES									5.39	3.84	1.56		1.56	
Glycine												13.77	13.77	
bis-Tris Propane														10.87
Total Flow Rate (L/min)	0.229	0.229	0.046	0.275	0.183	0.458	0.275	0.183	0.452	0.452	0.183	0.092	0.275	1.925

Concentrations													
Stream	DF1I-1	PCN-1	PCNI-2	PCGI-1	PCGI-2	PCHI-1	PCHO-1	PCWI-1	PCWI-2	PCWO-1	PCVI-1	PCVI-2	DF2I-1	DF2I-2
Pembrolizumab (g/L)	20.65	20.65		17.20		10.24	1.21	23.82		1.13	21.00		14.00	
$ZnCl_2$ (mM)			100.00	16.67		10.00	10.00	10.00		3.68	0.90		0.60	
PEG (wt%)					17.50	7.00	6.98			2.76	0.60		0.004	
HEPES (mM)									50.00	35.62	35.62		23.74	
Glycine (mM)												2.00	0.67	
bis-Tris Propane (mM)														20.00
Total Flow Rate (L/min)	0.229	0.229	0.046	0.275	0.183	0.458	0.275	0.183	0.452	0.452	0.183	0.092	0.275	1.925

Mass Flows (g/min)												
Stream	AXI-1	DF2O-1	AXI-2	AXI-3	AXI-4	AXO-1	AXO-2	AXO-3	DF3I-1	DF3I-2	CXI-1	DF3O-1
Pembrolizumab	3.72	0.13				0.17			3.55		3.43	0.12
$ZnCl_2$		0.02										
PEG		1.16										
HEPES		1.56								22.94	3.28	19.66
Glycine		13.77										
bis-Tris Propane	1.55	9.32	1.55	1.55	1.55	1.55	1.55	1.55	1.55			
Acetate Buffer @ pH 2.9				1.62		1.62						
NaOH					11.00	11.00						
Total Flow Rate (L/min)	0.275	1.925	0.275	0.275	0.275	0.275	0.275	0.275	0.275	1.925	0.275	1.925

Concentrations												
Stream	AXI-1	DF2O-1	AXI-2	AXI-3	AXI-4	AXO-1	AXO-2	AXO-3	DF3I-1	DF3I-2	CXI-1	DF3O-1
Pembrolizumab	13.52	0.07				0.61			12.90		12.46	0.06
$ZnCl_2$ (mM)		0.09										
PEG (wt%)		5.90E-04										
HEPES (mM)		3.40									50.00	
Glycine		95.29										
bis-Tris Propane		20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00			
Acetate Buffer (mM)				100.00		100.00						
NaOH (M)					1.00	1.00						
Total Flow Rate (L/min)	0.275	1.925	0.275	0.275	0.275	0.275	0.275	0.275	0.275	1.925	0.275	1.925

Mass Flows (g/min)													
Stream	CXI-2	CXI-3	CXI-4	CXO-1	CXO-2	CXO-3	VFI-1	UFI-1	DF4I-1	UFO-1	FFI-1	DF4I-2	DF4O-1
Pembrolizumab				0.16			3.27	3.24	3.11	0.13	3.00		0.11
HEPES	3.28	3.28	3.28	3.28	3.28	3.28	3.28	3.28	1.43	1.85			1.43
Acetate Buffer @ pH 2.9		1.62		1.62									
NaOH			11.00	11.00									
Total Flow Rate	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.120	0.155	0.120	0.840	0.840
Concentration													
Stream	CXI-2	CXI-3	CXI-4	CXO-1	CXO-2	CXO-3	VFI-1	UFI-1	DF4I-1	UFO-1	FFI-1	DF4I-2	DF4O-1
Pembrolizumab				0.57			11.89	11.78	25.90	0.85	25.00		0.13
HEPES (mM)	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00			7.15
Acetate Buffer (mM)		100.00		100.00									
NaOH (M)			1.00	1.00									
Total Flow Rate	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.275	0.120	0.155	0.120	0.840	0.840

5.7 Plant Location

Our manufacturing facility will be located in Norristown, Pennsylvania. A 49.5 acre lot will be purchased approximately 4 miles from Merck's master cell bank located in West Point, Pennsylvania. This close proximity to Merck's master cell bank will reduce costs related to working cell bank acquisition and storage while transporting. With Merck's Pembrolizumab patent expiring in 2028, we intend to work as a Merck contractor beginning production in 2025 to account for the projected increase in demand. Opportunities for expansion by offering new products and services of interest to Merck and other nearby facilities can also be considered. Our facility will introduce many job opportunities to the community and attract bright talent from local colleges and universities.

5.8 Process Economics

5.8.1 Plant Capital Costing

Capital costs are those purchases pertaining to upfront investment in construction of a new or existing chemical manufacturing plant and are one-time costs (Turton et al., 2018).

For this design capital costs include land, buildings, main equipment, piping and other needs listed in Table 5.8.1.1. According to Peters and Timmerhaus (1991) Capital costs can be estimated using budget allocations dependent on a calculated purchased main equipment cost as tabulated in Table 5.8.1.2 which totaled approximately $13.2 million and a Lang Factor used to estimate the total cost according to equation 5.8.1. A Lang Factor of 4.74 was chosen due to the facility being a fluid processing plant. Subsequent categories are given an estimated percentage of this total cost based upon the industry and knowledge of the designed chemical plant. For example, land cost was researched according to our plant location and thus was over the recommended range while piping was at the bottom of its range due to our facility's choice of single-use equipment throughout the process.

Additionally, $1 million was added to the Fixed Capital Investment to account for FDA validation of the newly constructed chemical plant to get process validation to ensure safety and quality of product and ensure efficacious drug product can be sold. However, due to the use of precipitation chromatography in our process which differs from traditional production methods longer validation could be required, but resources on this timeframe were unable to be located.

The prices for main capital equipment were gathered through product quotes, previous capstone groups, similar products, or custom estimates tabulated in Table 5.8.1.2.

Table 5.8.1.1 Fixed Capital Costs.

Capital Cost = (Lang Factor)*(Sum of Purchased Costs of All Major Equipment)
Equation 5.8.1. Estimating Capital Costs Based on Major Equipment Costs (Turton et al., 2018).

