To: AIChE National Review Committee

From: Group 1737

Subject: Completion of Neural Stem Cell Manufacturing Facility

To whom it may concern:

Group 1737 was organized in an attempt to design a manufacturing facility that produces neural stem cells. We are pleased to announce the completion of the design along with a detailed economic and safety analysis. The group recommends pursuing the construction of the manufacturing facility with plans to expand internationally when the economic opportunity is present.

We are looking forward to the review from your committee. With this transmittal, the group gives the committee permission for public review on the facility. The quality input will be used as a tool for improving the facility as the group looks to move into detailed design. We look forward to the honest and useful feedback from our work.

Design for Neural Stem Cell Commercial Manufacturing Facility

Group Number

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9 March 2016

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Abstract:

Over the past ten years, advances in stem cell therapy have progressed from low yield treatments on small organisms, to effective, targeted treatments on humans. One of the largest markets for stem cell therapy is treatment of bodily injury, particularly in the nervous system. Research has shown that induced pluripotent stem cells can expand and differentiate into neural stem cells, which has been clinically shown to relieve pain caused by spinal cord injuries.[16] Currently, over 250,000 Americans suffer from spinal cord injuries, with a 5% increase predicted over the next five years. Stem cell therapy is a rapidly developing field to provide treatment of these injuries, and with the technology available, a facility can be design to produce enough neural stem cells to treat the entire market.

Specifically, induced pluripotent stem cells have shown to grow rapidly with relatively high viability in vitro. By selectively differentiating induced pluripotent stem cells into neural stem cells, a large amount of neural stem cells can be provided to victims of spinal cord injury.[16] The facility outlined in this report is designed to take a batch of 100,000 induced pluripotent stem cells and create enough neural stem cells to treat the predicted American population over the next five years. The total amount of neural stem cells needed to treat the entire market is approximately 1.2 trillion cells per year. Batch processes, especially pharmaceutical processes, have relatively low service factors. A service factor of 0.75 was determine in order to account for the significant down time associated with batch processes because sterilization is key to maintaining the integrity of the facility and product.

Economically, the viability of the project has been evaluated over a 5 year time period with a minimum rate of return of 50%. It is very likely that international expansion will present an economic opportunity in a relatively short time frame. To account for potential expansion, the facility has the ability to incorporate overseas markets when the opportunity arises. It is recommended that the company moves forward with detailed design of the facility immediately to capitalize on the current financial opportunity.

Introduction:

Stem cell therapy is at the frontier of modern treatment methods. Research has shown that the efficiency of stem cell cultures has increased exponentially over the last five years; however, this advance is met with an increasing market size. Stem cell treatment has been validated on a small scale, but there are currently no large scale treatment options are available in the United States due to restrictions by the FDA.[27] On the other hand, other countries that implement stem cell therapy have produced promising results. Spinal cord injuries that are treated outside of the United States typically consist of a series of 12-16 injections, with each injection consisting of around three million stem cells[19]. The total number of people affected by spinal cord injuries in the United States over the next five years is predicted to be 310,000. In order to make enough stem cells to treat the entirety of the affected population in the United States, a facility needs to be able to have a high viability of stem cells, with extreme precision and efficiency within the process. Furthermore, the facility needs to have a high throughput in order to meet the market demand, but costs need to be minimized to ensure the economic viability of the project. Overall, recent stem cell research has shown potential for production of high throughput processes that allow the industry to reach a larger market. However, no current facility has been designed to provide therapy to the entire population.

Process Flow Diagram:

In the Process Flow Diagrams seen across the next few pages, the volumes given are the total volume for one batch. In particular, the media is changed more than once per batch; however, the replaced media is not of equivalent volume to the original media. Since cell concentration must be maintained within a certain range, a larger volume of replacement media is needed than the original volume due to an increased number of cells. Therefore, it is more applicable to display the volume per batch instead of the volume added when materials need to be replaced. The process is illustrated over three drawings for clarity and simplicity.

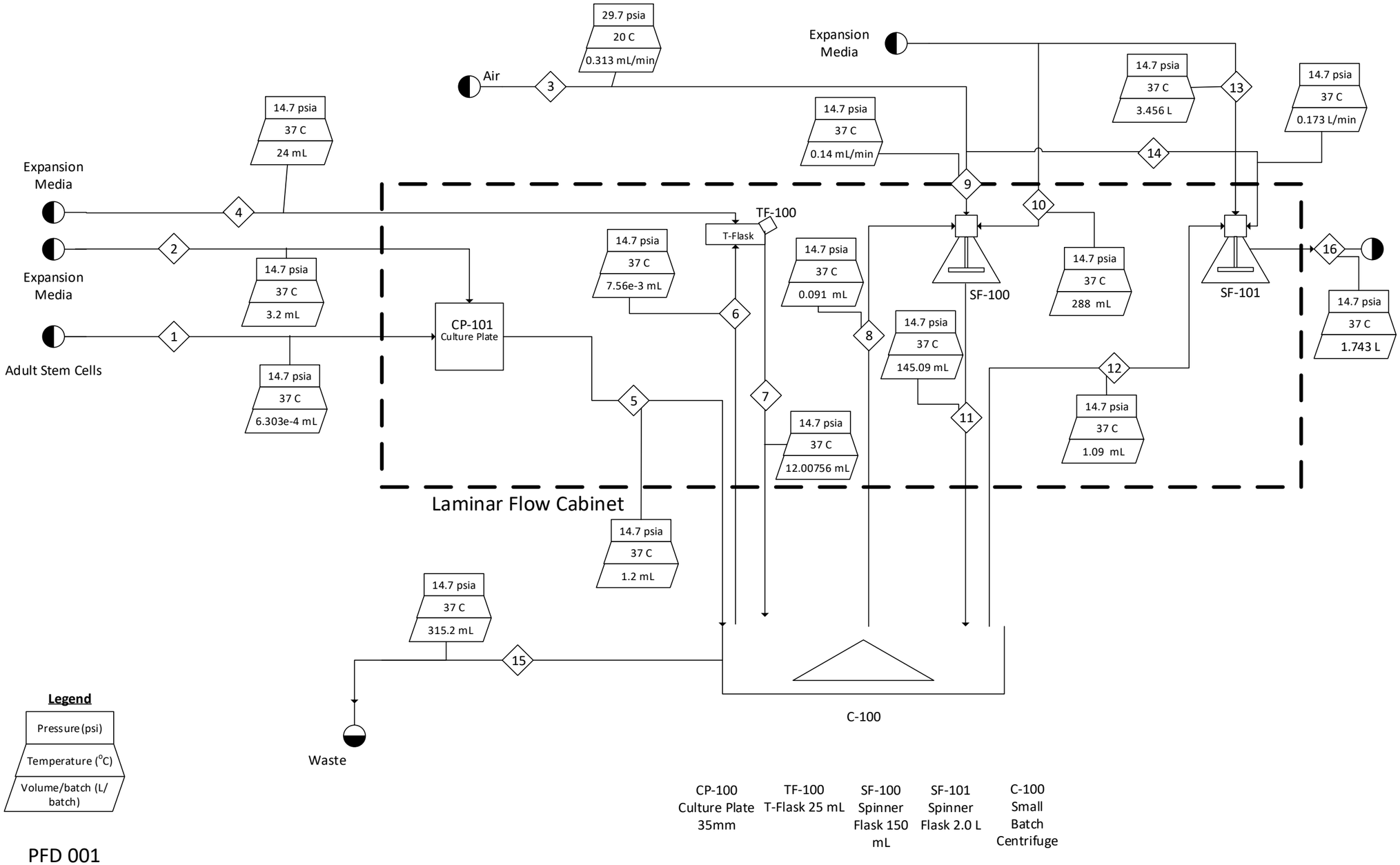
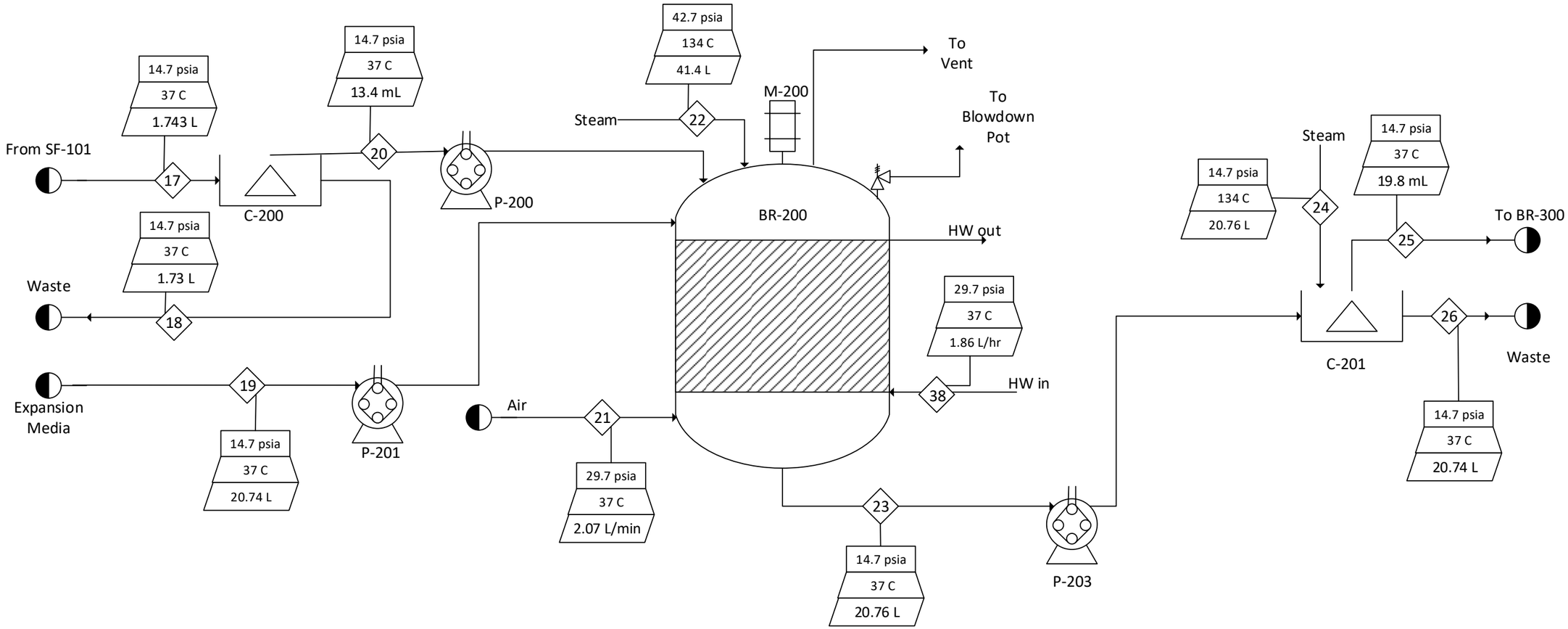


Figure 1-PFD of Neural Stem Cell Production Facility



Legend

Pressure (psi)

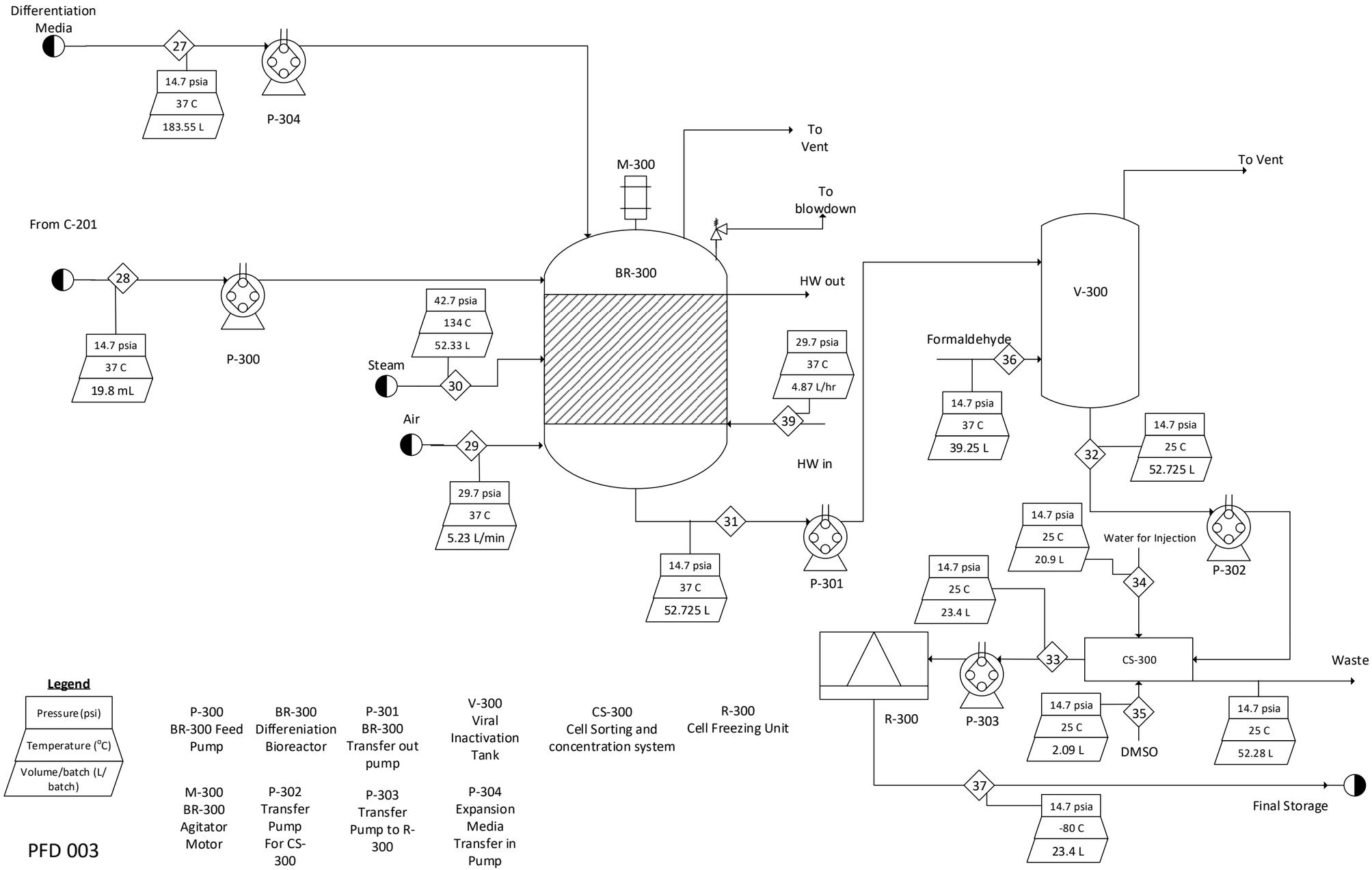
Temperature (^{o}C)

Volume/batch (L/ batch)

C-200	P-200	P-201	BR-200	P-203	C-201	M-200
2 L Centrifuge	Peristaltic Pump BR-200 Cell Pump	Peristaltic Media Pump for BR-200	Bioreactor for Final Expansion	BR-200 Transfer out Peristaltic Pump	Final Expansion Centrifuge	BR-200 Agitator Motor

PFD 002

Figure 2-PFD of Neural Stem Cell Production Facility



Legend

Pressure (psi)
Temperature (°C)
Volume/batch (L/batch)

PFD 003

P-300 BR-300 Feed Pump
BR-300 Differeniation Bioreactor
P-301 BR-300 Transfer out pump
V-300 Viral Inactivation Tank
CS-300 Cell Sorting and concentration system
R-300 Cell Freezing Unit
M-300 BR-300 Agitator Motor
P-302 Transfer Pump For CS-300
P-303 Transfer Pump to R-300
P-304 Expansion Media Transfer in Pump

Figure 3-PFD of Neural Stem Cell Production Facility

Stream Summary Table - Expansion and Differentiation of Induced Pluripotent Stem Cells to Neural Stem Cells													
Stream number	1	2	3	4	5	6	7	8	9	10	11	12	13
Phase	Solid/Liquid	Liquid	Gas	Liquid	Solid/Liquid	Solid	Solid/Liquid	Solid/Liquid	Gas	Liquid	Solid/Liquid	Solid/Liquid	Liquid
Pressure (psig)	0.00	0.00	15.00	0.00	0.00	0.00	0.00	0.00	15.00	0.00	0.00	0.00	0.00
Temperature (°C)	37.00	37.00	20.00	37.00	37.00	37.00	37.00	37.00	20.00	37.00	37.00	37.00	37.00
Heat Capacity (J/gC)	3.45	4.3	1.0005	4.3	4.3	3.45	4.3	3.45	1.0005	4.3	4.3	3.45	4.3
Actual Density (kg/m^3)	1125.00	1100.00	1.225	1100.00	1100.00	1125.00	1100.00	1125.00	1.225	1100.00	1100.00	1125.00	1100.00
Total Volumetric Flow Rate (mL/batch)													
iPSCs (mL)	6.30E-04	-	-	-	7.56E-03	7.56E-03	9.08E-02	9.08E-02	-	-	1.09E+00	1.09	-
Expansion Media (mL)	-	3.20	-	24.00	1.20	-	12.00	-	-	288.00	144.00	-	3456
Air (mL/min)	-	-	0.31	-	-	-	-	-	0.14	-	-	-	-
Differentiation Media (mL)	-	-	-	-	-	-	-	-	-	-	-	-	-
NSCs (mL)	-	-	-	-	-	-	-	-	-	-	-	-	-

Stream Summary Table - Expansion and Differentiation of Induced Pluripotent Stem Cells to Neural Stem Cells													
Stream number	14	15	16	17	18	19	20	21	22	23	24	25	26
Phase	Gas	Liquid	Solid/Liquid	Solid/Liquid	Liquid	Liquid	Solid/Liquid	Liquid	Gas	Solids/Liquids	Gas	Solid/Liquid	Liquid
Pressure (psig)	15.00	0.00	0.00	0.00	0.00	0.00	0.00	15.00	28.00	0.00	25.90	0.00	0.00
Temperature (°C)	20.00	37.00	37.00	37.00	37.00	37.00	37.00	20.00	134.00	37.00	127.00	37.00	37.00
Heat Capacity (J/gC)	1.0005	4.3	4.3	4.3	4.3	4.3	3.45	4.3	2.19	4.299	2.19	3.45	4.3
Actual Density (kg/m^3)	1.23	1100.00	1100.00	1100.00	1100.00	1100.00	1125.000	1100.00	1.58	1100.00	1.58	1125.00	1100.00
Total Volumetric Flow Rate (mL/batch)													
iPSCs (mL)	-	-	13.05	13.05	-	-	13.4	-	-	19.80	-	19.80	-
Expansion Media (mL)	-	315.2	1730	1730	1730	2.07E+04	-	-	-	2.07E+04	-	-	2.07E+04
Air (mL/min)	173.00	-	-	-	-	-	-	2.07E+03	-	-	-	-	-
Differentiation Media (mL)	-	-	-	-	-	-	-	-	-	-	-	-	-
NSCs (mL)	-	-	-	-	-	-	-	-	-	-	-	-	-
Steam (L)	-	-	-	-	-	-	-	-	41400	-	20760	-	-

Stream Summary Table - Expansion and Differentiation of Induced Pluripotent Stem Cells to Neural Stem Cells													
Stream number	27	28	29	30	31	32	33	34	35	36	37	38	39
Phase	Liquid	Solid	Gas	Gas	Solid/Liquid	Solid/Liquid	Solid/Liquid	Liquid	Liquid	Gas	Solid/Liquid	Liquid	Liquid
Pressure (psig)	0.00	0.00	15.00	28.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.00	15.00
Temperature (°C)	37.00	37.00	20.00	134.00	37.00	25.00	25.00	25.00	25.00	25.00	-80.00	37.00	37.00
Heat Capacity (J/gC)	4.3	3.45	1.0005	2.19	4.28	4.28	4.28	4.184	1.96	4.184	4.184	4.184	4.184
Actual Density (kg/m^3)	1100.00	1125.00	1.23	1.58	1100.000	1100.00	1100.00	1050.00	1050.00	1050.00	1050.00	1050.00	1050.00
Total Volumetric Flow Rate (mL/batch)													
iPSCs (mL)	-	1.98E+01	-	-	-	-	-	-	-	-	-	-	-
Expansion Media (mL)	1.84E+05	-	-	-	-	-	-	-	-	-	-	-	-
Air (mL/min)	-	-	5230	-	-	-	-	-	-	-	-	-	-
Differentiation Media (mL)	-	-	-	-	52330	52331	-	-	-	-		-	
NSCs (mL)	-	-	-	-	396	396	396	-	-	-	396	-	-
Water-(Steam/Water for Injection) (L)	-	-	-	52330	-	-	20.9	20.9	-	-	20.9	1.86 L/hr	4.87 L/hr
Dimethylsulfoxide (L)	-	-	-	-	-	-	2.09	-	2.09	-	2.09	-	-
Formaldehyde (L)	-	-	-	-	-	-	-	-	-	39.25	-	-	-

Table 1-Stream Summary Table for Neural Stem Cell Production Facility

Material Balances:

To verify the system meets constraints material and energy balances were performed. The validity was determined by stating that what enters a unit must be equals what comes out. In order to perform and accurate material balance, cell and media densities need to be approximated. The material balances were done on a mass basis and densities were assumed to be uniform. The values for the density of media, stem cells, and air are 1100 kg/m^3, 1125 kg/m^3, and 0.0012 kg/m^3, respectively[8]. If the difference between mass that enters and mass that exits is approximately zero then the system was considered balanced. Each expansion vessel as well as the differentiation reactor were balanced. A summary is seen in Table 1 and in the Appendix for all of the units.

CP-101	
Stream	Mass (g)
1	0.000709
2	1.1
5	1.10071
In-Out	-1E-06
Stream	Energy (J)
1	0.06150575
2-4	118.25
5	118.326325
In-Out	-0.01481925

Table 2-Sample Material Balance Calculation

The first expansion material balance was performed as follows. There are 7.1 x 10^{-4}g of cells entering the system and 1.1g of fresh media entering. 1.10071g of material leaves after 86 hours. This yields 1.0 x 10^{-6} as the difference between what comes in and what exits. This is close enough to zero; therefore, this system is balanced.

Similarly, the second expansion has 8.51 x 10^{-3}g of cells entering with 13.2 g of fresh media. 13.2021 g of spent media and expanded cells leaves the system after 84 hours. The difference between in and out was found to be 6.4 x 10^{-3}. This confirms the system is balanced.

The third expansion has 0.102g of cells entering. There is 158.4 g of media entering as well. With this system, air is fed to this expander and the total oxygen consumed is 0.867g per batch. The amount of material out from this batch is 159.3g. The difference between in and out of the system is 0.0721. This is small enough to consider the system balanced

Moreover, the fourth expansion has 1.23g of cells entering. The media entering is 1901g and the oxygen amount entering is 0.867g. The final mass of the leaving mixture is 1901.95g. The difference in material in and out is 0.95g. In comparison to the overall magnitude of the feed streams this difference is small enough to be considered zero. This is the last expansion carried out in the laminar flow cabinet.

Lastly, the fifth expansion is carried out in a 40L bioreactor with air sparged through the media. The mass of cells entering is 14.68g and the mass of media added is 22,814g. The amount of oxygen added is 2.19g. This make the overall difference 0.92g. This is low enough to qualify as balanced.

The differentiation is the last step in the process. The amount of cells entering is 22.4g. The amount of media charged is 57,560g. The amount of oxygen delivered is 63.2g. The final amount out of the system is 57,607g. The difference then from in and out is 42.11g. This is a little large compared to the other units but compared to the overall system this is within acceptable range. A possible explanation of this is the lack of information of growth rates and substrate consumption rate of the cells during differentiation. Overall though this unit was considered balanced taking into account the uncertainty of the kinetic data.

Process Description:

Stem cell culturing is a delicate process and caution must be used throughout the process. The process begins with a batch of 100,000 induced pluripotent stem cells stored in a 1mL plastic vial in the vapor phase of a liquid nitrogen tank. Specifically, a temperature indicator and low temperature alarm are set to notify operators of a significant decrease in the temperature of the tank. The stem cells need to be kept at cryogenic conditions until the facility is ready to operate in order to properly preserve the raw materials. Premature thawing of the cells significantly reduces the overall production[17]. Once the facility is ready to begin a batch, the induced pluripotent stem cells are transferred from cryogenic storage to a thaw bath for two minutes to fluidize the cells. Water at 40^{o}C is used to thaw the cells at an appropriate rate. After two minutes, the stem cells are then transferred by an operator to a 35mm square culture plate.

For all expansion chambers, the expansion media chosen is Essential 8 by Gibco. For the first expansion, 1.6mL is used each change in conjunction with the cell culture. The media in the culture is changed every three days and the cells stay in the culture plate for 85 hours. The cells are separated from the media via centrifuge and transferred to a 25mL T-Flask with fresh media for 85 hours. The cell culture goes through five expansion chambers in total, each with the same residence time. The size of the final three expansion chambers are 150mL, 2L, and 40L, respectively. At the end of the expansion phase, the number of cells is expected to grow from 100,000 to around 3.1 billion over the course of 15 days. In order to detach the cell aggregates, trypsin is added before the cells are removed. Moreover, all expansion chambers are kept at 37^{o}C via electrical heating by a hot plate. Before transferring the process into a larger expansion chamber, the process is concentrated and water for injection is used to fluidize the cells to allow for transportation. After the 5th expansion chamber, the cells are sent to a centrifuge to isolate

the stem cells from the media where they are then fed to a bioreactor. For each expansion chamber, an oxygen consumption rate of 15.4 pmol/hr is used to determine the amount of oxygen needed to be supplied for each batch. Similarly, a media consumption rate of 8.51 pmol/hr is used to determine how often the media needs to be changed in the permanent expansion chamber[26].

In order to determine how long to keep the induced pluripotent stem cells, solver was used to maximize the operating time throughout the year by changing the total residence time of the cells in the expansion phase by varying the number of batches per year with the constraint of meeting the need for the market. The result is the ideal residence time of the cells in the expansion phase. However, nothing regarding the number of chambers, size, or media volume is included. Based on research, the media needs to be changed every other day to prevent unviable conditions of the cells. Cell cultures have shown significant a significant drop off in viability at concentrations over 1.2 million cells/mL because cells will compete against each other for nutrients[17]. To prevent this, cells are transferred to a larger expansion chamber with a higher volume of media to dilute the concentration of the cells. To produce the number of cells that need to be produced for each batch and maintain the appropriate concentrations, five expansion chambers are used with increasing size to maximize the efficiency of the process. Also, multiple articles have shown the doubling time of induced pluripotent stem cells to be around 24 hours. This produces a logarithmic relationship between growth factor and residence time. A graph of this relationship can be seen in Figure 4.

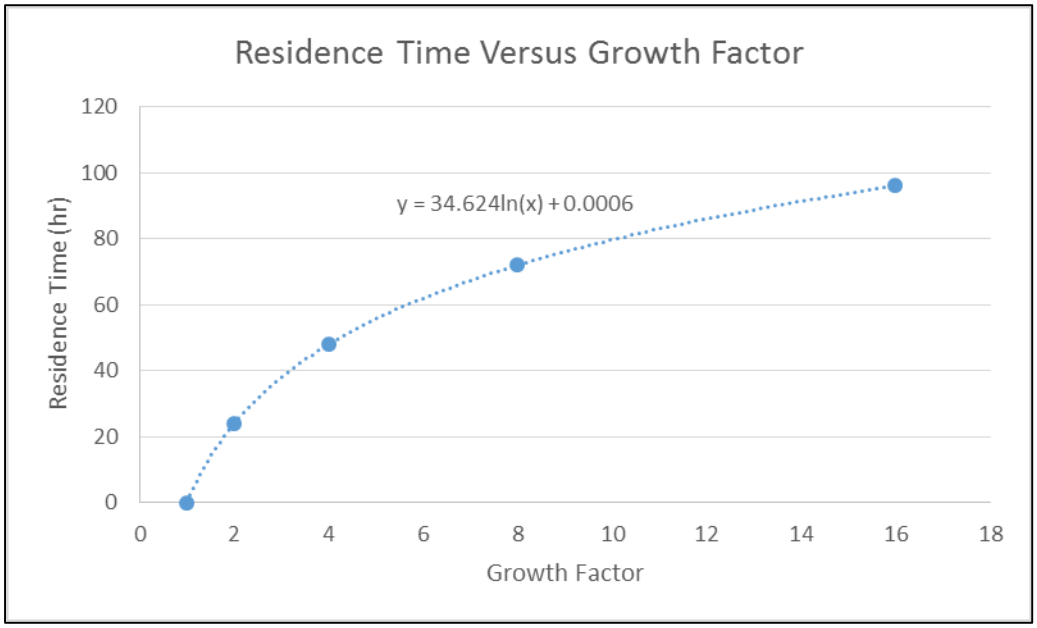


Figure 4-Graph of Residence Time versus Growth Factor

Additionally, number of cells versus residence time has an exponential relationship, which is consistent with standard cell growth. Similarly, this relationship is depicted graphically in Figure 5. Approximately three billion cells are sent to the reactor, which after differentiation produces around 58 billion neural stem cells per batch. This factor is specific to the neural cell media specification for growth factors. To meet market demand, 19 batches of 58 billion induced pluripotent stem cells are required.

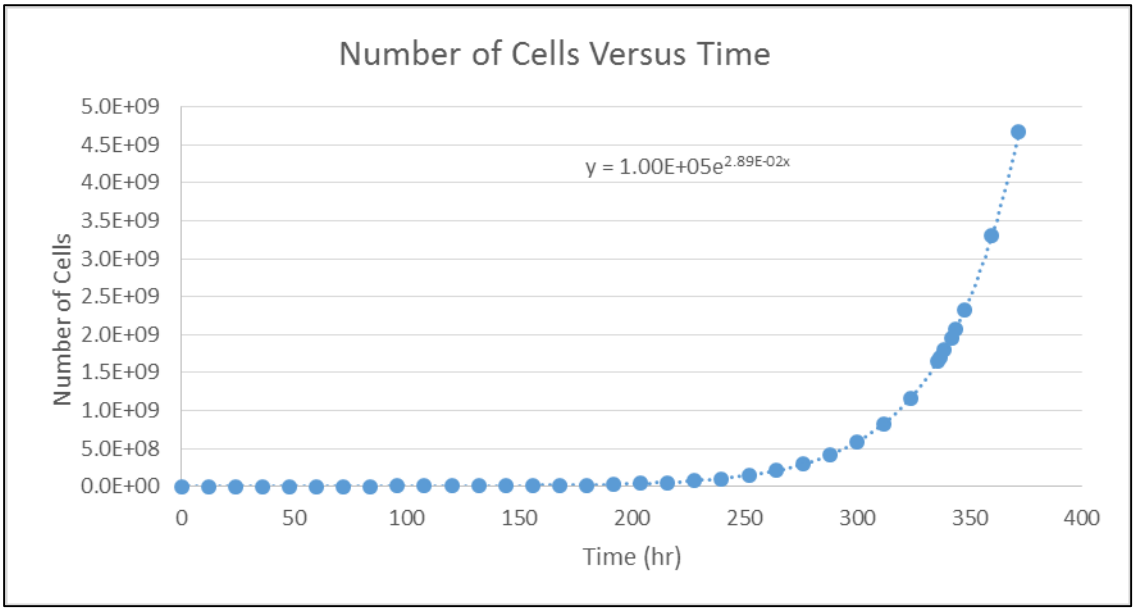


Figure 5-Graph of Number of Cells versus Residence Time

As stated previously, centrifuges are used after each expansion step to separate stem cells from media. For this process, centrifuges that handle very small volumes are placed in a vial and are separated in a closed system while large volumes are separated using an open flow system. The first three expansion chambers all use the same centrifuge because it can handle the small range of volumes. However, the other two centrifuges are open systems. For open system centrifuges, fluid is sent through the system, where stem cells stick to the wall, and then removed and transferred to the next unit. Each open system centrifuge is constructed from stainless steel to prevent contamination of the cells.

The bioreactor has a 200L reactor volume and the liquid level in the reactor is expected to range from 22 liters to 53 liters over the course of seven days. The bioreactor needs to be able to handle one trillion neural stem cells each year to meet market demands. Neural induction media is added to the process to initiate differentiation to neural stem cells. The media is changed every

other day to maintain the proper process conditions in the bioreactor. A low shear agitator is used to maintain a well-mixed composition to ensure maximum yield. Low shear is critical to ensure that the stem cells are not damaged during the differentiation process. Furthermore, three process variable are critical to control in the reactor: pH, temperature, and oxygen concentration[5]. A change in pH is directly associated with consumption of media. As the nutrients in the media are consumed, waste is produced and the pH will deviate from ideal conditions. Once the pH reaches unacceptable conditions, the media is changed to minimize cell death[22]. Controls systems are installed to notify operators when the media needs to be changed to prevent undesirable conditions. Likewise, temperature is controlled by pulling a slip stream off of the boiler to provide hot water to maintain a temperature of 37^{o}C inside the vessel[17]. Since the bioreactor is insulated, a minimal amount of heat is lost from the system. However, an automated temperature controller is necessary due to the economic consequences; the hot water feed is essential to maintaining the viability of the cells and needs to be controlled as precisely as possible. Lastly, the oxygen concentration is critical to the viability of the stem cells. Mitosis requires glucose and amino acids that the media provides as well as oxygen from a sparger to maximize the efficiency of cell division. Since oxygen concentration of the process is vital, an automated controls system is added to more precisely maintain the ideal operating conditions. Oxygen concentrations are set at 20% through a flow controlled gas sparger. After seven days in the bioreactor, the cells are sent to an inactivation chamber, where 37% gaseous formaldehyde is added at 1.31 liters per second for two hours. This step removes any viruses in the process, but does not harm the neural stem cells[10]. Gaseous formaldehyde targets the protein coat of the virus to inactivate it.

In order to separate the differentiated stem cells from the undifferentiated stems cells, a magnetic automated cell separator is used to sort the cells. Antigens with metal attached are injected into the process that bond to the neural stem cells and leaves the undifferentiated cells untouched. The batch stays in the separator for two hours to allow for complete separation. After two hours, the undifferentiated stem cells pass through the unit and the neural stem cells are removed from the separator by using water for injection. The cells are concentrated down to three million cells per milliliter using water for injection. In order to protect the cells, DMSO is added as a cryopreservative and the finished product is sent to cryogenic storage that is kept at -148^{o}C using liquid nitrogen[15]. Again, temperature control is critical to the viability of the product. The neural stem cells are kept in storage until it is shipped to the formulation group to prepare the product for packaging.

Energy Balance and Utility Requirements:

A large amount of operating costs each year is spent on energy consumption in the form of heating and cooling. Balances on all of the units was necessary to confirm the validity of the design calculations. The balance was performed in similar fashion to the mass balance by finding the energy into the unit and subtracting the energy out of the system and adding the energy either transferred into or out of the system. This sum should add to be zero if the system is truly in balance. Due to uncontrollable losses, however, none of the energy balance summed to exactly zero. The physical data of the system can be seen below in Table 3. These heat capacity values were estimated based on medium properties as well as cell properties. The heat of reaction for the cell growth and differentiation were determined to be negligible due to the low amount of heat needed for temperature control as well as the heat transferred in the laminar flow cabinet as

the cabinet has such a controlled environment. All energy balances are found in the Appendix. A sample is located at the end of this section.

For Streams 1, 2, and 5 the energy was calculated by Equation 1 below. Where C_p is the heat capacity above and T is the temperature of the stream entering.

$$h = mC_pT \quad (1)$$

Where:

h = enthalpy of the material (J)

m = Mass of the Material (g)

Cp = Heat Capcity of the Material (J/gC)

T = Temperature of the Material (C)

The energy into the system was calculated as 0.062 Joules (J) for Stream 1 using Equation 1. For Stream 2 the amount of heat entering the first expansion is calculated as 118.25 J. The heat exiting the system in Stream 5 then is calculated as 118.33 J. Finding the difference then becomes -0.0148 J: small enough to be considered balanced.