Component	Recommended Range %	Our Percentages	Cost
Direct Costs			
Purchased Equipment	15-40	21	$13,208,694
Equipment Installation	6-14	8	$5,008,737
Instrumentation and Controls	2-8	6	$3,756,553
Piping (Installed)	3-20	3	$1,878,276
Electrical (Installed)	2-10	7	$4,382,645
Buildings (inlcluding services)	3-18	15	$9,391,381
Yard Improvements	2-5	2	$1,252,184
Service Facilities (installed)	8-20	9	$5,634,829
Land	1-2	6	$3,500,000
Indirect Costs			
Engineering and Supervision	4-21	9	$5,634,829
Construction Expense	4-16	6	$3,756,553
Contractor's Fee	2-6	3	$1,878,276
Contigency	5-15	5	$3,130,460
Total Fixed Capital Investment			$62,609,208
Total with Validation			$63,609,208

Table 5.8.1.2. Main Equipment Costs.

Process	Upstream/Downstream	Equipment	One Time Quantity	Cost	Total Cost	Seller
Master Cell Bank	Upstream	Merck Licensed Cell Bank	1	$600,000	$600,000	Merck
WCB Storage	Ancillary	VIP ECO Model MDF-DU702VH-PA Freezer	2	$20,927	$41,854	Henry Schien
WCB Thawing	Ancillary	Thermo Fisher Precision GP 02 Water Bath	2	$1,184	$2,368	Thomas Scientific
Ferm	Upstream	WAVE 25 L Rocker Reactor	2	$35,000	$70,000	Cytiva
Ferm	Upstream	Xcellerex XDR 50/2000	6	$20,000	$120,000	Cytiva
TFF	Upstream	Repligen KrosFlo KR2i	6	$8,616	$51,696	Repligen
Precip Chrom	Upstream	Custom	1	$200,000	$200,000	Estimate
Washing	Upstream	StaMixCo HT-50-1.50-6-C	2	$2,500	$5,000	StaMixCo
Viral Inactivation	Upstream	Custom	1	$70,000	$70,000	Estimate
Diafiltration	Downstream	Pall Delta Cadence Inline Diafiltration Module	6	$16,700	$100,200	Pall
AEX/CEX	Downstream	ÄKTA ready single-use system	10	$277,000	$2,770,000	Cytiva
UF	Downstream	Sigma 3-section SPTFF system with Pellicon®	2	$5,525	$11,050	Millipore Sigma
Form/Fill	Ancillary	SA25 Aseptic Filling Workcell	1	$7,000,000	$7,000,000	
Form/Fill	Ancillary	Sartorius 50 L Palletank	6	$4,000	$24,000	Sartorius
Form/Fill	Ancillary	Pall Allegro Mixer	4	$48,229	$192,916	Pall
Pumps	Ancillary	Masterflex Ismatec	26	$3,671	$95,446	VWR
Pumps	Ancillary	Masterflex I/P	28	$1,938	$54,264	VWR
Autoclave	Ancillary	Custom	1	$564,000	$564,000	
Heat Exchange	Ancillary	SS Counter Current Double-Pipe HE	1	$1,000	$1,000	
WFI	Ancillary	MECO Masterpak Ultra System	1	$554,900	$554,900	Millipore Sigma
Holding Tanks	Ancillary	50 L	1	$20,000	$20,000	
Holding Tanks	Ancillary	100 L	2	$20,000	$40,000	
Holding Tanks	Ancillary	200 L	6	$20,000	$120,000	
Holding Tanks	Ancillary	500 L	2	$20,000	$40,000	
Holding Tanks	Ancillary	1000 L	2	$20,000	$40,000	
Holding Tanks	Ancillary	2000 L	11	$20,000	$220,000	
Holding Tanks	Ancillary	5000 L	8	$20,000	$160,000	
Holding Tanks	Ancillary	20,000 L	2	$20,000	$40,000	
			Total:	$9,595,190	$13,208,694	

5.8.2 FDA Approval and Validation Costs

Keytruda is already an FDA approved product. This pembrolizumab manufacturing facility need not include upfront costs for clinical trials and only process validation of the manufacturing process to produce efficacious and safe product according to critical quality attribute will be required. The approach to process validation as described by the FDA is in three stages: process design, process qualification, and continued process verification (U.S. Food and Drug Administration, 2011). Process design is the defined manufacturing process in the stated facility which will be based upon previous Merck development and scale-up during Keytruda's

initial commercialization, these will have been accumulated and documented followed by establishing process control strategies in accordance with FDA guidance documents. For the validation process to meet CGMP requirements the guidelines state, "[t]here shall be written procedures for production and process control designed to assure that the drug products have the identity, strength, quality, and purity they purport or are represented to possess..." (U.S. Food and Drug Administration, 2011). We as manufacturers will impose operations and controls that result in a product to meet those attributes from the design to meet the regulations and guidelines.

Process qualification is where the process design of this optimized facility will be evaluated to ensure that it meets the stated critical processing parameters (CPPs). According to the FDA the qualification process is in four steps. First, the selection of utilities and equipment and verification of these utility systems with design specifications. Second, process performance qualification (PPQ), the verification that these operate in the proper CPP operating ranges. Third, the PPQ protocol that defines operating conditions, data collection, testing methodology, and criteria for acceptance, and risk assessment in addition to several other technical details. Finally, these study activities will be planned, cumulative data recorded, and clear conclusions conveyed that indicate the process met the established criteria and is in a state of control. Justification for approval and release of lots will be determined by this compilation of data and documents in addition to appropriate review and approvals by the established quality unit. The FDA will review these documents and protocols prior to the beginning of operation of the protocol. Following the conclusion and acceptance of these commercial manufacturing can commence.

Continued process verification is maintaining ongoing compliance with the FDA following initial validation to ensure routine production maintains the same level of quality and control throughout the facility's lifetime. This ensures the process remains in a state of control in the validated state. The quality unit will maintain a system or systems for detecting departures

from process design and maintaining CGMP requirements. This will be accomplished by monitoring process trends, quality of raw and other materials, in-process materials, and finished product. The collected information will be used by statisticians or trained individuals to verify the critical quality attributes (CQAs) are controlled. This continued data collection will provide a means to detect process variability and evaluate areas for improvement. The PPQ protocol will be renewed annually and a related report is mandated to be delivered no later than 60 days following the initial approval date. This will ensure the facility will remain in compliance during its lifetime.