For TF-100, the same process was applied by using Equation 1 across all of the streams. For Stream 6 the amount of energy entering is found to be 0.738 J. For Stream 4 the energy is 2100.1 J for the media entering. For the exiting mixture in Stream 7, the heat is 2100.46 J. This leaves the difference of the in and out as 0.40 J with neglecting heat transfer from the box. The value of the difference is low enough to be accepted.

The first stirred flask, SF-100, has 8.857 J entering with the cells, 25201.4 J with the fresh media. This is the first expansion to require air so the energy entering with Stream 9 is 21.8 J. The final mixture exits with 25226 J of energy. This leaves a difference between energy in and out at 5.8 J. This is small enough when compared to the overall amount of energy in the system.

This step is still contained in a laminar flow box so the energy transferred by the system is considered negligible.

The second stirred flask, SF-101, the cells bring in 106.7 J in Stream 12. The media is Stream 13 brings in 302,417 J. The air brings in 21.7 J in stream 14. The exit mixture has an energy amount of 302,529 J. These values give a balance of 16.4 J. This is a close enough value in comparison with the stream values to justify a balance closing.

The fifth expansion chamber is the first vessel to be outside of a laminar flow cabinet. This required a jacketed to maintain the temperature in the vessel. To calculate the amount of heat needed to maintain the internal temperature, the vessel dimensions of length 0.587 m and diameter 0.147 m were found by using Excel Solver to find the L and D that satisfy an L/D ratio of four.[25] The heat transfer area was estimated as the surface area of a cylinder with the dimensions given above. This gives a surface area of 0.271 m^2 for the inner surface the outer was found by adding a half inch of insulation to the outer thickness of the vessel. The outer surface area was found to be 0.277 m^2. The heat transfer rate could then be found by using the resistance method detailed in Perry's Chemical Engineers Handbook[24]. The heat transfer rate to the environment was found to be 1.19 Watts or 4.056 BTU/hr. This amount although small is non-negligible. Using this rate and a hot water stream the flow rate of water needed to keep the internal temperature was calculated to be 1.85 kg/hr. This ensures there is no significant temperature change in the reactor. To perform the energy balance, Stream 19, the fresh media feed was found to carry 3.63 MJ. The cells carry 1,880 J of energy and the air has 55 J of energy. The heating from the water over the cycle introduces 6,260 J of energy. The heat loss to the environment is 6,260 J The final amount of energy out of the system is found to be 3.63 MJ. This

gives a difference of 1,092 J. This is close enough to zero given the magnitude of the other streams.

The differentiator was sized in a similar manner to the final expander with an L/D of 4 and a final vessel size of 108 liters. The heat loss with a half inch of insulation was found to be -3.1 Watts. This means that water at a flow rate of 4.85 kg/hr at 1 degree temperature drop. This gives a total amount of heat needed to be transferred at 1.87 MJ. The cells bring in 19.4 kJ from Stream 28. The media brings 9.1583 MJ in stream 27 and the air brings in 1,590 J with Stream 29. The final amount of heat coming out of the system is found to be 9.16 MJ. This gives a total difference of 1,940 J. This is low enough when compared to the stream magnitudes.

The final unit is the refrigeration unit for final storage. This unit needs a large amount of energy due to the large temperature drop the cells require to remain viable long term. The energy for the system was found using Equation 2 below.

$$Q = mC_p(T - T_f) + m\lambda + mC_{ps}(T - T_{final}) \quad (2)$$

Where:

Q = heat transfer needed

m = total mass being chilled (kg)

T = initial temperature of mixture (^oC)

T_f = fusion temperature (^oC)

λ = heat of fusion of mixture (J)

C_{ps} = heat capacity of the solid mixture (J/kg ^oC)

T_{final} = final temperature of the mixture (^oC)

Most of the energy needed to chill comes from the freezing process as would be expected from any phase change. The constant values are seen below in Table 4. Using Equation 2 with

the constants below the energy needing to be transferred was found to be -12,867 kJ. This is handled by Asymptotes VIA freeze QUAD system. The cycle time for a batch was found to be 117 minutes with a maximum cooling rate of 1 C/min.

Energy Requirements for Freezing	
Mass In (g)	25645
Cp (J/gC)	4.545
Heat of Fusion (J/g)	333.55
Cps (J/gC)	1.4
Initial Temperature (C)	25
Freezing Temperature (C)	0
Final Temperature (C)	-80
Energy Required (kJ)	14340

Table 4-Refrigeration Constants

CP-101	
Stream	Energy (J)
1	0.06150575
2	118.25
5	118.326325
In-Out	-0.01481925

Table 5-Example Energy Balance

Once the energy balances confirmed the validity of the equipment design, utility costing was performed. The overall utility costs were relatively low due the process consisting of batch reactors with residence times under three minutes. Additionally, much of the equipment did not require utilities as the glucose within the cellular media provided the energy for the reactions to occur as opposed to heat or high pressures. It is important to note that the process is a batch process so the cost of utilities per year is determined by calculating the utility cost per batch and multiplying the cost per batch by the total number of batches. The majority of units which

required utility input were support systems for the process itself, such as the boiler and laminar flow cabinets.

The utility costs for predesigned equipment was determined by converting the set voltage and electrical currents to power in kilowatts and using the provided costing information of $0.05 per kW-hr. This method was applied to R-300, the laminar flow cabinet, the air filtration systems, and the centrifuges: C-100, C-200, C-300. A sample of this calculation can be seen below. All other calculations of this method can be found in the Appendix.

Purifier Utilities	
Volts V	230
Amps A	5.4
Power kW	1.242
Energy Used per Batch kW-hr	10335.92
Price of Energy Per Batch $/kW-hr	0.05
Price to Run $/Year	9818.59

Table 6-Purchased Equipment Utility Costs

For all other units, hand calculations using data and engineering heuristics were used to approximate energy consumption. One such unit this for which this method was used is the boiler unit. Both the costs of electricity to heat the coils and the boiler feed water were calculated using by hand calculation found in Analysis, Synthesis, and Design of Chemical Processes.[25] An example of the costing process can be seen below equations for the sample calculation can be found in the Appendix.

Operational Costing	
Boiler Feed Water $/kg	0.00245
Electricity $/kW-hr	0.05
Boiler Feed Water $	0.010995

Electricity $	586.8408
Total $	586.8518
Total/year	11150.18

Table 7-Boiler Utility Costs

Water proved to be an important utility in the process because it is needed for sterilization in between batches.[21] For both the final expansion chamber, BR-200, and the differentiator, BR-300, water is needed to heat the jacketed reactors to isothermal conditions. Instead of installing a heat exchanger to heat the water before feeding it to the reactor jacket, excess boiler feed water is fed to the reactor. Boiler feed water is used because the low cost of the utility makes slightly oversizing the boiler more economic than purchasing two new heat exchangers.[25] Additionally, water for injection for the final product vials is needed to prepare the stem cells for injection into a patient. The total weight of the water for injection need was calculated by determining the necessary concentration for therapy in the vials with the cells and replacing the media with water for injection.[20] Finally, boiler feed water was also used in the warm water bath to thaw the frozen iPSCs after cryostorage. Boiler feed water was selected to warm the cells due to the low cost and because the boiler feed water is treated, making it less harsh on the cell vials. An example calculation of the energy calculations for the warm water bath can be seen in Table 8.

Water Heat Calculations	
Heat Required by Water (J)	0.85
Water Heat Capacity (J/kgK)	4.108
ΔT water (K)	1.11
Mass of Water (kg)	0.19
Volume of Water (L)	0.19
Volume of Water (gal)	0.05
Volume of Tank (gal)	0.10
Time in Thawing (min)	2
Energy Transferred (mJ)	7.10437875

Table 8- Energy Transfer for Thawing of Stem Cells

Equipment List and Unit Descriptions:

Boiler:

To prevent contamination, the process equipment is sterilized between each batch. Due to its low operating cost, steam-in-place was chosen as the method of sterilization. A boiler is used to generate the necessary 132 ºC saturated steam for steam-in sterilization. 132 ºC saturated steam was chosen for sterilization due to its common use in CDC sterilization techniques.[21] The boiler must generate enough steam to contact the all of the surface area of the process vessels with steam for three minutes. The total volume of all the process vessels is 0.272 m^3; however, the boiler was sized to produce ten times the volume of steam necessary to both meet industry standards and ensure enough steam is produced to fill the process vessels for the full 3 minute sterilization time.[21] In order to generate 2.72 m^3 of steam, 4.30 kg of boiler feed water is fed to the boiler for each steam-in process. The boiler has a duty of 62 kW to convert the boiler feed water to saturated steam. Due to the relatively small duty of the boiler and lack of corrosive or caustic materials, plain carbon steel was used as the MOC to avoid unnecessary cost. After use,

the steam vents to atmosphere so a full boiler system is not needed. This was done because the steam generated is so small, it is more cost effective to generate more steam than to install an entire boiler system. Heating coils were used to generate steam because electricity is a cheaper utility than fuel gas.[25]

Cryogenic Storage/Preparation:

Both the purchased adult iPSCs and the final NSCs must be kept in cryogenic storage until use or shipping, respectively.[3] Liquid nitrogen is used to keep the stem cells at the desired temperature. The stem cells are stored in a 24K Cryostorage System by MidSci.[1] This system was chosen due to the low cost compared to other products and its large storage capacity of 24050 2 mL vials. The storage units use SS as a material of construction and have a liquid nitrogen capacity of 365 L. Three storage units were selected to account for large hold up of the inlet adult iPSCs; the NSCs and iPSCs are stored in separate storage units to avoid cross contamination of stem cells. Due to a significant evaporation rate, excess liquid nitrogen for the storage units is kept in an exterior tank. The exterior tank was sized based on the need to refill the Cryostorage tanks every 52 days.[1] This is a common heuristic used for liquid nitrogen storage. This calculation led to a necessary size of 1500 L; however, nitrogen storage tanks have nominal sizing so a tank with a volume of 1893 L (3000 gal) was used. The exterior tank was assumed to be pressurized to 200 psig, based on literature values.[11] Due to the incredibly low temperature of the nitrogen, insulated stainless steel was used for the MOC. A refrigeration vessel was also incorporated to prepare the NSCs for cryogenic storage. The CryoMed Controlled Rate Freezer was used because of its FDA certification and stainless steel construction. The freezer has a max liquid hold up of 34 L which is the smallest unit than can

hold the entirety of the product in one batch. The freezer also can full chill to cryogenic conditions at -150^{o} C and has a small electrical requirement of 120 V and 60Hz.

Viral Inactivation Tank:

A major issue with pharmaceutical manufacturing is the potential for the presence of viruses in the medical treatment. To reduce this potential, a viral inactivation tank was placed before the cell separation. The tank uses a sparger to distribute gaseous formaldehyde throughout the NSCs to kill off any viruses. The tank maintains a formaldehyde concentration 0.2 g/L for 2 hours to ensure complete virus inactivation. A sparger was necessary to ensure even distribution of formaldehyde throughout the media due to the natural mixing inherent to a sparger. Also, a sparger avoids having to spend extra money on an agitator for the tank. In order to reduce batch time, the inactivation tank was sized identically as differentiator to a volume of 108 L so the entirety of the 52 L batch could fit in the tank and only half way fill the tank. The tank was assumed to be at atmospheric pressure and is constructed from stainless steel to prevent corrosion and contamination.

Pumps:

Eight pumps were added throughout the process to ensure consistent volumetric flow rates. A major issue with the use of pumps is the shear stress caused by the impeller damaging the stem cells. To avoid cell damage, peristaltic pumps were used in place of traditional centrifugal pumps. The low shear of peristaltic pumps make them ideal for pumping cell media through the process. Since peristaltic pumps do not significantly increase the pressure of the

working fluid, sizing was based entirely off the volumetric flow rate of 1.75 lpm. The Vector 2004 model peristaltic pump is used for all 8 pumps.

Cell Separator:

While the cell differentiator provides nearly 100% conversion from iPSCs to NSCs, separation of undifferentiated cells from NSCs is still necessary. A CliniMACS Prodigy cell separator is used to purify the cell product stream before being chilled. The cell separator uses magnetic-activated cell sorting to differentiate the neural stem cells. This is done by using magnetic antigens with metal attached to cause to allow a magnetic field to separate the NSCs from other cells. The device has a MOC of HDPE (high density polyethylene) and is design in accordance with the Miltenyl Biotec company standards.

Expansion Chambers:

Due to the limited feed stock, multiple expansion chambers are needed to produce the requisite number of cells before differentiation. The expansion is done in 5 parts with 4 expansions done on a bench top scale and the final expansion done in a large tank. Each expansion chamber was sized to start expansion with an initial cellular density of 100,000 cells/mL and end with a cellular density of 1.2 million cells/mL.[8] These densities were chosen based to coincide with the original starting density and the maximum cellular density possible while still ensuring cellular vitality.[8] Residence time in each chamber was determined by using a logarithmic function to model the time needed for the cell density to change from the starting density to the final density. The residence time for each expansion chamber can be seen in the Appendix**.** The original minimum number of chambers was only 4; however a 5th expansion

chamber was added to ensure none of first four chambers would be undersized and to account for future international market expansion. The first four expansion chambers are placed in laminar flow cabinet, along with a single bench-scale centrifuge for separating the concentrated stem cells from used media. The final expansion chamber feeds to an automated centrifuge which concentrates and separates the expanded stem cells from the media.

Bioreactor:

The GE XDR-200 Bioreactor Complete by GE was chosen as the cell differentiator for the process. This reactor type was chosen because of the known cost of the vessel and that it satisfactorily meets requirements to differentiate iPSCs into NSCs. A reactor size of 200L was chosen because it was the smallest nominal reactor volume that was still at least double the size of the process fluid.

It was deemed appropriate that the reactor be at least double the 52L volume of the cells and media to ensure over-pressure events and backflow through the vents or reactors could be avoided. The differentiator is a batch reactor which uses an agitator to stir the suspended cells in the differentiation media and operates at 37ºC and atmospheric pressure respectively. The batch reaction has a residence time of seven days based on media specifications which illustrate the time necessary for iPSCs to differentiate into NSCs.

Centrifuges:

Three centrifuges were placed in the process to concentrate and separate cells from media. The first centrifuge is a bench top-scale size with a volume of $0.03m^3$ and has individual vials in which the media and cells can be placed during centrifugation. The second and third

centrifuges follow the final expansion chamber and the bioreactor, respectively. Centrifuges were sized to not operate over 3000 RPM and 300 RCF to prevent damage to cells. The requirement of low shear is satisfied at these conditions. All centrifuges were sized by assuming an L/D of 4 and using solver by changing settling velocity to determine an overall volume and a distance away from the center of the expansion chamber. The final volumes of the Centrifuges 2 and 3 are 0.05 m^3 and 0.08m^3, respectively.

Equipment Specification Sheets:

Equipment Specification Sheets-Vessels						
Equipment Tag	Category	Description	Extreme Temperature	Max Pressure	Volume/Surface Area	MOC
CP-101	Expansion Chamber	Culture Plate First Expansion Takes $1.0*10^5$ Cells to $1.2*10^6$ Cells	37 °C	1.01 Bar	962.11mm	Plastic
TF-100	Expansion Chamber	T Flask Second Expansion Takes $1.2*10^6$ Cells to $1.44*10^7$ Cells	37 °C	1.01 Bar	19mL	Plastic
SF-100	Expansion Chamber	Spinner Flask Third Expansion Takes $1.44*10^7$ Cells to $1.73*10^8$ Cells	37 °C	1.01 Bar	250mL	Plastic
SF-101	Expansion Chamber	Spinner Flask Fourth Expansion Takes $1.73*10^8$ Cells to $2.07*10^9$ Cells	37 °C	1.01 Bar	3L	Plastic
BR-200	Expansion Chamber	Spinner Flask Fifth Expansion Takes $2.07*10^9$ Cells to $3.14*10^9$ Cells	37 °C	1.01 Bar	40L	SS
BR-300	Differentiator	Converts iPSCs to NSCs	37°C	1.01 Bar	200L	SS
V-300	Inactivation Tank	Kills Viruses without damaging cells	37°C	1.01 Bar	200L	SS
R-300	Media Prep Tank	Prepares Cells for Cryostorage	-150°C	1.01 Bar	20L	SS
WWB	Warm Water Bath	Thaws iPSCs out of cryostorage	-150°C	1.01 Bar	5L	SS
CS-300	Cell Sorter	Sorts out undifferentiated cells from NSCs	25 °C	1.01 Bar	25L	SS

Table 9-Vessel Equipment Specification Sheets

Equipment Specification Sheets-Pumps					
Equipment Tag	Category	Description	Pressure Rise	Flow Rate	MOC
P-200	Pump	Peristaltic Pump Feeds concentrated cells to BR-200	~0 psi	1.75 L/min	SS
P-201	Pump	Peristaltic Pump Feeds media to BR-201	~0 psi	1.75 L/min	SS
P-203	Pump	Peristaltic Pump Feeds cells and media to C-201	~0 psi	1.75 L/min	SS
P-300	Pump	Peristaltic Pump feeds concentrated cells to BR-300	~0 psi	1.75 L/min	SS
P-301	Pump	Peristaltic Pump feeds differentiated cells to V-301	~0 psi	1.75 L/min	SS
P-302	Pump	Peristaltic Pump feeds cells to CS-300	~0 psi	1.75 L/min	SS
P-303	Pump	Peristaltic Pump feeds sorted cells to R-300	~0 psi	1.75 L/min	SS
P-304	Pump	Peristaltic Pump feeds media to BR-300	~0 psi	1.75 L/min	SS

Table 10-Pump Equipment Specification Sheet

Equipment Specification Sheets-Centrifuges					
Equipment Tag	Category	Description	Diameter	RPM	MOC
C-100	Centrifuge	Peristaltic Pump Feeds concentrated cells to BR-200	0.05	1976	SS
C-200	Centrifuge	Peristaltic Pump Feeds media to BR-201	0.1	1329	SS
C-201	Centrifuge	Peristaltic Pump Feeds cells and media to C-201	0.13	1159	SS

Table 11-Pump Equipment Specification Sheet

Equipment Specification Sheets-Support Systems					
Equipment Tag	Category	Description	Temperature	Pressure	MOC
LFC	Sanitation	Provides clean air for first 4 expansion chambers	25°C	1.01bara	SS
Boiler	Sanitation	Boils water for steam-in sanitation	121°C	2.05bara	SS
HEPA Filter	Sanitation	Provides clean air for manufacturing facility	25°C	1.01bara	SS
Cyrostorage	Storage	Keeps NSC product cold until shipping	-150°C	2.02bara	SS
Refrigerator	Storage	Cools NSCs for Cryostorage	-150°C	2.02bara	SS

Table 12-Pump Equipment Specification Sheet

Equipment Cost Summary:

Generally, biomedical facilities have smaller capacities than standard chemical plants, but the price per area is significantly more expensive due to the need for highly specialized equipment and tight physical constraints on the product.[7] In particular, the neural stem cell production facility must be consistent with the current good manufacturing practices as outlined by the FDA.[9,18] Maintenance of the facility is paramount not only to manufacture a quality product, but to ensure the health and safety of the employees on site. A distribution of the fixed capital investments can be seen in Figure 6.

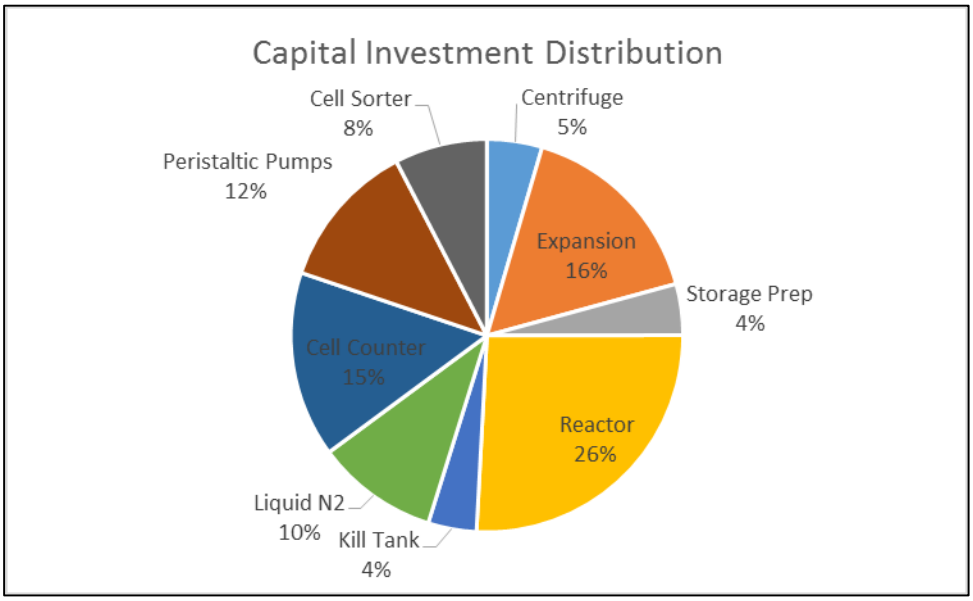


Figure 6-Distribution of Fixed Capital Investment

Figure 6 does not include the capital investment for the boiler unit or the cost of facility construction due to the magnitude of each item. Proper cleanliness/manufacturing of the facility is vital to maintain the integrity of product because a significant portion of the process is open to atmosphere. For example, high efficiency particulate air resistance (HEPA) systems are used throughout the facility in order to keep the air quality consistent with current good manufacturing

practices. Multiple HEPA standard air filters are installed in the air lines to ensure that the air does not contaminate the process. However, compared to the construction cost of the facility, the cost of the ventilation system is relatively insignificant. The cost of the air purifiers is estimated at $14,400 for the entire system.

Similarly, the most important piece of equipment in the process is the bioreactor because it is the source of revenue generation. The reactor takes induced pluripotent stem cells and differentiates them into neural stem cells using a neural induction medium. Tight parameters such as pH, oxygen, and temperature must be stable in order to ensure maximum viability of the stem cells.[23] These process variables are maintained by installing control systems. Furthermore, the reactor needs to be constructed from stainless steel to provide enhanced corrosion and fouling protection. Altogether, the reactor estimate is $259,600.

Although the product of the bioreactor is what ultimately makes the process economical, the expansion chambers allow the process to generate the volume of stem cells needed to meet market demands. The process starts at 100,000 cells and expands to over three billion cells based on the doubling time of the cells.[16] This process is time consuming and needs to be done as efficiently as possible. However, a single expansion chamber cannot be used because the process cannot be controlled appropriately. Therefore, five expansion chambers are used to slowly grow the process from a volume of 1 mL to 25 L. The first four of these chambers are disposable and range in size from a 35mm cell culture plate to a 3 L plastic vessel. Since the chambers are disposable, it is more economical to replace them after each batch rather than adding downtime to steam treat the chambers. Each year it is estimated that the disposable expansion chambers costs $7600. However it is not economical to replace the 40L expansion chamber after every batch. Therefore, a permanent vessel is installed to minimize the capital

investment of the project. However, the vessel needs to be steam treated after each batch. Stainless steel is used in place of plastic to provide a more structurally sound and durable vessel. The last expansion chamber is expected to cost $153,000.

Subsequently, centrifuges provide separation of materials with different densities. For pharmaceutical processes, centrifuges are invaluable because they allow for fine separation between the product and other materials. With regard to neural stem cells production, the unit consists of three centrifuges that vary in size to handle different volumes of fluid. The first centrifuge is a closed system, where the process is put into a vial compatible with the centrifuge. The last two are open systems where the stem cells stick to the outside of the unit while the waste passes through. The cells are then washed to force them into the next step of the process. The cost of the three centrifuges combined is estimated at $38,700.

The kill tank can be modeled as a vessel even though it reacts with protein coating on viruses to inactivate them. Formaldehyde is bubbled through the vessel using a sparger to ensure the stem cells do not have viruses in solution. The viral inactivation chamber is made of stainless steel for quality and durability and is estimated at $40,100.

After viral inactivation, the product goes to a cell separation unit that removes undifferentiated stem cells and excess media from the neural stem cells. This unit is estimated at $76,700 and gives a high purity product.

Lastly, the cells are sent to a vessel that adds dimethylsulfoxide to ensure proper cryopreservation of the neural stems cells.[15] These cells are then refrigerated in a different unit to bring them to cryogenic storage conditions. The combined cost of both of these units is estimated to be $42,000.

Storage conditions are key to maintaining the cells in cryogenic state and liquid nitrogen is used to keep the cells at these conditions. Storage tanks are utilized to store the cells in the vapor phase of liquid nitrogen.[15] The initial storage tank holds 100,000 induced pluripotent stem cells. The final storage tank holds 365L of liquid nitrogen to preserve the final volume of cells before shipping. Also, a large liquid nitrogen storage tank needs to be present on site to provide fresh liquid nitrogen to account for heat losses in the Dewar. Altogether, there are three liquid nitrogen storage tanks that have been estimated to cost $101,000.

Steam needs to be generated to sterilize all non-disposable equipment and hot water is needed to maintain a constant temperature in the bioreactor. To do this, a boiler must be installed to produce steam. The estimated cost of the boiler has been estimated to be $16,800,000. Lastly, there needs to be work done on the process in order for the fluid to be transferred for automated parts of the facility. Eight pumps are placed in locations where material needs to be transferred from one location to another where manual transfer is either impractical or inefficient. Each pump provides the same volumetric flow rate and the same discharge pressure; therefore, the cost of each pump is the same. In total, the pumps are estimated to cost $122,000.

The total equipment cost is approximately $18 million. This accounts for units in the process, and all units needed to maintain the integrity of the process as well as utilities.

Fixed Capital Investment Summary:

The most expensive part to the entire facility is the building in which the facility is enclosed. It is also the largest portion of the capital investment. For this particular project, the building must be incompliance with Class 1000 GMP Production Facility protocol to meet

standards.[8] The price of the lab can be estimated based on an average laboratory size with above average cost per gross square foot (gsf). In 2014, the average cost per gross square foot was $725-834 and facility size ranged from 100,000-200,000gsf. By choosing a 150,000gsf facility that costs $800/gsf, the facility can be estimated to cost around $100 million.[8] The relatively high estimation stems from two key factors: the large space required for the pharmaceutical process and the high standard for cleanliness. In comparison, the cleanliness of the facility is that of a hospital operating room. This increases the cost of the facility considerably.

Moreover, the equipment listed above describes the bare modulus cost. However, there are additional costs to consider that are independent of the cost of production. Turton et. al. generalizes these costs into a "contingencies and fees" category. Included in this category are costs for shipping, installation, and labor that arise after the piece of equipment is built.[25] The result of these additional costs is the total installed cost. An 18% rate of contingencies and fees is assumed for this project in accordance with heuristics in Turton et al. The total installed cost is a function of the bare modulus cost as seen in Equation 3.

$$C_{TM} = 1.18 C_{BM} \quad (3)$$

Where:

C_{TM} = Total Installed Cost

C_{BM} = Bare Modulus Cost

Stainless steel was chosen for this process because the higher quality metal provides the corrosion resistance needed to keep the process clean while still being relatively inexpensive. Moreover, carbon steel introduces rust into the process over time; therefore, a higher quality metal is installed in piping to keep the process clean. Other exotic metals would perform better, but stainless steel is compatible with the system and the most economical option. Since piping is

in contact with the process, stainless steel is used to prevent contamination. The cost is not estimated in this report because not enough information is known regarding the physical layout of the facility, but an upgrade in piping metallurgy needs to be taken into economic consideration when the detailed design phase begins. A summary of the fixed capital investment can be seen in Table 13. The costs included in the table are the total installed cost of each piece of equipment.

Fixed Capital Cost Summary		
Bioreactor	$	259,600
Permanent Expansion Chamber	$	153,400
Small Centrifuge	$	12,900
Medium Centrifuge	$	15,300
Big Centrifuge	$	17,500
Kill Tank	$	40,100
Cryopreparation Tank	$	17,500
Liquid Nitrogen Storage Tank	$	84,300
Boiler Unit	$	19,722,000
Refrigeration Unit	$	24,780
Cryo Storage Unit	$	16,190
Cell Counter	$	153,400
Laminar Flow Cabinet	$	14,285
HEPA Filters	$	19,580
Warm Water Tank	$	3,500
Peristaltic Pumps	$	122,000
Cell Sorter	$	76,700
Facility Construction	$	101,422,000

Table 13-Summary of Fixed Capital Cost

When batch size is small the process needs to be controlled manually, but when the batch is large enough, it can be controlled more precisely through automation, introducing an extra cost in the fixed capital. Similarly, automated parts of the process are controlled via a distributed control system. Specifically, the bioreactor and the final expansion chamber have control systems designed to keep the process at optimal conditions. These conditions are very sensitive

and directly affect the viability of the stem cells. Also, error is increased when operators have more interaction with the process. However, the process cannot be fully automated due to the small volume of the early processes. Automating the process would cause some material to not be transferred, causing a significant decrease in the yield of a batch. On the same hand, extreme delicacy is required for the early stages of the batch process, which cannot be accomplished with automation. This becomes favorable when the batch is larger because human control becomes more difficult as the capacity increases.

To estimate the cost of the control systems for the facility, a standard estimation was used and added onto the installed cost of the equipment. This includes the control valve, logic controller, measurement device, and the electrical wiring. Automated controls are critical on the bioreactor, where temperature, oxygen concentration, and pH need to be tightly controlled. Ultimately, significant extra expenses apart from the cost of equipment need to be considered when determining the economic viability of a project.

Safety, Health, and Environmental Considerations:

A unique set of parameters are required for facilities with biological components are required to satisfy OSHA and FDA requirements. Keeping all employees safe and protecting the environment is the most important priority for the facility. Failure to do either of these results is unacceptable in operation. Material that is not sent to the packaging and formulation group needs to be treated properly in order to comply with environmental regulations. From an environmental standpoint, biological waste needs to be handled properly to prevent widespread contamination, but non-biological waste is still treated with the normal precautions. Biological material needs to be inactivated before other disposal methods can be implemented. However,

there are some materials that are not compatible with the sewer system regardless of the presence of biological organisms.[9] Specifically, DMSO is not compatible with the sewer system and needs to be disposed in an organic waste container. Ultimately, an operating procedure detailing the disposal methods for each material in the process is created to prevent incidents in the facility.

Health of employees is maintained through proper operating and safety procedures. Employees handling hazardous chemicals will wear the proper personal protective equipment as outlined by the materials safety and data sheet. Proper handling of biological waste is critical because it presents a unique hazard. All biological waste is inactivated via formaldehyde or steam treatment. A table outlining the material properties present in the facility can be seen below. Most materials do not present high risk hazard and are handled by the process design. A list of key properties of chemicals used in the process is seen in Table 14.

Material	Molecular Weight (g/mol)	Normal Boiling Point (°C)	Flammability Limit (%)	Flash Point (°C)
Induced Pluripotent Stems Cells	-	-	-	-
Neural Stem Cells	-	-	-	-
Expansion Media	-	-	-	-
Reactor Media	-	-	-	-
Trypsin	23.3 kDa	Denatures at 50°C	N/A	N/A
Dimethylsulfoxide	78.13	189	2.6-3.5	50
Formaldehyde	30.03	-19	7.0-71.0	60
Nitrogen	28.02	-195.8	None	N/A
Water for Injection	18.02	0	None	None
EDTA	292.24	614.2	None	93.3

Material	Autoignition Temperature (°C)	Liquid Density (kg/m^3)	Reactivity with Water	Toxicity Limits (ppm)
Induced Pluripotent Stems Cells	-	1125	-	-
Neural Stem Cells	-	1125	-	-
Expansion Media	-	1100	-	-
Reactor Media	-	1100	-	-
Trypsin	N/A	N/A	Soluble	None
Dimethylsulfoxide	N/A	1101	Hygroscopic	7920
Formaldehyde	430	1080	Soluble	42
Nitrogen	None	807	None	N/A
Water for Injection	None	1000	N/A	N/A
EDTA	N/A	860	Soluble	N/A

Table 14-Material Properties Summary

Since very low temperatures are used for cell preservation, proper protective equipment is used to prevent skin damage due to contact from liquid nitrogen. Likewise, DMSO is an incredibly miscible solvent and diffuses through skin upon contact. Other hazards originate from low pressure steam and other cleaning procedures. Low pressure steam generated from the boiler unit causes burns when exposed to open skin. Therefore, procedures are placed around the stream outlet. All materials safety and data sheets are onsite and readily available in an effort to keep all operators safe and knowledgeable. The material safety and data sheets are also included in the Appendix.

Moreover, the process is designed to keep all material inside of the pipes through units that are closed systems. The controls system is designed to keep the process variables at the desired set points, prevent contamination, and maintain the integrity of the facility. However, some parts of the process have to be performed manually. Operators and lab technicians will be

trained on how to perform the critical, manual steps in the process as well as emergency shutdown procedures. The NOAA provides an interaction matrix where a hazards associated with chemicals can be simulated. Table 15 shows the results of the interaction matrix.

	FORMALDEHYDE (P)			
DIMETHYL SULFOXIDE	**Caution** Potentially hazardous	DIMETHYL SULFOXIDE		
NITROGEN, REFRIGERATED LIQUID (CRYOGENIC LIQUID)	**Caution**	**Compatible**	NITROGEN, REFRIGERATED LIQUID (CRYOGENIC LIQUID)	
EDTA	**Incompatible** Generates gas Generates heat Intense or explosive reaction Polymerization hazard	**Compatible**	**Compatible**	EDTA
WATER	**Caution** Polymerization hazard	**Compatible**	**Compatible**	**Caution** Corrosive

(P) = Potentially self polymerizable

Table 15-Interaction Matrix Depicting Material Compatibility

The results indicate that the only incompatible materials are formaldehyde and EDTA; therefore, these materials need to be separate to prevent incompatible mixing of chemicals. EDTA is used in conjunction with trypsin to remove the cells from the bioreactor to send it to the inactivation tank, which has formaldehyde. However, the reaction does not occur at the concentrations present in the tank. If the concentrations are ever increased, EDTA is removed from the system in order to keep the process safe.

A key part of maintaining the scheduled production rate is the downtime, where operators need to sterilize and clean equipment efficiently to minimize the shutdown time. To ensure minimal downtime, all personnel are trained on FDA cGMP practices and facility procedures prior to starting work.[4] In addition to the procedures in place to prevent a workplace injury, inherent safety considerations have been included in the design to minimize the potential for an accident. A summary of these inherent safety procedures can be seen in Table 16. The hazards

seen below address designs within the system and outline the action needed to safely deal with each hazard in the facility.

Hazard	Inherent Safety Concept	Action
Extreme storage temperatures	Simplification	Implement concise operating procedure
Biological material	Attentuation	Maximize automation of process/dilute material before treatment
Toxic Cryopreservative	Minimization	Automated Injection Volume
Low Pressure Steam	Moderation	Calculate volume to sterilize equipment
Toxic Waste	Attenuation	Dilute waste before disposal
Atmospheric Contamination	Substitution	Utilize high purity air/water instead of tap water/ambient air
Mechanical	Simplification	Sufficient training in operation of centrifuges/pumps

Table 16-Summary of Inherent Safety Designs

Particularly, pinch hazards arise when maintenance is performed on centrifuges and peristaltic pumps. Therefore, sufficient training in maintenance and repairs is needed to simplify the process. Furthermore, the steam used for cleaning is an open system and extra protective layers are installed to keep operators safe. Hot work gloves are worn at all times to prevent burns to the skin in addition to standard PPE. Implementing inherent safety concepts within the design of the facility minimizes the risk of recordable injuries.

All cleaning procedures are in compliance with FDA standards and provide the company with a safe working environment as well as sterile biological products. Any vessel that contains biological material is steam cleaned for 3 minutes as per FDA guidelines[21]. Any material sent to the sewage system is subject to the constraints set by the city. Regardless of regulations, no living organisms can be sent to the sewer. Therefore, living waste is treated by inactivation via a detergent before it is properly disposed. Table 17 below summarizes the potential consequences associated with failing to prevent hazards present in the process. The consequences are assessed on a “Low-Medium-High” Scale.