It is estimated that the initial validation process (steps 1-2) will take approximately 1.5 years to complete considering the contracting status and use of single-use equipment incorporated in the design. Additionally, a capital cost of $1 million was factored into the capital investment cost (Kilduff et al., 2022).

5.8.3 Operating Expenses

To produce pembrolizumab continuously there are numerous annual expenses incurred to produce drug product such as disposable or single-use equipment, chemicals, raw materials, labor, and other such needs to keep the plant operational and/or maintain high levels of production or product quality depending on the unit of interest. Table 5.8.3.1 summarizes the required annual equipment for operation and the associated costs. Table 5.8.3.2 summarizes the required annual raw chemicals and costs. The majority of the costs are the highlighted items including single-use ready-to-use chromatography columns, HF filters, chemicals for buffer formulation, and vials for filling. These costs were sourced from various vendors and are USP grade or higher.

Labor costs were estimated using equation 5.8.3.1 to determine the number of required operators, where P is the number of particulate steps, and N_{np} are the number of non-particulate

steps. It was determined that there are 6 particulate steps: inoculation trains (2), buffer formulation (2), and buffer autoclaving (2). There are 15 particulate steps: 2, 4 column IEX lines (8); 3, 2 stage formulation lines (6), and a filling unit (1). Thus using the equation it was determined that 35 operators were required and due to the 24/7 operating nature of this facility, the need for vacation, sick days, and personal days 4.5 times the 35 operator number is required. Thus, 158 full-time operators will be employed by this facility as of 2023 the glassdoor average salary for a pharmaceutical operator is $69,817. There are 13 campaigns at 25 days each; it is expected that 325 days of necessary operation are required, so each operator will be paid for a full year's salary due to the expectation of issues or unplanned shutdowns. Thus the labor costs for operators total $11,031,086.

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

Equation 5.8.3.1. Labor Costs.

Utilities for this manufacturing facility include steam, compressed air, chilled ethylene glycol, and power. For these calculations water was included in the chemicals list instead of utilities. Air conditioning and electricity for layout specific needs such as lighting and other operator/employee needs were not considered and deemed outside the scope of this project. The water requirements and cost are summarized in Table 5.8.3.2. These costs relative to others in the analysis are nearly negligible so a cost of $4000 was assumed for the calculations.

Using the Turton et al. (2018) method of operating cost calculation a number of other expenses were calculated derived from the FCI, labor costs (C_OL), utilities (C_UT), waste treatment (C_WT), and raw materials (C_RM) (Turton et al., 2018). Thus the total operating cost is approximately $128,240,000 and is summarized in Table 5.8.3.3.

Table 5.8.3.1 Annual Equipment Requirements and Costs

Process	Upstream/Downstream	Equipment	Annual Quantity	Cost per Unit	Total Cost	Seller
Ferm	Upstream	WAVE single use bag	26	$333	$8,658	Cytiva
Ferm	Upstream	Xcellerex XDR 50/2000	0	$20,000	$120,000	Cytiva
TFF	Upstream	Repligen KrosFlo KR2i	0	$8,616	$51,696	Repligen
Depth Filtration	Upstream	Millistak+ A1HC Pod Depth Filter	26	$222	$5,772	Millipore Sigma
Washing	Upstream	MiniKros hollow fiber membrane module with 0.2 µm	1	$944	$944	Repligen
Washing	Upstream	Sartorius double-layer Sartoclear depth filter	13	$1,244	$16,172	Sartorius
AEX	Downstream	ReadyToPro-cess Capto Q 1 L column by Cytiva	65	$12,589	$818,285	Cytiva
CEX	Downstream	ReadyToPro-cess Capto Q 1 L column by Cytiva	65	$12,589	$818,285	Cytiva
Viral Filtration	Downstream	Virosart HF filter	1950	$733	$1,429,350	Sartorius
Form/Fill	Ancillary	Sartorius 50 L Palletank	0	$4,000	$24,000	Sartorius
Form/Fill	Ancillary	50 L Single Use Bags	975	$393	$383,175	Sartorius
Form/Fill	Ancillary	200 L Single Use Bags	975	$531	$517,238	Pall
Form/Fill	Ancillary	PharmD Peristaltic Tubing	2000	$10	$20,000	Dupont
Form/Fill	Ancillary	4 mL Vials	7722000	$0.30	$2,316,600	Thomas Scientific
50 L CSTR Bag	Ferm	Single-Use Mixer BioProcess Containers	13	$393	$5,109	Cytiva
100 L CSTR Bag	Ferm	Single-Use Mixer BioProcess Containers	26	$495	$12,870	Cytiva
200 L CSTR Bag	Ferm	Single-Use Mixer BioProcess Containers	65	$531	$34,483	Cytiva
500 L CSTR Bag	Ferm	Single-Use Mixer BioProcess Containers	13	$531	$6,897	Cytiva
2000 L CSTR Bag	Ferm	Single-Use Mixer BioProcess Containers	26	$531	$13,793	Cytiva
				Total Cost	$6,603,326	

Table 5.8.3.2 Annual Chemical Requirements and Costs

Process	Material	Quantity Per Campaign (kg or L)	Price/Unit ($)	Cost per Campaign ($)
Fermentor	Process Water	20,952.0	$0.003	$63.48
	Media (L-Glutamine + Glucose)	39,858.9	$85.083	$3,391,318.05
Precipitation	0.1M ZnCl2	22.8	$97.000	$2,214.51
	17.5 wt% PEG	288.2	$48.000	$13,835.04
	Process Water	3,033.8	$0.003	$9.19
Washing	50mM HEPES	48.5	$785.000	$38,048.95
	2M Glycine	123.8	$71.350	$8,829.56
	Process Water	11,845.8	$0.003	$35.89
Diafiltration to AEX	20mM bis-Tris Propane	290.8	$1,170.000	$340,189.09
	Process Water	69,480.0	$0.003	$210.52
AEX	0.1M Acetate	2.7	$38.680	$104.64
	0.1M NaOH	2.0	$21.460	$42.47
	bis-Tris Propane	9.0	$1,170.000	$10,496.02
	Process Water	4,617.1	$0.003	$13.99
Diafiltration to CEX	50mM HEPES	827.9	$785.000	$649,865.55
	Process Water	69,480.0	$0.003	$210.52
CEX	0.1M Acetate	2.7	$38.680	$104.71
	0.1M NaOH	2.0	$21.460	$42.49
	50mM HEPES	25.6	$785.000	$20,062.77
	Process Water	4,620.0	$0.003	$14.00
Final Diafiltration	Process Water	69,480.0	$0.003	$210.52
Formulation/Fill	L-Histidine	3.3	$1,120.000	$3,749.76
	Sucrose	151.2	$47.800	$7,227.36
	Polysorbate - 80	0.4	$98.800	$42.68
			Total Per Campaign:	$4,486,941.78
			Annual Cost:	$58,330,243.12