Hazard	Equipment Damage	Environmental Compliance	Loss of Life	Disruption of Other Business Units	Legal/PR	Community Impact
Biological Waste in Sewer	-	Medium	-	-	High	High
DMSO Release	-	Low	Low	Low	Low	-
Formadehyde Release	-	-	Low	Medium	Medium	-
Contamination of Process	-	Medium	-	High	-	-
Steam	-	-	Low	-	Medium	-

Table 17-Potential Consequences Summary Table

The first measure that needs to be taken in order to comply with current good manufacturing practices is HEPA quality air within the facility. Air circulating in the facility is required to be medical grade to prevent stem cell contamination and ensure maximum growth. Furthermore, water for injection is required for any application where water comes in contact with cells to prevent contamination as well. The water needs to be as pure as possible since it will be injected into patients with the stems cells. FDA standards require an increase in the purity of water from that of tap water[4]. Moreover, existing safeguards are used in order to minimize the risk within the process. Stainless steel is used in place of carbon steel for all permanent process equipment to prevent corrosion or contamination and plastic or glass is used for all disposable parts. Also, operators are required to wear special personal protective equipment to prevent contact with the cells. This prevents contamination of both the operator as well as the stem cells. All of these materials are the result of an inherently safe facility[9].

In conclusion, there is no potential for project termination based solely on safety concerns. However, the process needs to be approved in the United States in order for the project to begin. Currently, any treatment involving the usage of stem cells is not permitted by the FDA. Operator training is critical because of the delicacy of the process and must be significant. As a whole, the facility present no significant safety dangers to the employees or surrounding community. Overall, proper safety and environmental procedures will be followed to ensure a safe, efficient work environment.

Other Important Considerations:

In order to meet production needs multiple batches a year are needed. This requires finding the proper amount of batches as well as the scheduling of each batch. To do this, each step in the batch must be timed and the next step ready as soon as the previous is done. The trick in scheduling is finding when to start the next batch. To accomplish this task, Intelligen SuperPro Designer is used. The software allows for input of process and time steps in a batch operation. The chart tools can then find a schedule that allows for the calculated number of batches to be achieved. After building a simulation a Gantt chart was generated using SuperPro. This chart is seen below in Figure 6. From Figure 6, it can be seen that there is an overlap of 169 hours that was determined by the software. This accounts for the time to set up and clean the vessels from the previous batch as well as start the next. There is also a service factor of 0.75 accounted for in this batch timing for FDA inspections and equipment maintenance during the year to comply with cGMPs. Figure 7 below also shows the time steps for each process in the batch with time amounts in hours next to each step. The longest by far is the differentiation but the early expansions take a considerate amount of time of the batch as well.

Another important decision made during evaluation of this project was the choice of expansion medium and differentiation medium. The expansion medium was chosen to be Essential 8 from Thermo Fisher. This media was selected because of the success in growing large numbers of pluripotent stem cells through multiple passages with no karyotype issues found. In conjunction this media does not require extra CO_2 for the buffering system. The differentiation media was chosen to be Gibco PSC Neural Induction Medium. This medium was selected for availability and functionality of turning pluripotent cells into neural stem cells. This media is widely available for purchase from Thermo Fisher in large amounts that are needed.

This media also has the functionality that is needed to induce PSCs to neural stem cells. This makes it a top choice for induction. Also there is no CO_2 needed for buffering thereby reducing the cost of operation. Another reason is that while the cells are being differentiated they are still able to expand by a factor of 20 with this media over the course of 7 days. This is also a shorter time span than other mediums used for induction. The properties described above make the two choices clear when compared to other forms of media.

		Task	Duration (h)	Start Time (h)	End Time (h)	Desc
1		Complete Recipe	537.68	0.00	537.68	
53		Complete Recipe (Batch #2)	537.68	368.39	906.07	
105		Complete Recipe (Batch #3)	537.68	736.78	1274.46	
157		Complete Recipe (Batch #4)	537.68	1105.17	1642.85	
209		Complete Recipe (Batch #5)	537.68	1473.57	2011.24	
261		Complete Recipe (Batch #6)	537.68	1841.96	2379.64	
313		Complete Recipe (Batch #7)	537.68	2210.35	2748.03	
365		Complete Recipe (Batch #8)	537.68	2578.74	3116.42	
417		Complete Recipe (Batch #9)	537.68	2947.13	3484.81	
469		Complete Recipe (Batch #10)	537.68	3315.52	3853.20	
521		Complete Recipe (Batch #11)	537.68	3683.92	4221.59	
573		Complete Recipe (Batch #12)	537.68	4052.31	4589.99	
625		Complete Recipe (Batch #13)	537.68	4420.70	4958.38	
677		Complete Recipe (Batch #14)	537.68	4789.09	5326.77	
729		Complete Recipe (Batch #15)	537.68	5157.48	5695.16	
781		Complete Recipe (Batch #16)	537.68	5525.87	6063.55	
833		Complete Recipe (Batch #17)	537.68	5894.27	6431.94	
885		Complete Recipe (Batch #18)	537.68	6262.66	6800.33	

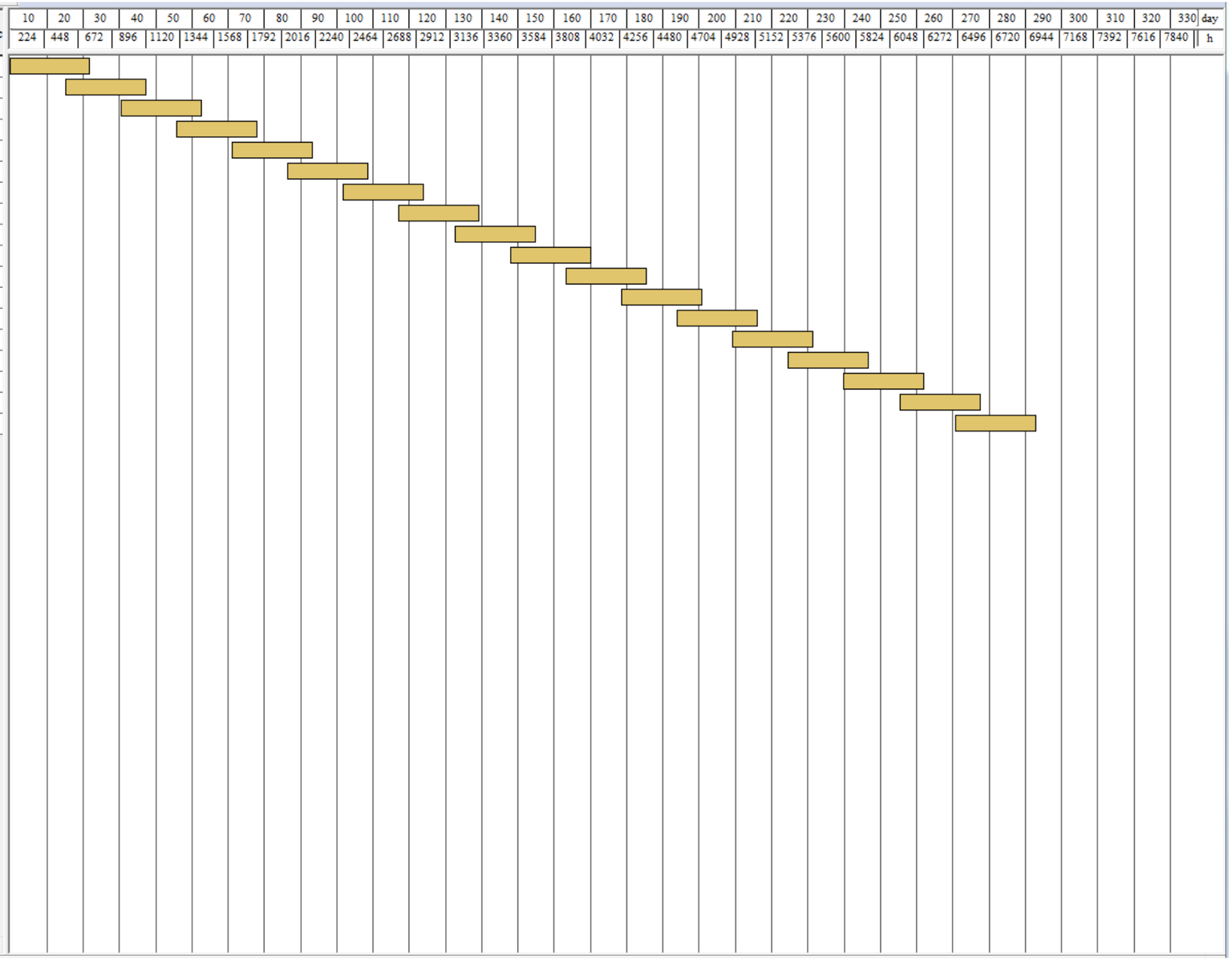


Figure 6-Gantt chart for Yearly Production

Duration (h)	Start Time (h)	End Time (h)	
537.68	0.00	537.68	
84.75	0.00	84.75	T-Flask
363.39	0.00	363.39	Media Prep
88.75	84.75	173.50	T-Flask
0.03	173.25	173.28	Centrifugation
86.75	173.28	260.03	Shake Flask
0.08	259.78	259.87	Centrifugation
86.76	259.87	346.62	Shake Flask
0.08	346.62	346.71	Centrifugation
15.49	346.62	362.12	Seed Cell Culture
0.08	362.12	362.20	C1
168.93	362.20	531.13	Cell Culture
4.00	531.13	535.13	Cell Sorting
2.55	535.13	537.68	Freeze-Thaw Module

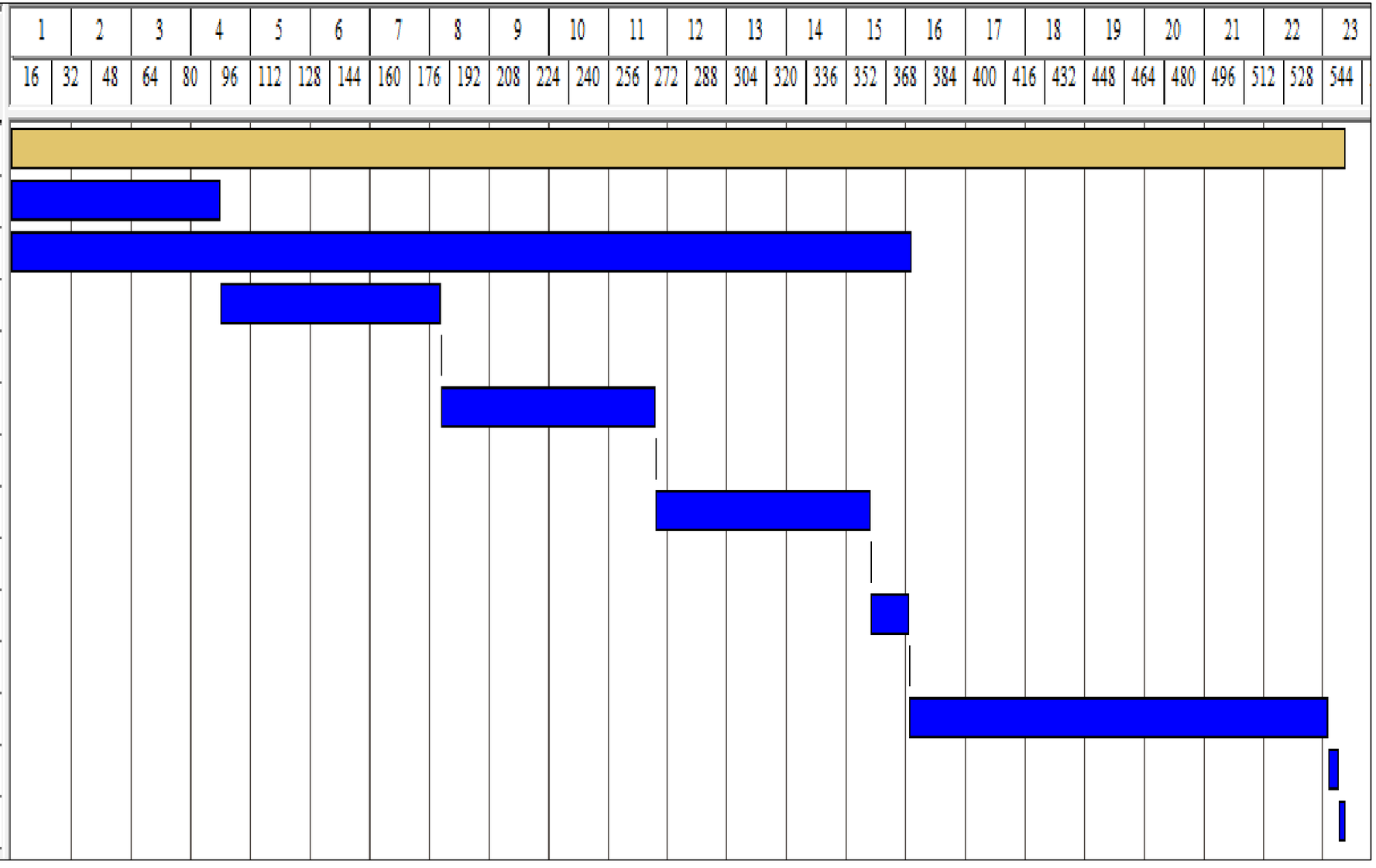


Figure 7-Batch process time sheet

Manufacturing Costs:

Cost of manufacturing can be divided into three categories: direct costs, fixed costs, and general expenses. Direct costs and fixed costs make up operating expenses that change with the rate of production and costs that are independent of changes in production rate, respectively. While general expenses represent the overhead required to perform everyday business function.

Total costs can be estimated as the sum between the three types of cost seen above. The formula for calculating the total costs is given by Turton et. al. is seen in Equation 4.[25]

$$Total\ Cost = C_{RM} + C_{WT} + C_{UT} + 2.215C_{OL} + 0.19COM + 0.146FCI + depreciation \quad (4)$$

Where:

C_{RM} = Cost of Raw Materials

C_{WT} = Cost of Waste Treatment

C_{UT} = Cost of Utilities

C_{OL} = Cost of Operating Labor

COM = Cost of Manufacturing

FCI = Fixed Capital Income

By estimating total manufacturing costs, the day to day plant operation costs can be estimated.

Operating labor provides a significant capital and personal investment. Highly skilled operators are required for this process, which makes their services more expensive. Simply having the skills is not enough; operators must be trained specifically to the process. The number of operators can be calculated from Equation 5.

$$N_{OL} = (6.29 + 31.7P^2 + 0.23N_{NP})^{0.5} \quad (5)$$

Where:

N_{OL} = Number of Operators per shift

P = Number of Processing Steps that Involve Handling of Particulate Solids

N_{NP} = Number of Nonparticulate Processing steps

The pieces of equipment can be summed and plugged into Equation () to show a need for four operators per shift. For the stem cell manufacturing facility, there are no steps that involve handling of particulate solids. Moreover, pumps and vessel are not included in account for N_{NP} in Equation 5.

Operators for this process need to be extensively trained and highly skilled in order to operate the process efficiently and precisely. Therefore, it is assumed that their average salary is significantly higher than that of an average operator. A salary of $110,000 per year is used in order to estimate the total cost of operating labor. Lastly, Equation 5 produces a need for four operator on site at all times. Turton et. al[25] uses Equation 6 to estimate the total number of operators needed to be hired for the manufacturing facility.

$$Total\ Hired\ Operators = 4.5\ N_{OL} \quad (6)$$

Eighteen operators will be hired to run the facility at all times. Therefore, the total cost of operators per year is estimated to be $2,000,000. As seen in Figure 8, the cost of operating labor is a large percentage of the total manufacturing cost associated with a facility.

The utilities costing was done with information given in the AIChE National Design competition memo. Utilities for the neural stem cell manufacturing consist of boiler feed water,

water for injection, electricity, sewer water, and liquid nitrogen. Boiler feed water is used to provide low pressure steam to sterilize all non-disposable equipment after each batch as well as maintain isothermal conditions in the bioreactor and expansion chamber. Electricity is used to power the boiler unit, peristaltic pumps, centrifuges, and agitators in the bioreactors. Furthermore, water for injection is added to the stem cells in order to store them at the appropriate concentration. Usage of the sewer is used for disposal of waste that is compatible with city sewer systems; other waste must be treated before other disposal method are incorporated. Lastly, liquid nitrogen is used to keep stem cells at cryogenic storage conditions. A summary of the utilities can be seen in Table 18 below.

Utility	Cost Data	Total Yearly Cost
Electricity (per kWhr)	$ 5.00	$ 10,678
Sewer (per 1000 gal)	$ 5.00	$ 5.22
Water for Injection (per 1000 liters)	$ 1,000	$ 7,600
Liquid Nitrogen (per liter)	$ 0.25	$ 1,734
Boiler Feed Water (per 1000 kg)	$ 2.45	$ 0.20

Table 38-Estimated Yearly Utility Costs

All yearly costs are in 2016 dollars and account for all utilities needed to perform the process. Utilities that are used outside of the process are not included due to lack of knowledge. For example, the electricity used for lighting in the facility is not included because not enough information is known presently. Ultimately, utility costs make up a relatively small amount of the total yearly manufacturing costs.

Pharmaceutical manufacturing facilities tend to have more raw materials than standard chemical plant due to the number of additive for the process. Although the induced pluripotent stem cells are relatively cheap, other raw materials are significantly more expensive such as media. Table 19 below summarizes the yearly cost for raw materials.

Raw Material	Yearly Cost	
Induced Pluripotent Stem Cells	$	1,753
Expansion Media	$	328,028
Differentiation Media	$	4,600,620
Trypsin w/ EDTA	$	1,313
Formaldehyde	$	33,526
Dimethylsulfoxide	$	180,804

Table 19-Summary of Yearly Raw Material Costs

The other factor taken into consideration in Equation 4 includes estimations based on the cost of manufacture and fixed capital investment. These play a significant role in the manufacturing cost of the process, and have been calculated in previous sections. The Figure below illustrates the proportions of the three types of cost present in the facility.

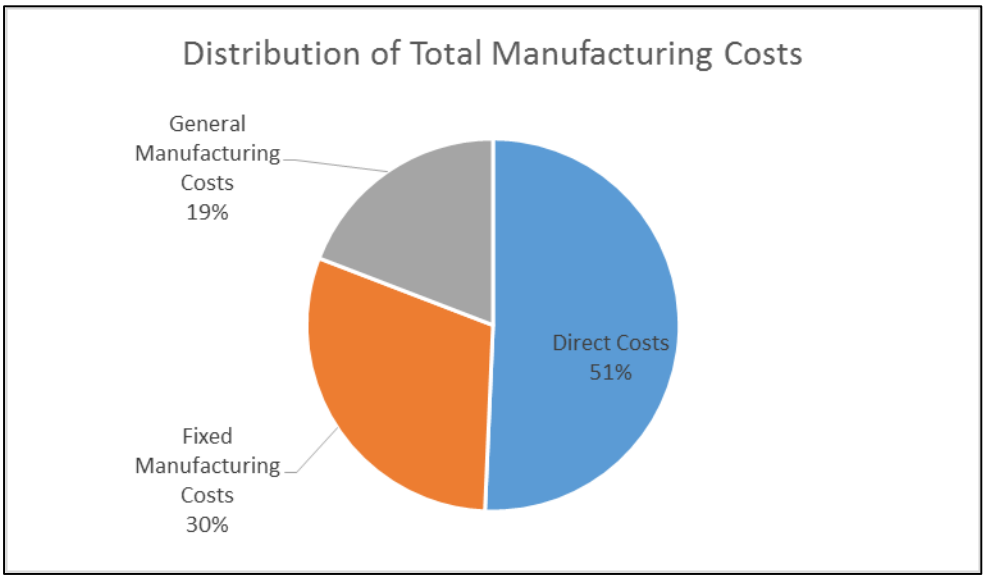


Figure 8-Distribution of Manufacturing Costs

Even though depreciation is included in the formulation of these costs, Figure 8 does not include depreciation because it changes independent of escalation.

Economic Analysis:

The driving force behind this project is the economic benefit to the company undertaking the risk of pursuing it. The minimum rate of return given that the project must pass to be profitable is 50%. This represents the minimum amount of return that the project could support before outside investment would be more profitable to pursue.

The following section details the capital estimation of the equipment that could not be directly cost from the AICHE GE spreadsheet. The methods used are consistent with Turton et. al.[25] for costing equipment. A list of equipment not costed from GE are seen below in Table 20 with the classification for sizing with key sizing parameter.

Equipment	Classification	Key Parameter
Boiler	Electric Heater	kW
Peristaltic Pumps	Positive displacement pumps	kW

Table 20-4EquipmentCcosted from Turton et. al.

For costing these pieces of equipment, Equation 7 below was used from Appendix A.1 in Turton[25]. This equation calculates the equipment free onboard cost or the manufacture cost without shipping included in 2001. To move from 2001 to 2016 dollars the CEPCI was used. The values for the CEPCI is seen below as well. The CEPCI is used by dividing the current by the past and multiplying this ratio by the C_p amount.

Equipment	K1	K2	K3	CEPCI 2001	CEPCI 2016
Centrifuges	4.7681	0.974	0.024	397	560
Electric Heater	6.9617	-1.48	0.3161		

Table 215-Costing factors for equipment

$$\log_{10}(C_p) = K_1 + K_2 \log_{10}(A) + K_3(\log_{10}(A))^2 \quad (7)$$

Where:

C_p = Bare Bones Cost

K_i = Cost Factor

A = Key Sizing Parameter

The next step to cost these pieces was to account for the MOC and the pressure requirements of the equipment. Since pressure was low for both pieces of equipment the pressure factor was determined to be 1. The costing equation for total installed cost is seen below for both pieces. Equation 8 is used for centrifuge sizing while Equations 9 and 10 is for the steam boiler. The boiler has special considerations as the steam superheat temperature is critical to cost of manufacture. The bare module cost takes into account the piping, controls, and instrumentation needed for these pieces of equipment as well as the cost of the unit and shipping.

$$C_{bm} = C_p F_{bm} \quad (8)$$

$$C_{bm} = C_p F_{bm} F_{\Delta T} \quad (9)$$

Where:

C_{bm} = bare module cost

F_{bm} = costing factor for C_{bm}

$F_{\Delta T}$ = degree of overheat factor from equation 9

and

$$F_{\Delta T} = 1 + 0.00184\Delta T - 0.00000335(\Delta T)^2 \quad (10)$$

Where:

$$\Delta T = amount\ of\ super\ heating\ in\ C^o$$

Using these equations the three centrifuges and boiler unit were costed. The boiler was the most expensive by far due to the nature of a boiler unit and that it needed to be a certain size over required to meet regulations.

The final cost used in discounted cash flow analysis was calculated from C_{bm} and is detailed below in Equation 11. This is the total cost with contingencies and fees. This takes into account a buffer of 15% overages on construction or cost of materials and 3% of cost of the project in fees. These numbers were chosen as a representative sample of normal project planning guidelines discussed in Turton et. al[25]. This gives a total of 18% for the over estimation of the overall cost of each piece of equipment.

$$C_{tm} = 1.18C_{bm} \quad (11)$$

Where:

C_{tm} = total cost with contingency and fees

C_{bm} = bare module cost

The application of these equations to three centrifuges and the steam boiler is seen below in Table 22. The centrifuges are of average to small size for bioprocesses. The first one is a small bench top model that uses disposable vials. The second is a larger bench top model that uses larger half liter to liter centrifuge vials but is still manual. The third centrifuge process the cells produced by the fifth expansion chamber and is made of stainless steel and is not disposable. The MOC for the boiler is carbon steel and the thickness is consistent with the pressure. The C_p, C_{bm}, C_{tm}, and key costing parameter are seen below in Table 22.

Equipment	Cp	Cbm	Ctm	Key Paramter
C-100	$12,561.00	$19,721.00	$32,855.00	Diameter= .2 m
C-200	$5,967.00	$9,369.00	$15,608.00	Diameter= .09 m
C-201	$16,088.00	$25,258.00	$42,079.00	Diameter= .26
Boiler	$3,949,624.00	$11,848,871.00	$19,722,252.00	Duty (kW)=26349

Table 22-Cost of Equipment

Incorporating cost calculated with the costs sourced from the GE data gives a total capital estimate of $21,260,000; this does not include the building cost. The boiler was the largest individual cost at $19,720,000. This can be attributed to the cost of all of the controls and piping needed for this system. A summary of equipment costs can be seen in Table 23 below; the items highlighted are costed from Turton et al[25]. All others were costed using GE data as well as internet data.

Fixed Capital Cost Summary		
Bioreactor	$	259,600
Permanent Expansion Chamber	$	153,400
Small Centrifuge	$	12,900
Medium Centrifuge	$	15,300
Big Centrifuge	$	17,500
Kill Tank	$	40,100
Cryopreparation Tank	$	17,500
Liquid Nitrogen Storage Tank	$	84,300
Boiler Unit	$	19,722,000
Refrigeration Unit	$	24,780
Cryo Storage Unit	$	16,190
Cell Counter	$	153,400
Laminar Flow Cabinet	$	14,285
HEPA Filters	$	19,580
Warm Water Tank	$	3,500
Peristaltic Pumps	$	122,000
Cell Sorter	$	76,700
Facility Construction	$	101,422,000

Table 236-Total Equipment cost

It is worth noting that no equipment was spared because all equipment is easily ordered with quick lead times on shipping.

Revenue:

The revenue for this project comes from the sale of the stem cells as therapy. An assumption made is that the stem cells can be sold at a bulk price not seen in the current market. This means that the current rate of $520.00 is much higher than the therapy cells would sell for. Using the current selling price as a reference a final sale price of $250 per million cells to ensure a reasonably affordable treatment option for patients. Multiplying this by the number of million cells produced a year yields a sales revenue of $230,000,000.

In order to determine the minimum sale price of the neural stem cells to achieve a rate of return of 50%, a breakeven analysis was performed. The result of this analysis was a sale price of $150 per million neural stem cells. This confirms that the breakeven analysis is accurate since the minimum rate of return given in the memo is 50%.

DCFROR:

Combining the revenue and utilities cost with capital investment gives a basis to perform a discounted cash flow rate of return analysis (DCFROR). This analysis takes into account the tax rate of 40% normally seen as well as depreciation and escalation of equipment and operating costs. An escalation rate of 0.86% was determined from the Department of the Treasury, using factors published each month. The depreciation rate was done using MACRS for a 5 year depreciation life with a half year assumed in year 1.

The major factors that affect the DCFROR are building cost, and boiler cost. These two costs contribute the most to the initial capital investment that in turn has a large effect on the rate

of return and net present value. These costs were minimized then to increase the viability of the project. To determine how viable the project is, the DCFROR was compared to the given i* of 50%. Any DCFROR below this point was deemed unviable for continuation. The project was determined to have a NPV of $95,730,000. This gives a DCFROR of 97.8% which is greater than i* therefore the project is viable.

The cash flow for the project is seen below in Table 25. The company situation was assumed to be stand alone as the statement stated that this is being pursued by a small to midsized biomedical company. This means that there is loss forward in the event of negative taxable income.

A return on investment comes when the project starts returning money and no longer paying off debts. There are two commonly reported times, which are undiscounted and discounted payback periods. The only difference is the discount to account for the time value of money. The payback time for the discounted period is found to be 1.27 years while the undiscounted is 0.995 years. These both confirm that the project is economically attractive to pursue as the return on investment comes before the project is over. A summary of this data is shown below in Table 24.

Estimated Payback Periods	
Discounted Payback Period	0.995
Undiscounted Payback Period	1.270

Table 24-7Payback Periods

Project Title:	Neural Stem Cell Therapy					
Corporate Financial Situation	Stand Alone					
¡*	0.5					
Other Relevant Info	MACRS	*Depreciation				
1= $1	1/1/2017	1/1/2018	1/1/2019	1/1/2020	1/1/2021	1/1/2022
End of Year	0	1	2	3	4	5
Sales Revenue	0	$ 232,180,000	$ 234,180,000	$ 236,190,000	$ 238,220,000	$ 240,270,000
+Salvage Value	0	0	0	0	0	0
-Royalties	0	0	0	0	0	0
Net Revenue	0	232,180,000	234,180,000	236,190,000	238,220,000	240,270,000
- Expansion Media Cost	0	$ (331,000)	$ (334,000)	$ (337,000)	$ (339,000)	$ (342,000)
- Reactor Media Cost	0	$ (4,633,000)	$ (4,673,000)	$ (4,713,000)	$ (4,753,000)	$ (4,794,000)
- DMSO	0	$ (182,000)	$ (184,000)	$ (186,000)	$ (187,000)	$ (189,000)
- Formaldehyde	0	$ (33,800)	$ (34,100)	$ (34,400)	$ (34,700)	$ (35,000)
- Induced Pluripotent Stem Cell	0	$ (1,800)	$ (1,800)	$ (1,800)	$ (1,800)	$ (1,800)
- Liquid Nitrogen	0	$ (6,700)	$ (6,800)	$ (8,100)	$ (6,900)	$ (6,900)
- 0.05% Trypsin w/ EDTA	0	$ (21,720)	$ (21,900)	$ (22,140)	$ (22,280)	$ (22,470)
- Disposable Expansion Chambers	0	$ (9,157)	$ (9,239)	$ (9,322)	$ (9,393)	$ (9,475)
-Cell Sorting Kits	0	$ (616,292)	$ (621,592)	$ (626,938)	$ (632,329)	$ (637,767)
- Boiler Feed Water	0	$ (10,800)	$ (10,900)	$ (11,000)	$ (11,100)	$ (11,100)
- Operating Labor	0	$ (2,420,640)	$ (2,441,458)	$ (2,462,454)	$ (2,483,631)	$ (2,504,990)
- Manufacturing Costs	0	$ (22,690,000)	$ (22,890,000)	$ (23,080,000)	$ (23,280,000)	$ (23,480,000)
- Utilities	0	$ (19,800)	$ (19,900)	$ (20,100)	$ (20,300)	$ (20,500)
Building Utility Costs	0	$ (229,961)	$ (231,938)	$ (233,933)	$ (235,945)	$ (237,974)
- Depreciation	0	$ (6,849,313)	$ (9,401,047)	$ (6,679,197)	$ (5,046,087)	$ (5,046,087)
- Amortization	0	0	0	0	0	0
- Depletion	0	0	0	0	0	0
- Loss Forward	0	0	0	0	0	0
- Writeoff	0	0	0	0	0	$ (89,665,477)
Taxable Income	$ -	$ 194,120,000	$ 193,300,000	$ 197,760,000	$ 201,160,000	$ 113,270,000
- Tax at 40%		$ 77,648,000	$ 77,320,000	$ 79,104,000	$ 80,464,000	$ 45,308,000
Net Income	$ -	$ 116,472,000	$ 115,980,000	$ 118,656,000	$ 120,696,000	$ 67,962,000
+ Depreciation		$ 6,849,313	$ 9,401,047	$ 6,679,197	$ 5,046,087	$ 5,046,087
+ Writeoff		0	0	0	0	$ 89,665,477
-Fixed Capital	$ (122,687,208)	0	0	0	0	0
Cash Flow	(122,687,208)	123,321,313	125,381,047	125,335,197	125,742,087	162,673,565
Discount Factor ($P/F_{i^*,n}$)	1.000	0.667	0.444	0.296	0.198	0.132
Discounted Cash Flow	(122,687,208)	82,214,208	55,724,910	37,136,355	24,837,943	21,422,033
NPV @ i*=	98,648,241					
DCFROR =	99.1%					

Table 258-Cash Flow Table for Neural Stem Cell Project Life

Sensitivity:

To determine the stability of the project a sensitivity analysis on capital investment, operation cost, and selling price. These parameters have the most variability and have a large impact on DCFROR. The initial capital investment was varied ±40%. This variance was chosen as many of the pieces of equipment are highly variable in cost depending on where it is sourced. The operational cost was varied at ±15%. This was chosen as many of the materials are sourced from limited vendors with limited stocks. Finally the revenue was varied ±25%. This was chosen to be representative of the market as more stem cell therapies are approved so competition will increase.

To graphically represent this analysis tornado charts are used. They show the variation in both the NPV and DCFROR of each situation. Overall this tool is useful to gage the variability in the profitability of the project in a dynamic world. Figures 9 and 10 show the NPV and DCFROR Tornado Charts, respectively.

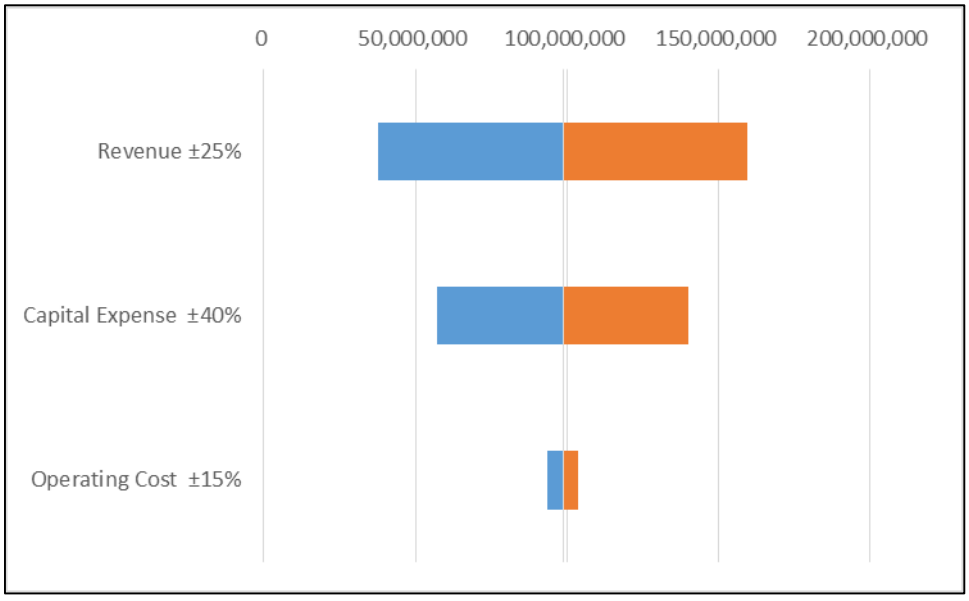


Figure 9-NPV Tornado Chart

The revenue variance has the largest effect on the overall NPV of the project. This is normal as revenue is the source of positive cash flow in the project. The capital expense has an odd shape around the center, because as at higher estimations the building cost dominates the overall expense.

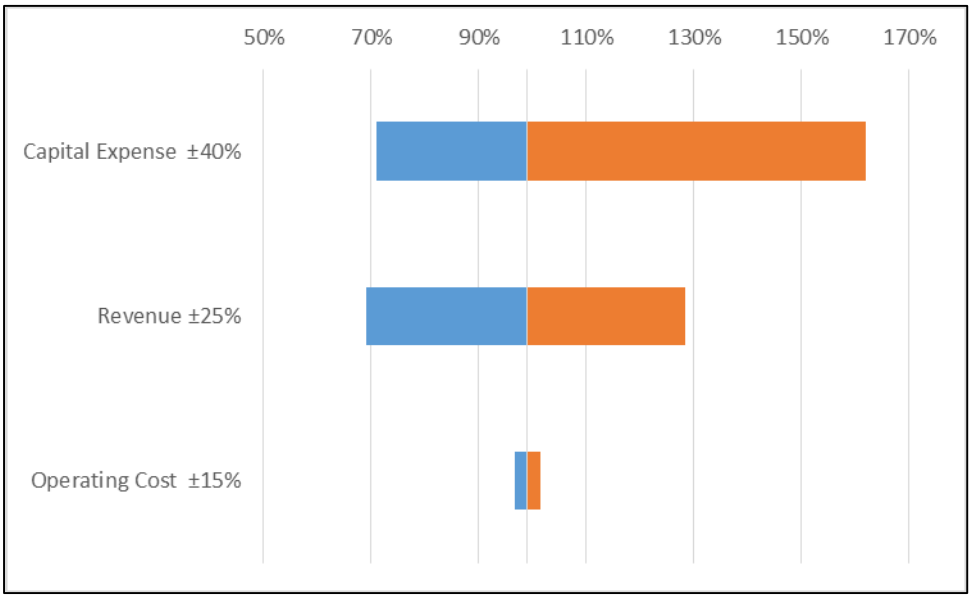


Figure 10-DCFROR Tornado Chart

The DCFROR tornado shows a much larger dependence on capital expense as DCFROR is sensitive to large changes in costs or revenues occurring early in the project. Overall, the minimization and negotiation of operating costs and fixed capital investment is key to keeping the project profitable moving forward into the future.