Table 5.8.3.3 Total Annual Operating Cost

Direct Costs	Nomenclature	Cost
Raw Materials	C_RM	$64,933,569
Waste Treatment	C_WT	$150,000
Utilities	C_UT	$4,000
Operating Labor	C_OL	$11,031,086
Direct and supervisory and clerical labor	0.18*C_OL	$1,985,595
Maintenance and repairs	0.06*FCI	$3,816,553
Operating supplies	0.009*FCI	$5,724,829
Laboratory charges	0.15*C_OL	$1,654,663
Patents and royalties	0.03*C_OM	$3,839,495
Fixed Costs		
Depreciation	0.1*FCI	$6,360,921
Local taxes and insurance	0.032*FCI	$2,035,495
Plant overheard costs	0.708*C_OL+0.036*FCI	$10,099,940
General Costs		
Administration	0.177*C_OL+0.009*FCI	$2,524,985
Distribution and selling costs	0.11*C_OM	$14,078,147
Total Operating Cost		$128,239,277

5.8.4 Economic Analysis using Discounted Cash Flow

An economic analysis using discounted cash flow was performed to determine the go/no go decision on the design of this facility. Capital costs, operating costs, and expected revenues of the pembrolizumab finished product were utilized in this analysis. As stated previously, FDA validation is estimated at 1.5 years for this analysis, but this time frame is expected to be longer due to the use of precipitation chromatography in the design. Under this assumption it is assumed construction will take 1 year and validation will happen simultaneously for the 1.5 years and validation materials will be manufactured in the 6 months prior to year 0 for quality testing. The plant will be operational for 15 years following validation for a total lifetime of 16.5 years following which the equipment will be salvaged and the plant shutdown. The important values for economic analysis are tabulated in Table 5.8.4.1.

Table 5.8.4.1. Important Values for Economic Analysis.

Capital Cost	$62,609,208
Validation Cost	$1,000,000

The capital cost, validation cost, annual operating costs were all discussed in previous sections. The value for annual revenue was determined by multiplying the expected manufactured dose number of 7 million times our sell price of $2817.52 chosen from the current dose price of $10,897.12 to be approximately 26% of the original cost in order to keep up with generics as the pembrolizumab comes off patent and in order to increase product availability to lower income populations (Cost Information and Financial Help with Keytruda, n.d.). The tax rate of 30% was determined from the federal income tax rate of 21% and the 8.99% corporate tax rate for the state of Pennsylvania where the plant is located (Corporate Net Income Tax, n.d.).

To perform the discounted cash flow analysis a discount rate of 15% was chosen for several reasons such as the stability of Keytruda in the marketplace, its place as an established drug with growing indications, and Merck's position as a public large scale company. In the 1.5 years prior to year 0, the capital cost was taken as an expense of $62.6 million and in the year prior to year 0 half of the operating cost was taken as an expense for the production of quality testing materials, $76.9 million. In the years following a constant cash flow of $12.3 billion dollars is assumed before the discount rate. This net cash flow post-tax is tabulated in Table 5.8.4.2 along with the present value (PV) of the cash flow and the cumulative discounted cash flow. The discounted cash flow can be seen graphically in Figure 5.8.4.1. The equation for discounted cash flow can be seen below in Equation 5.8.4.1 where TCF_t is taxed cash flow in year t, DCF_t is discounted cash flow in year t, and cumulative is the summed values.

Table 5.8.4.2. Discounted Cash Flow Analysis.

Year	Net Cash Flow after-tax	PV of Cash Flow	Cumulative Discounted Cash Flow
-1.5	-$62,609,208.42	-$79,893,201	-$79,893,201
-1	-$76,943,565.97	-$90,521,842	-$170,415,044
0	$12,335,495,706	$12,335,495,706	$12,165,080,663
1	$12,335,495,706	$10,485,171,350	$22,650,252,013
2	$12,335,495,706	$8,912,395,648	$31,562,647,661
3	$12,335,495,706	$7,575,536,301	$39,138,183,962
4	$12,335,495,706	$6,439,205,856	$45,577,389,817
5	$12,335,495,706	$5,473,324,977	$51,050,714,794
6	$12,335,495,706	$4,652,326,231	$55,703,041,025
7	$12,335,495,706	$3,954,477,296	$59,657,518,321

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

Equation 5.8.4.1. Discounted Cash Flow in Year.

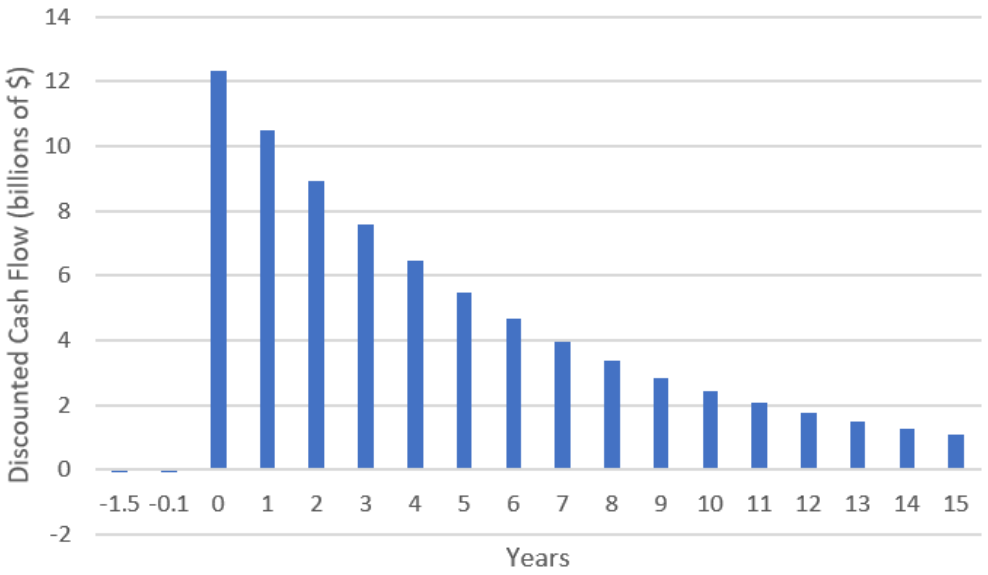


Figure 5.8.4.1. Cumulative Discounted Cash Flow Analysis.

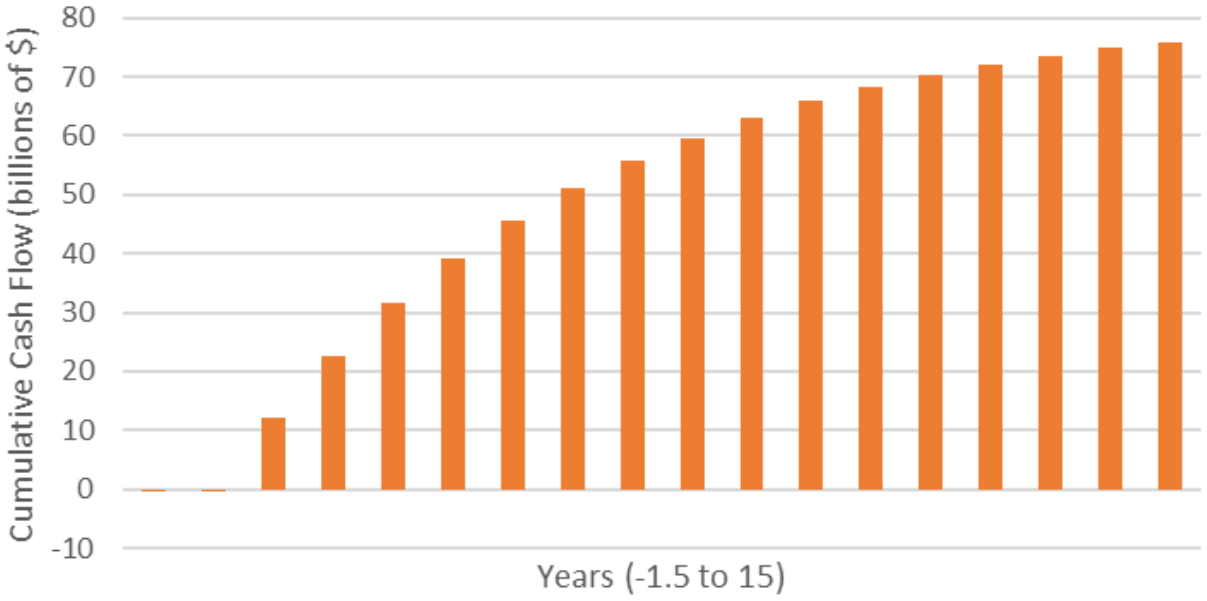


Figure 5.8.4.2. Discounted Cash Flow over Plant Lifetime.

Following the discounted cash flow analysis, net present value of the facility and internal rate of return were calculated using equations 5.8.4.2 and 5.8.4.3 respectively. NPV is net present

cash flow, CCF is cumulative cash flow, i is the discount rate of 15%. P is the principle, the initial capital investment, i is the discount rate of 15%, and R is the internal rate of return (IRR). IRR is indicative of the discount rate necessary for the facility to break even. Our NPV was found to be $61.4 million and our IRR to be 1132%. These values indicate that the project is highly profitable.

$$NPV_{15} = CCF_{15}(1 + i)^{15}$$

Equation 5.8.4.2. Net Present Value at Year 15.

$$P(1 + R)^{15} - P(1 + i)^{15} = NPV_{15}$$

Equation 5.8.4.3. Internal Rate of Return at Year 15.

5.8.5 Risk Analysis

This facility is anticipating a drop in price of the drug Keytruda upon its patent expiration in 2028 and the rise of generics as competition and thus a more efficient facility needed to be developed. Thus in comparison to the current price of Keytruda this facility is well positioned to succeed based on the economic analysis and margins attained as seen by the economic analysis. Additionally, as this facility will operate as a Merck contractor it is assumed that distribution and the brand name will allow for seamless entry into the market upon validation. The current output of Keytruda does not meet the expected demand currently so it is not expected for any product to go unsold even with the 20% market share assumption. Several other assumptions were made to determine the economic viability of this facility, a discount rate of 15% was assumed given the construction and validation time assumed and popularity of pembrolizumab as a product in the market. If some of these assumptions were to vary in practice such as a longer validation period of 3-5 years the success of the product could differ as well as the incurred costs prior to the production of commercial product.

In analyzing a more conservative economic estimate such as a discount rate of 50% the NPV of the facility drops to $60.1 million and the IRR to 719%. However, even with the reduced future cash flows the plant is still highly profitable and would be worth moving forward with. The NPV of these cash flows are depicted in Figure 5.8.5.1, the initial cash flows from the first few years of operation make up the greatest portion of the cumulative profits and later years provide very few contributions.