Conclusions:

Based on the information given in the report it can be concluded that the neural stem cell manufacturing facility is economically attractive. Currently, utilization of stem cells as a type of therapy is not allowed in the United States; the only legal application is biomedical research[27].

Legalization of stem cells therapy will inevitably introduce competition into the domestic market. Proper training is critical to the success of the facility, especially for operator training. Although this presents a challenge, the project is economically attractive. Sensitivity analyses and uncertainty calculations produce a rate of return greater than the minimum rate of return. Moreover, due to the nature of the process, downtime plays a significant factor in the production of the facility. In order to satisfy market demand, optimization of downtime is critical.

As with any project, increasing capacity creates an opportunity to capitalize financially. The memo states that the facility is looking to expand into European and Asian markets in the future, but meeting the demands of the United States is a primary objective. As designed, the facility can meet the demands of the United States over the project evaluation lifetime. It has also been concluded that international expansion in the next 10-15 years is going to produce a significant financial opportunity. However, the facility is designed to handle the additional needs of foreign markets. An example of this is the over sizing of the bioreactor. The unit has the ability to handle double the current capacity, while additional expansion can be performed without disturbing the original process.

Recommendations:

Based on the previous information detailed in the report, it is in the best interests of the company to pursue the project. Small scale testing needs to be done to validate the safety and applicability of the project to the Food and Drug Administration. Also, the company needs to wait for legal rulings before significant capital is invested in the project. Once the patents are obtained for the process, the company needs to act quickly to maximize the 20 year life on the patent. Based on the success of the company, an increase in competition is expected once the

therapy is approved. Lastly, significant upfront cost needs to be avoided prior to approval of the project by the court. Based on the company's size, it cannot afford to invest on a project with a significant up front cost. Overall, proceeding with the project is highly recommended; however, detailed design of the facility needs to occur after the therapy is legalized.

Additionally, the manufacturing facility will most likely need to be scaled up to keep up with market demand. This will call for an increase in capacity, whether that comes from modification of the current process or construction of a new facility. Location of the facility also plays a significant factor in the viability of the project. Regardless of the legal status on the project, location provides the company with an economic edge. Research into the most profitable location is highly recommended in order to maximize the viability of cells during shipping. Moreover, legal issues will decrease when the project is expanded into foreign markets. Stem cell therapy is legal is countries across the world and expansion into these particular markets is highly recommended.

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Appendix

SuperPro Simulation:

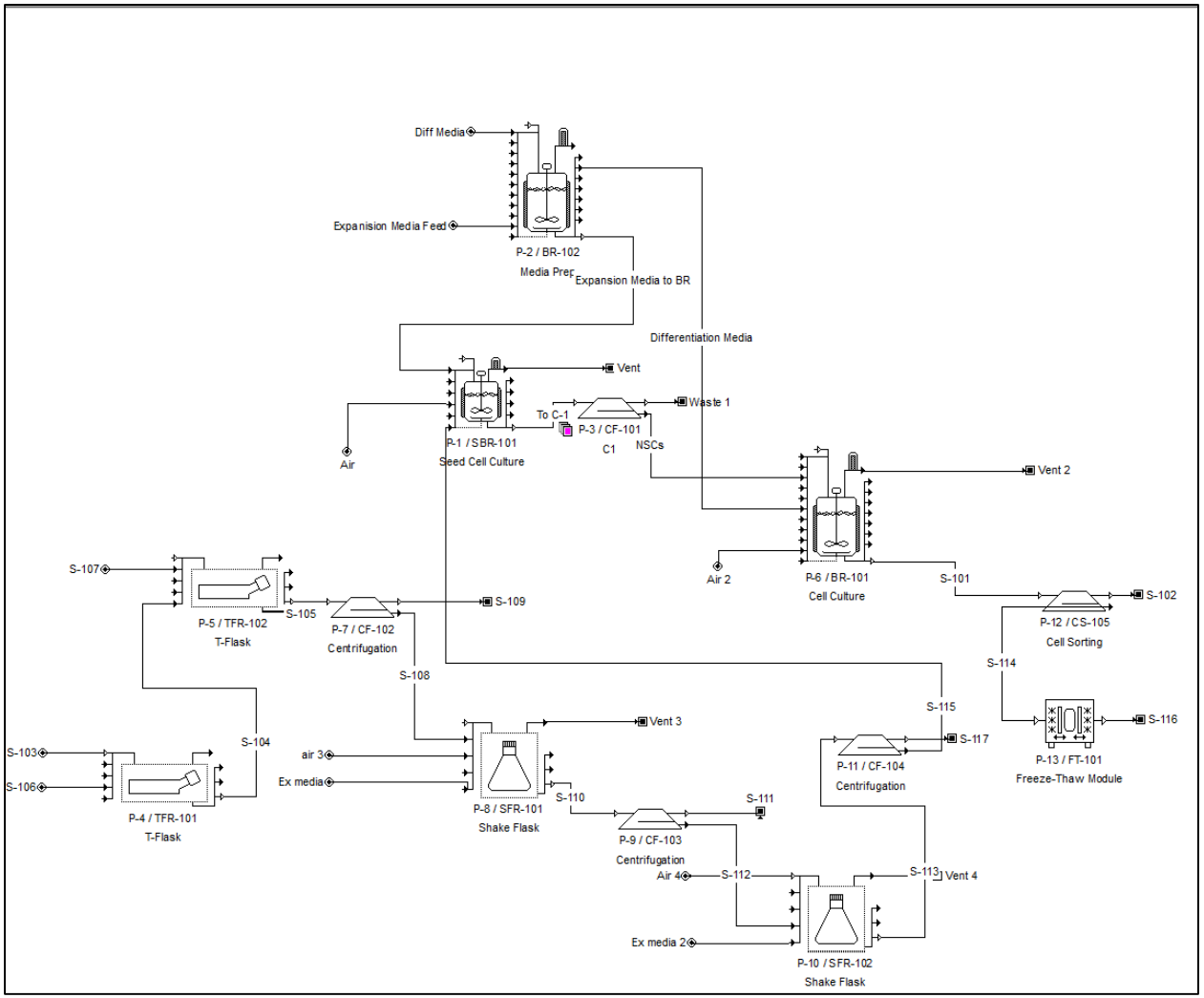


Figure 11-Super Pro Simulation Setup

Material Balances/Energy Balances:

CP-101	
Stream	Mass (g)
1	0.00
2	1.10
5	1.10
In-Out	0.00
Stream	Energy (J)
1	0.06
2	118.25
5	118.33
In-Out	-0.01

TF-100	
Stream	Mass (g)
6	0.0085
4	13.2000
7	13.2021
In-Out	0.0064
Stream	Energy (J)
6	0.7382
4	2100.1200
7	2100.4555
In-Out	0.4027

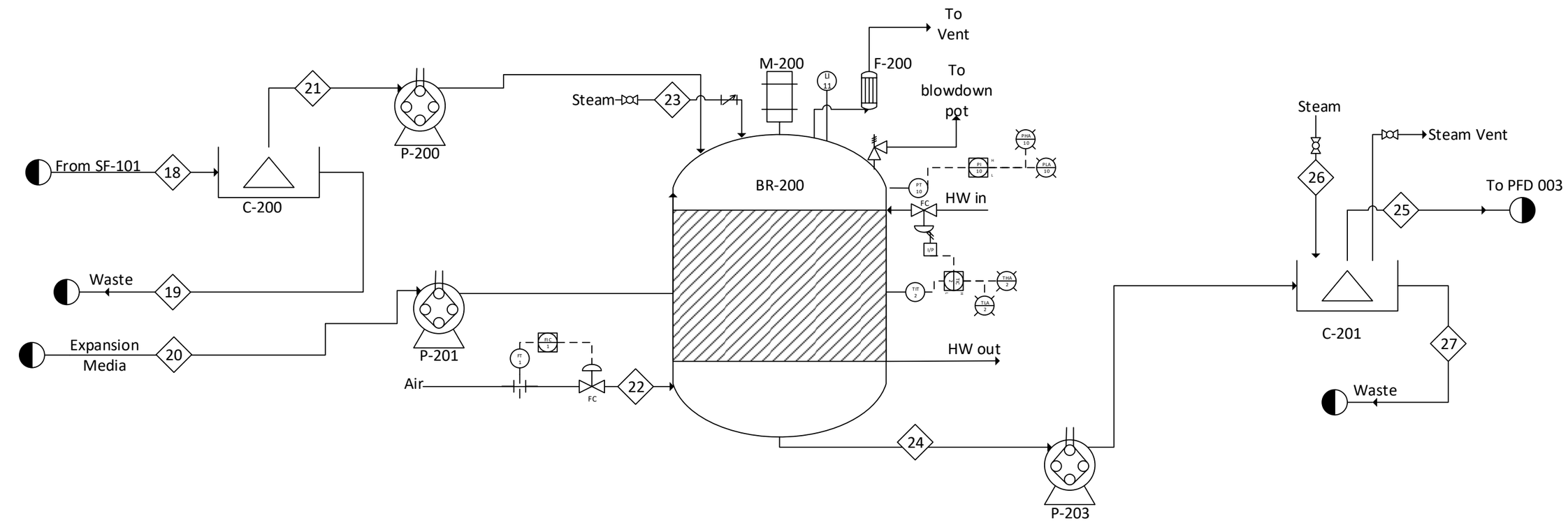
SF-100	
Stream	Mass (g)
8	0.102
10	158.400
9	0.867
11	159.297
In-Out	0.072
Stream	Energy (J)
9	8.857
10	25201.440
13	21.783
11	25226.273
In-Out	5.808

SF-101	
Stream	Mass (g)
12	1.230
13	1900.800
14	0.867
16	1901.947
In-Out	0.950
Stream	Energy (J)
12	106.703
13	302417.280
14	21.783
16	302529.396
In-Out	16.370

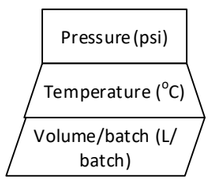
BR-200	
Stream	Mass (g)
19	22814.00
20	14.68
21	2.19
23	22829.95
In-Out	0.92
Stream	Energy (J)
19	3629707.40
20	1884.77
21	55.02
23	3631569.28
Heat Lost	62640.00
Heat added	62640.00
In-Out	77.91

BR-300	
Stream	Mass (g)
27	57563.00
28	22.40
29	63.20
31	57606.49
In-Out	42.11
Stream	Energy (J)
27	9158273.30
28	1943.20
29	1587.90
31	9161569.11
Heat Lost	-1874880.00
Heat added	1874880.00
In-Out	235.29

R-300	
Stream	Energy (kJ)
Heat	-12867
Mass in (g)	25645
Cp (J/gC)	4.545
Heat of Fusion (J/g)	333.55
Cp,s (J/gC)	1.4
T (C)	25
Tf (C)	0
Final T (C)	-80
Rate of Cooling (C/min)	1
Time to cool (min)	117
Power needed (kW)	-1.83

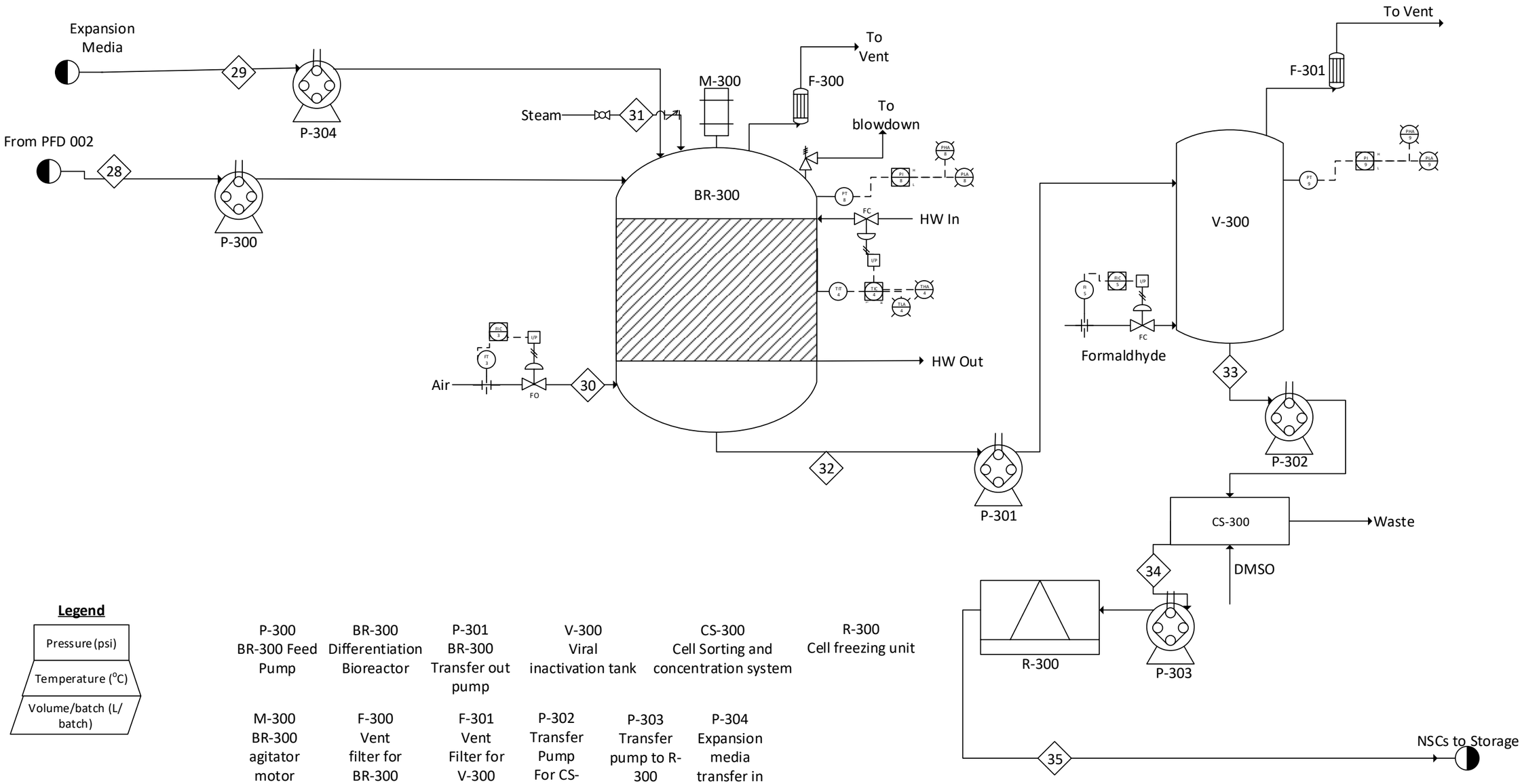


Legend



C-200 2 L centrifuge	P-200 Peristaltic Pump BR-200 Cell Pump	P-201 Peristaltic Media Pump for BR-200	BR-200 Bioreactor for final expansion	P-203 BR-200 transfer out peristaltic pump	C-201 Final expansion centrifuge	M-200 BR-200 agitator motor	F-200 Vent filter for BR-200

Figure 12-Basic Control Scheme of Stem Cell Manufacturing Facility



Legend

Pressure (psi)

Temperature (^{o}C)

Volume/batch (L/ batch)

P-300 BR-300 Feed Pump	BR-300 Differentiation Bioreactor	P-301 BR-300 Transfer out pump	V-300 Viral inactivation tank	CS-300 Cell Sorting and concentration system	R-300 Cell freezing unit

M-300 BR-300 agitator motor	F-300 Vent filter for BR-300	F-301 Vent Filter for V-300	P-302 Transfer Pump For CS-300	P-303 Transfer pump to R-300	P-304 Expansion media transfer in pump

Figure 63Basic Control Scheme of Manufacturing Facility

Costing Information: (All Total Manufacturing Costs are in 2016 Dollars)

Sized Equipment Costing Summary

Final Expansion Centrifuge	
Cp	$12,561
K1	4.7681
K2	0.974
K3	0.024
A	0.2
Fp	1
Fbm	1.57
Cbm	$19,721
Ctm	$32,855

Small Scale Centrifuge	
Cp	$5,967
K1	4.7681
K2	0.974
K3	0.024
A	0.09
Fp	1
Fbm	1.57
Cbm	$9,369
Ctm	$15,608

Pre-differentiation Centrifuge	
Cp	$16,088
K1	4.7681
K2	0.974
K3	0.024
A	0.26
Fp	1
Fbm	1.57
Cbm	$25,258
Ctm	$42,079

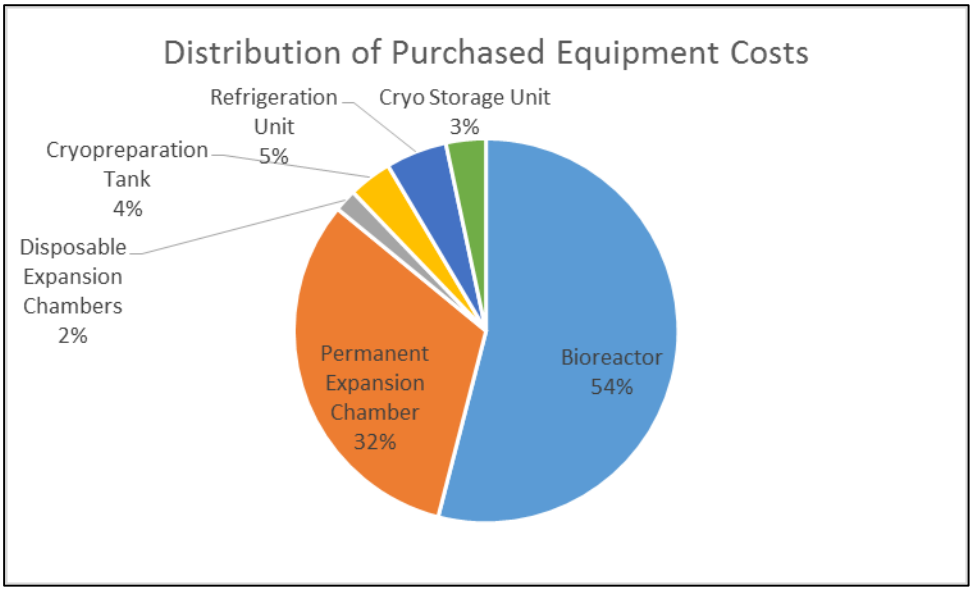
Warm Water Tank	
Cp	$2,081
K1	3.5565
K2	0.3776
K3	0.0905
A	0.0004
Fp	1
Fm	1
Cbm	$2,081
Ctm	$3,468