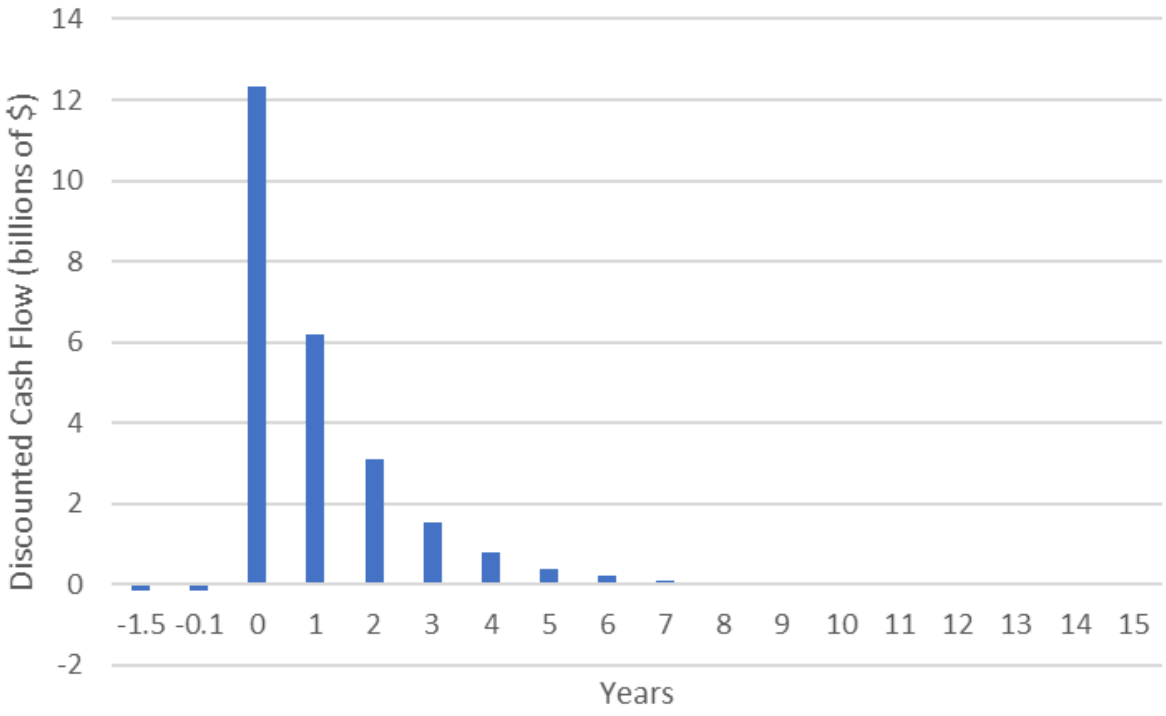


Figure 5.8.5.1. Discounted Cash Flows with a 50% Discount Rate.

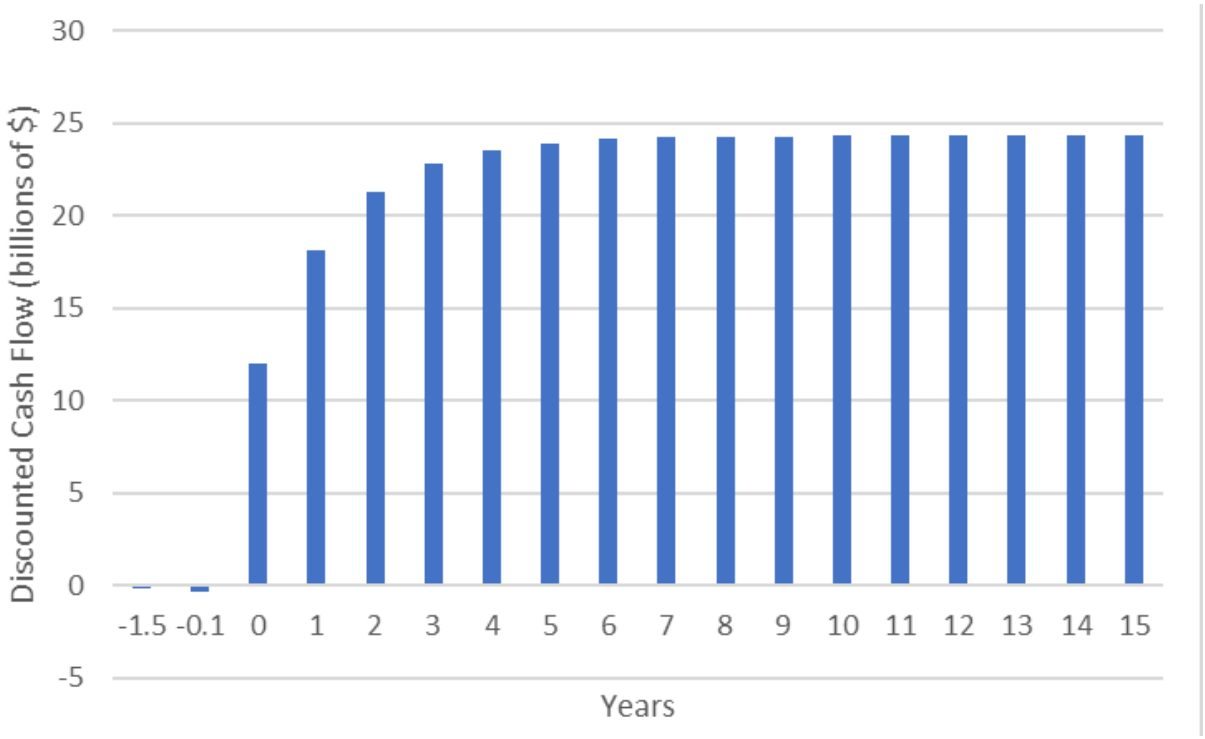


Figure 5.8.5.2. Cumulative Discounted Cash Flow at 50% Discount Rate.

5.9 Quality Control

In order to conform with regulatory requirements of the United States and other regulatory bodies such as the FDA to remain in compliance and abide by U.S. and international pharmacopeias a quality control (QC) unit will be in use at this facility to ensure current Good Manufacturing practices (cGMP). QC will ensure patients are receiving safe and effective medicine from this facility. As stated by the FDA a QC unit is responsible for approving and rejecting all "components, drug product containers, closures, in-process materials, packaging material, labeling, and drug products, and the authority to review production records to assure that no errors have occurred or, if errors have occurred, that they have been fully investigated" (U.S Food and Drug Administration, 2023). To meet these requirements a variety of labs, equipment, and inspection will be required. Also standard operating procedures (SOP) will be adapted from other Merck facilities to meet the same quality control attributes (CQA) for testing

of various criteria at each step in the manufacturing process from raw materials to finished product to ensure potency, purity, and chemical and physical properties.

The CQAs in the manufacturing of pharmaceutical products and mAbs specifically can be divided into several categories: physical, chemical, biological, or microbiological (Reason et al., 2014). Each of these have specific limits and ranges which must be met to continue forward processing. The CQAs are vital to both the efficacy and safety of such complex molecules (Reason et al., 2014). The equipment required involves chromatography, mass spectrometry, spectroscopy, in addition to standard analytical scales and tracking softwares to meet time or weight requirements (Pharmaceutical Quality Control Testing, n.d.).

Specific analytical methods for characterizing mAb CQAs at this facility will include capillary zone electrophoresis, isoelectric focusing, ion exchange, size exclusion chromatography (SEC), reversed phase (RP), ultraviolet (UV), fluorescence, LC-MS, amino acid analyzer, peptide mapping, GC-MS, immunoassays and cellular assays, IEX chromatography, SDS-polyacrylamide gel electrophoresis, and HPLC chromatography (Alhazmi, 2023). These characterization techniques will confirm structural and physicochemical, immunological, biological, purity, and quantification of the mAb product (Alhazmi, 2023). PCR testing will be performed for the detection of host-cell impurities. Results of these methods will be compared to a lot verified with proper characteristics through successful testing and release according to FDA regulations. Any detections of impurities or failed lots will be investigated to determine the source and cause of a failed batch release to optimize the process and prevent future issues at all points of testing whether in-process or for the final product.

Necessary tests will be performed hourly before and after each unit operation for in-process tests as well as sampling for continuous processes such as perfusion or fed-batch operations to ensure no contamination within those processes. Rigorous and frequent testing and

compilation of data is necessary for batch release and continued validation from the FDA as well as for internal investigations into batch related issues or product recalls. Also process monitoring will be in place to monitor flows, temperatures, pressures, and other process variables that must be kept within critical or non critical process parameters for optimum product performance and safety.

As specified elsewhere in this document disposal units and kill tanks have been designed in the event of nonconforming materials which need to be immediately disposed of to prevent further downstream contamination and for analysis and investigation. Also, all material produced between the last successful test and the failed test will be sent to waste streams as well. Additionally, backup fermentors, pumps, chromatography units, and other equipment are in place to resume operations and allow resumption of full capacity and production. This will prevent significant disruption of operations. All employees will be trained to identify these scenarios and investigate the issues to maintain GMP requirements.

6. Regulatory, Safety, Health, and Environmental Considerations

Our pembrolizumab production facility and supply chain will adhere to current Good Manufacturing Practices (cGMP) to meet FDA regulations and ensure the safety of the patients receiving our treatment. This entails strict documentation of contaminants, operating parameters, storage conditions, and quality deviations to ensure that the product meets the strength, quality, and purity advertised to the consumer. Likewise, cGMPs demand that raw materials meet quality standards and that both the equipment and people on the production floor are appropriately contaminant-free so as to not compromise the product. These protocols will be enforced through extensive training modules, standard operating procedures (SOPs), formal cGMP documentation, and a top-down culture of safety and integrity (FDA, 2021).

Reagent safety concerns in this process arise from caustic chemicals, mainly NaOH, NaCl, HCl, and bis-Tris propane. These buffers can cause severe burns, as well as eye and organ damage if workers come into contact with them. These buffers are mainly used in the downstream process, specifically in precipitation and AEX and CEX chromatography, as well as in diafiltration steps associated with these processes. Workers will wear PPE as designated by OSHA and undergo the appropriate training to handle contact with these chemicals, minimizing exposure to these chemicals through physical touch, inhalation and ingestion. Floor drains, chemical showers, and eyewash stations will be implemented throughout the facility to minimize the outcomes of a chemical exposure. Non-slip flooring will also be used on the manufacturing floor.

This facility will output both solid and liquid waste warranting environmental impact consideration. Specifically, single-use bags and piping will be used in each step of the inoculum train and will be a major source of plastic solid waste. Nonetheless, elimination of the plastic materials is more energy efficient on an industrial scale than the extra heat treatment and solvents that traditional process methods would entail according to Flanagan et al. (2014). To sanitize the bioreactor through the single-use method, an electric heater can be used instead of steam. According to the same study, clean-in-place and sanitize-in-place systems, the WFI system, media production, and HVAC made cleaning one of the most environmentally demanding steps. The other two steps of the process with the largest environmental impact will be maintenance of the bioreactor and precipitation chromatography - both require significant fluid input and output and temperature maintenance (Flanagan et al., 2014). NaOH and HCl used in the disposal process and the reagents listed above will pose the greatest liquid environmental hazard. Biological materials remaining in the liquid waste will be inactivated using 1.0 M NaOH. This

mixture of buffers will then be neutralized to a pH of 7.0 with 1.0M HCl and further treated as necessary.

7. Social and Ethical Considerations

For the well-being of the company's reputation, the facility, the employees, the surrounding community, the product quality, and the drug patients, it is important to recognize and mitigate social and ethical concerns regarding the pembrolizumab production plant and the impact on the surrounding community.

As mentioned in the safety section (Section 6), all employees, including outside contractors, will undergo extensive safety training both during the onboarding process and at regular intervals during their employment. Our business will uphold a healthy company culture that inspires respect among employees at all levels, integrity, and safe practices. Employees will be offered competitive salaries, insurance and retirement benefits, and services to facilitate location changes.

Materials, equipment, processes, products, and distribution methods will be ethical or will be sourced ethically as well as held to quality standards enforced by the FDA and CDC to patient safety and product consistency. Public concerns regarding the safety of the drug product can be freely reported via an anonymous feedback form and will be promptly addressed.

Construction of the plant may be somewhat disruptive to the surrounding community due to noise and traffic; however, no harm or substantial impact will be made by the plant during its construction or ongoing use. Our disposal system, detailed in Section 4.6, and employment of single-use systems will ensure that environmental damages are limited to a minimum. Likewise, the efficiency of the continuous production will lower the required amount of natural resources input to the process.

In addition to lowering environmental harm, single-use and continuous production will drive down the expenses of pembrolizumab production and enable a drastic reduction in the cost of each dose to patients. Considering that this is a unique life-saving therapeutic against cancer, it is morally obligatory that it be made more economically accessible.

8. Conclusions and Recommendations

This report demonstrates the profitability of increasing the scale of Keytruda production using continuous technology, single use equipment, and precipitation chromatography. The proposed manufacturing facility would allow for a more efficient production process through the use of a continuous perfusion bioreactor. The addition of precipitation chromatography will decrease downstream production costs, as it is more cost effective than protein A chromatography but with similar yields.

By reducing production costs, this manufacturing facility will increase profitability while reducing costs to patients. The construction of this facility and the addition of competition into the market would reduce the cost of Keytruda by 50%. By lowering the price per dose, we expect to capture 20% of the projected increase in market demand, producing 1400 kg of Keytruda per year. Based on the economic analysis, Merck's investment in this manufacturing facility would be highly profitable, with the facility's net present value being $61.4 million and an internal rate of return of 1132% for 15 years of operation.

It is our recommendation that Merck move forward with the proposed project. Given the patent expiration and pembrolizumab's recent FDA approval as a first line treatment, this manufacturing facility will be a good long term investment for the company. We have found that the kinetics for this fermentation lead to very long seed train incubation times, so we recommend that future researchers devote more time to determining the optimal number of bioreactors and bioreactor configuration for their upstream process, as well as optimizing the kinetics based on

emerging research. We also recommend future researchers research new technologies that would make the downstream chromatography process more continuous and rely less heavily on batch scheduling to achieve continuous downstream processing.

For more sustainable processes or ones that would provide easier access to mAbs for low-income countries, we recommend looking into a different expression system, such as tobacco, instead of CHO cells, and optimizing water and electricity usage for a G-Con pod, that would allow mAbs to be produced in a foldable sterile environment, facilitating mAb production within low-income countries. As with all plant designs, there are inherent risks associated with safety and product quality, but our research suggests that the proposed facility design is ready for validation and construction.

9. Acknowledgements

Many individuals have contributed their time and expertise to aid this team throughout the design of this capstone project. We would like to sincerely thank Professor Eric Anderson, Professor George Prpich, Professor Giorgio Carta, Professor Michael King, and Dr. Blair Okita for their assistance. Professor Anderson ensured we stayed on track with finishing our capstone project and advised us through any difficulties that occurred. Professors Prpich, Carta, and King and Dr. Okita provided their expertise into the area of pharmaceutical manufacturing, and were generous in taking the time to aid us in successfully completing our capstone. We would also like to thank the University of Virginia community for supporting us in our academic endeavors and enabling the completion of this project.

10. Tables of Nomenclature

10.1 Symbols

Table 10.1.1 Table of symbols.

Symbol	Definition	Units

α	Flow removal factor	N/A
A (UF/DF)	Membrane area	m^2
A (Heat Exchanger)	Heat transfer area	m^2
A_t	Bioreactor cross sectional area	cm^2
C_F	Feed concentration of pembrolizumab	g/L
C_P (UF/DF)	Permeate concentration of pembrolizumab	g/L
C_P (Heat Exchanger)	Specific heat capacity	J/g-^{O}C
C_R	Retentate concentration of pembrolizumab	g/L
C_{O2}	Minimum oxygen concentration	mg/L
C_{O2}^*	Oxygen solubility at 37℃	mg/L
D	PFR tube diameter	m
DBC_{10}	Dynamic binding capacity at 10% breakthrough	mg/mL
D_i	Impeller diameter	cm
d_p	Particle diameter	m
D_t	Bioreactor tank diameter	cm
ε	Bed porosity fraction	N/A
H_i	Impeller height in bioreactor	cm
H_L	Bioreactor working height	cm
H_t	Bioreactor total height	cm
k_La	Oxygen mass transfer coefficient	h^{-1}
L (AEX/CEX)	Column length	m
L (viral inactivation)	PFR length	m

q_{DF}	DF buffer flow rate	L/min
q_F	Feed flow rate	L/min
m	Mass of fluid	g
N (filtration)	Diavolumes	N/A
N (perfusion)	Impeller speed	rpm
N (precipitation)	Number of stages	N/A
η	Liquid viscosity	Pa-s
N_a	Aeration number	N/A
n_i	Number of impellers	N/A
N_P	Power number	N/A
Δp	Viral inactivation pressure drop	kPa
ΔP	Chromatography pressure drop	kPa
P	Power requirement	kW
ρ	Density	kg/m^3
P_g	Gassed system power input	kW
Q	Heat transfer	J/s
Q_B	Crossflow rate	L/min
Q_g	Aeration rate	m^3/s
Q_{O2}	Cell oxygen consumption rate	mmol/g-h
Q_P	Permeate flow rate	L/min
Q_R	Retentate flow rate	L/min
Q_W	WFI flow rate	L/min
R	Contaminant reduction factor	N/A
Re	Reynolds number	N/A
S	Solute sieving coefficient	N/A

ΔT	Cooling stream temperature change	^{O}C
T_C	Cool stream temperature	^{O}C
T_H	Hot stream temperature	^{O}C
ΔT_{lm}	Log mean temperature difference	^{O}C
σ	Rejection coefficient	N/A
u	Superficial liquid velocity	m/s
$u_{p,avg}$	Average permeate flux	L/m^2-min
μ	Cell growth rate	h^{-1}
μ	Viscosity	kg/m-s
U_0	Heat transfer coefficient	W/m^2-K
V (perfusion)	Bioreactor volume	L
V (viral inactivation)	PFR volume	mL
V_{col}	Column volume	N/A
V_{load}	Load volumes	L
v	Fluid velocity	m/s
v_s	Superficial velocity	m/h
X	Cell concentration	g/L
Y	Flow conversion fraction	N/A
$Y_{X/O2}$	Cell oxygen yield coefficient	g-cell/g-O2

10.2 Acronyms

Table 10.2.1. Table of acronyms.

Acronym	Definition
AEX	Anion Exchange Chromatography
CDC	Center for Disease Control

CEX	Cation Exchange Chromatography
cGMP	Current Good Manufacturing Practices
CHO	Chinese Hamster Ovarian
CIP	Clean in Place
CSTR	Continuous Stirred Tank Reactor
CV	Column Volumes
DBC	Dynamic Binding Capacity
DF	Diafiltration
dMMR	Mismatch Repair Deficient
EDI	Electrodeionization
FDA	Food and Drug Administration
HCCF	Harvested Cell Culture Fluid
HCP	Host Cell Protein
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
HVAC	Heating, Ventilation, and Air Conditioning
IEX	Ion Exchange Chromatography
LMIC	Low to Middle Income Countries
MAb	Monoclonal Antibody
MSI H	Microsatellite Instability-high
NSCLC	Non-small Cell Lung Cancer
OSHA	Occupational Safety and Health Administration
PD-1	Programmed Death Receptor-1
PEG	Polyethylene Glycol
PFR	Plug Flow Reactor
pI	Isoelectric Point

PPE	Personal Protective Equipment
RO	Reverse Osmosis
RTD	Residence Time Distribution
SIP	Sanitize in Place
SPTFF	Single-pass Tangential Flow Filtration
SOP	Standard Operating Procedure
TFF	Tangential Flow Filtration
TMP	Transmembrane Pressure
UF	Ultrafiltration
VDF	Variable Drive Frequency
VRF	Volume Reduction Factor
WFI	Water for Injection

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