Inactivation Tank	
Cp	$2,251
K1	3.7957
K2	0.4593
K3	0.016
A	0.1
Fp	1
Fm	3.1
Cbm	$17,766
Ctm	$29,597

Liquid Nitrogen Storage Tank	
Cp	$2,985
K1	3.4974
K2	0.4485
K3	0.1074
A	0.89
Fp	2.2
Fm	3.6
Cbm	$49,747
Ctm	$82,877

Boiler Capital Costing	
Cp°	$3,949,624
K1	6.9617
K2	-1.48
K3	0.3161
A	26348
Cbm	$11,848,871
Fbm	3
Ctm 2001	$13,981,668
Ctm 2016	$19,722,252

Purchased Equipment Costing Summary		
Equipment	Cost	Quantity
Bioreactor	$259,600	1
Permanent Expansion Chamber	$153,400	1
Disposable Expansion Chambers	$9,074	4
Cryopreparation Tank	$17,500	1
Refrigeration Unit	$24,780	1
Cryo Storage Unit	$16,190	1
Liquid Nitrogen Dewer	$325	2
Cell Counter	$153,400	1
Laminar Flow Cabinet	$14,285	2
HEPA Filters	$19,580	2
Cell Sorter	$76,700	1



Utilities Summary		
Equipment	Utility Consumed	Price per Year
Cryogenic Storage	Liquid Nitrogen	$6,642
Refrigeration System	Electricity	$26
Boiler Unit	Boiler Feed Water	$4
Boiler Unit	Electricity	$1,317
HEPA Filter	Electricity	$1,034
Laminar Flow Cabinet	Electricity	$5,742
Agitator	Electricity	$64

Materials Cost	
Induced Pluripotent Stem Cells	$1,820.20
Trypsin w/ EDTA	$21,531
Expansion Media	$328,028
Differentiation Media	$4,600,620
DMSO	$180,804
Oxygen	$0

Utilities Consumed:

Utilities Summary		
Equipment	Utility Consumed	Price per Year
Cryogenic Storage	Liquid Nitrogen	$6,642
Refrigeration System	Electricity	$26
Boiler Unit	Boiler Feed Water	$4
Boiler Unit	Electricity	$1,317
HEPA Filter	Electricity	$1,034
Laminar Flow Cabinet	Electricity	$5,742
Agitator	Electricity	$64

Utilities System Capital Investment		
Equipment	Utility Consumed	Amount Consumed
Cryogenic Storage	Liquid Nitrogen	2562 L
Refrigeration System	Electricity	529 kW hr
Boiler Unit	Boiler Feed Water	34455 kg
Boiler Unit	Electricity	26340 kW hr
HEPA Filter	Electricity	10335 kW hr
Laminar Flow Cabinet	Electricity	57421 kW hr
Agitator	Electricity	1277 kW hr

Boiler Operational Costing*	
Boiler Feed Water $/kg	0.00245
Electricity $/kW-hr	$0.05
Boiler Feed Water $	$4.44
Electricity $	$1,317.00
Total $	$1,321.44
Total/year	$25,107.41
*Per Steamout	

Process Summary Table:

Basic Process Information	
Initial Number of Cells	100000
Initial Batch Size (mL)	1.00
Initial Concentration(Cells/mL)	100000
Growth Factor	31163
Maximum Cell Concentration (Cells/mL)	1.2×10^{6}
Expansion Batch Size (Cells)	3.14×10^{9}
Total Expansion Residence Time (hrs)	358.25
Individual Expansion Residence Time (hrs)	84
Neural Stem Cells (Cells/Batch)	6.27×10^{10}
Batches per Year	18.3
Total Neural Stem Cells (Cells/Year)	1.151×10^{12}

Market Demand	
Dose Size (mL)	0.3
Dosage Concentration (Cells/mL)	3.0×10^{6}
Doses per Patient	16
People Treated per Year	62000
Total Cell Demand (Cells/Year)	1.151×10^{12}

Batch Processing Information	
Manual Error %	3.0
Service Factor	0.75
Total Working Days Available	273.75
Stem Cell Viability %	80

Expansion Phase Summary Table				
Expansion	Cells	Volume (mL)	Residence Time (hrs)	Total ResidenceTime (hrs)
1	1.20×10^{6}	1	86.0	86.0
2	1.44×10^{7}	12	84.0	174.0
3	1.73×10^{8}	144	84.0	260.0
4	2.07×10^{9}	1728	84.0	346.0
5	3.14×10^{9}	20736	14.5	360.5

Expansion Phase Components			
Phase 1	Volume (mL)	Temperature (^{o}C)	Pressure (bar)
Air (Oxygen)	N/A	20	1
Media	1	37	1
Trypsin	0.5	20	1
Phase 2	Volume (mL)	Temperature (^{o}C)	Pressure (bar)
Air (Oxygen)	N/A	20	1
Media	12	37	1
Trypsin	6	20	1
Phase 3	Volume (mL)	Temperature (^{o}C)	Pressure (bar)
Air (Oxygen)	0.14 mL/min	20	1
Media	144	37	1
Trypsin	72	20	1
Phase 4	Volume (mL)	Temperature (^{o}C)	Pressure (bar)
Air (Oxygen)	173 mL/min	20	1
Media	1728	37	1
Trypsin	1000	20	1
Phase 5	Volume (mL)	Temperature (^{o}C)	Pressure (bar)
Air (Oxygen)	2.07 L/min	20	1
Media	20736	37	1
Trypsin	10000	20	1

Reactor Summary Table	
Cells In	3.14×10^{9}
Neural Stem Cells Out	6.27×10^{10}
Conversion	0.8
Total Volume (L)	200
Residence Time (hrs)	168
Glucose Consumption Rate (g/(cell*hr))	2.15×10^{-10}
Hot Water Flow Rate (L/hr)	4.87
Material of Contruction	Stainless Steel
Other Features:	Low Shear Agitator
	Pressure Relief Valve

Reactor Process Variables	
Temperature (^{o}C)	37
Pressure (bar)	1
O_2 Concentration (%)	20
Liquid Volume (L)	52.725
pH	7.2

Sparger Sizing Table	
Total Sparger Contact Area (mm^2)	33.35
Number of Holes	30
Diameter per Hole (mm)	1.190
Diamter of Sparger (m)	0.133

Inactivation Chamber Specification Table	
Diameter (m)	0.233
Height (m)	0.934
Liquid Level Fraction	0.625
Volume of Tank (m^3)	0.1
Liquid Volume (L)	52.7
Material of Construction	Stainless Steel

Inactivation Chamber Process Variables	
Temperature (^{o}C)	37
Pressure (bar)	1.01
Formaldehyde Concentration (g/L)	0.2
Residence Time (hrs)	2.0
Liquid Density (kg/m^3)	1200
Gas Density (kg/m^3)	1.38
Formaldehyde Amount (L)	39.25

Cryostorage Sizing Table	
Product Cryostorage Container	MIDSCI 24K Cryostorage
Capacity (1 mL vials)	24050
Liquid N_2 Capacity (l)	365
Holding time (days)	52
Diameter (m)	0.956
Height (m)	1.11
Refigerator Storage Volume (m^3)	45.31
NSC CryoPrep Volume (m^3)	0.21

Liquid Nitrogen Storage Tank	
Diameter (m)	1.22
Height (m)	3.05
Pressure (barg)	13.79
Volume (liters)	1892.71
Dewar Volume (liters)	1.00

BATCH SHEET NO.	
PRODUCT	**Main simulation**
SPD Case File	H:\Spring Design\Main simulation.spf
Created By	CEAT ITS

OPERATING INSTRUCTIONS

The following procedures/operations must be performed in order.

P-4 (in TFR-101)

Repeat the following operations 1 time(s).

CHARGE-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Charge 0.00 kg (or 0.00 L) via Port S-103 to TFR-101. **Comments**		0.00	0.25		
CHARGE-2: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**

Charge 0.00 kg (or 0.00 L) via Port S-106 to TFR-101. **Comments**		0.25	0.50		
REACT-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Allow the mixture to react in TFR-101. The exit temperature should not exceed 37.0 °C and the exit pressure 1.0 bar. Make sure that the final working vessel volume does not exceed the maximum allowable working to vessel volume ratio (90.0 %). **Comments**		0.50	84.50		
TRANSFER-OUT-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Transfer 0.00 kg (0.00 L) of material out of TFR-101 into TFR-102 at a rate of 600.00 kg/h (604.16 L/h). Make sure that the final working vessel volume does not exceed the maximum allowable working to vessel volume ratio (90.0 %). **Comments**		84.50	84.75		

P-2 (in BR-102)

Repeat the following operations 1 time(s).

CHARGE-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Charge 20.81 kg (or 20.78 L) via Port Expanision Media Feed to BR-102. **Comments**		0.00	0.31		
HEAT-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Heat the contents of BR-102 for 30.98 min to a final temperature of 37.00 °C. **Comments**		0.31	0.91		
TRANSFER-OUT-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Transfer 20.81 kg (21.01 L) of material out of BR-102 into SBR-101 at a rate of 297.09 kg/h (300.00 L/h). Make sure that the final working vessel volume does not exceed the maximum allowable working to vessel volume ratio (90.0 %). **Comments**		0.91	1.23		
CHARGE-2: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**

Charge 52.33 kg (or 52.24 L) via Port Diff Media to BR-102. **Comments**		362.11	362.45		
HEAT-2: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Heat the contents of BR-102 for 30.99 min to a final temperature of 37.00 °C. **Comments**		362.45	363.05		
TRANSFER-OUT-2: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Transfer 52.33 kg (52.84 L) of material out of BR-102 into BR-101 at a rate of 600.00 kg/h (605.85 L/h). Make sure that the final working vessel volume does not exceed the maximum allowable working to vessel volume ratio (90.0 %). **Comments**		363.05	363.39		

P-5 (in TFR-102)

Repeat the following operations 1 time(s).

CHARGE-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Charge 0.01 kg (or 0.01 L) via Port S-107 to TFR-102. **Comments**		84.75	85.00		
PULL-IN-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Transfer 0.00 kg (0.00 L) of material into TFR-102 from Source Equipment via … at a rate of 600.00 kg/h (604.16 L/h). Make sure that the final working vessel volume does not exceed the maximum allowable working to vessel volume ratio (90.0 %). **Comments**		85.00	85.25		
REACT-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Allow the mixture to react in TFR-102. The exit temperature should not exceed 37.0 °C and the exit pressure 1.2 bar. Make sure that the final working vessel volume does not exceed the maximum allowable working to vessel volume ratio (90.0 %). **Comments**		85.25	173.25		
TRANSFER-OUT-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**

Transfer 0.01 kg (0.01 L) of material out of TFR-102 into CF-102 at a rate of 600.00 kg/h (605.17 L/h). Make sure that the final working vessel volume does not exceed the maximum allowable working to vessel volume ratio (90.0 %). **Comments**		173.25	173.50		

P-7 (in CF-102)

Repeat the following operations 1 time(s).

CENTRIFUGE-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Centrifuge in CF-102 at a rate of 0.41 L/h for 2.00 min. **Comments**		173.25	173.28		

P-8 (in SFR-101)

Repeat the following operations 1 time(s).

CHARGE-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Charge 0.14 kg (or 0.14 L) via Port Ex media to SFR-101. **Comments**		173.28	173.53		
PULL-IN-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Transfer 0.00 kg (0.00 L) of material into SFR-101 from Source Equipment via … at a rate of 600.00 kg/h (592.53 L/h). Make sure that the final working vessel volume does not exceed the maximum allowable working to vessel volume ratio (90.0 %). **Comments**		173.53	173.78		
REACT-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Feed SFR-101 via Port Stream Name from Source Equipment. Allow the mixture to react. The exit temperature should not exceed 37.00 °C and the exit pressure 1.01 bar. Make sure that the vessel volume does not exceed 7.27 % of the total vessel volume. **Comments**		173.78	259.78		
TRANSFER-OUT-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**

Transfer 0.14 kg (0.14 L) of material out of SFR-101 into CF-103 at a rate of 600.00 kg/h (605.55 L/h). Make sure that the final working vessel volume does not exceed the maximum allowable working to vessel volume ratio (90.0 %). **Comments**		259.78	260.03		

P-9 (in CF-103)

Repeat the following operations 1 time(s).

CENTRIFUGE-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Centrifuge in CF-103 at a rate of 1.75 L/h for 5.00 min. **Comments**		259.78	259.86		

P-10 (in SFR-102)

Repeat the following operations 1 time(s).

CHARGE-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Charge 1.72 kg (or 1.73 L) via Port Ex media 2 to SFR-102. **Comments**		259.86	260.11		
PULL-IN-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Transfer 0.00 kg (0.00 L) of material into SFR-102 from Source Equipment via … at a rate of 600.00 kg/h (599.75 L/h). Make sure that the final working vessel volume does not exceed the maximum allowable working to vessel volume ratio (90.0 %). **Comments**		260.11	260.36		
REACT-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Feed SFR-102 via Port Stream Name from Source Equipment. Allow the mixture to react. The exit temperature should not exceed 37.00 °C and the exit pressure 1.79 bar. Make sure that the vessel volume does not exceed 43.61 % of the total vessel volume. **Comments**		260.36	346.36		
TRANSFER-OUT-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**

Transfer 1.98 kg (1.99 L) of material out of SFR-102 into CF-104 at a rate of 600.00 kg/h (605.61 L/h). Make sure that the final working vessel volume does not exceed the maximum allowable working to vessel volume ratio (90.0 %). **Comments**		346.36	346.62		

P-11 (in CF-104)

Repeat the following operations 1 time(s).

CENTRIFUGE-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Centrifuge in CF-104 at a rate of 23.98 L/h for 5.00 min. **Comments**		346.62	346.70		

P-1 (in SBR-101)

Repeat the following operations 1 time(s).

PULL-IN-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Transfer 20.81 kg (21.01 L) of material into SBR-101 from Source Equipment via … at a rate of 600.00 kg/h (605.85 L/h). Make sure that the final working vessel volume does not exceed the maximum allowable working to vessel volume ratio (50.0 %). **Comments**		346.62	346.90		
PULL-IN-2: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Transfer 0.09 kg (0.09 L) of material into SBR-101 from Source Equipment via … at a rate of 300.00 kg/h (300.52 L/h). Make sure that the final working vessel volume does not exceed the maximum allowable working to vessel volume ratio (50.0 %). **Comments**		346.90	347.15		
REACT-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Feed SBR-101 via Port Stream Name from Source Equipment. Allow the mixture to react. The exit temperature should not exceed 37.00 °C and the exit pressure 1.01 bar. Make sure that the vessel volume does not exceed 49.98 % of the total vessel volume. **Comments**		347.15	361.65		
TRANSFER-OUT-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**

Transfer 20.92 kg (21.12 L) of material out of SBR-101 into CF-101 at a rate of 100.00 kg/h (100.97 L/h). Make sure that the final working vessel volume does not exceed the maximum allowable working to vessel volume ratio (90.0 %). **Comments**		361.65	362.11		

P-3 (in CF-101)

Repeat the following operations 1 time(s).

CENTRIFUGE-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Centrifuge in CF-101 at a rate of 84.49 L/h for 5.00 min. **Comments**		362.11	362.20		

P-6 (in BR-101)

Repeat the following operations 1 time(s).

PULL-IN-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Transfer 52.33 kg (52.84 L) of material into BR-101 from Source Equipment via … at a rate of 600.00 kg/h (605.85 L/h). Make sure that the final working vessel volume does not exceed the maximum allowable working to vessel volume ratio (90.0 %). **Comments**		362.20	362.53		
PULL-IN-2: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Transfer 0.24 kg (0.24 L) of material into BR-101 from Source Equipment via … at a rate of 600.00 kg/h (603.02 L/h). Make sure that the final working vessel volume does not exceed the maximum allowable working to vessel volume ratio (50.0 %). **Comments**		362.53	362.78		
REACT-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Feed BR-101 via Port Stream Name from Source Equipment. Allow the mixture to react. The exit temperature should not exceed 37.00 °C and the exit pressure 1.01 bar. Make sure that the vessel volume does not exceed 49.88 % of the total vessel volume. **Comments**		362.78	530.78		
TRANSFER-OUT-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**

Transfer 52.72 kg (53.21 L) of material out of BR-101 into CS-105 at a rate of 600.00 kg/h (605.59 L/h). Make sure that the final working vessel volume does not exceed the maximum allowable working to vessel volume ratio (90.0 %). **Comments**		530.78	531.12		

P-12 (in CS-105)

Repeat the following operations 1 time(s).

CENTRIFUGE-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Centrifuge in CS-105 at a rate of 13.30 L/h for 240.00 min. **Comments**		531.12	535.12		

P-13 (in FT-101)

Repeat the following operations 1 time(s).

PULL-IN-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Transfer 5.29 kg (5.32 L) of material into FT-101 from Source Equipment via … at a rate of 600.00 kg/h (603.22 L/h). Make sure that the final working vessel volume does not exceed the maximum allowable working to vessel volume ratio (99.0 %). **Comments**		535.12	535.38		
FREEZE-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Freeze the contents of FT-101 for 117.00 min to a final temperature of -80.00 °C. **Comments**		535.38	537.41		
TRANSFER-OUT-1: **INSTRUCTIONS FOR MANUFACTURING**	**DATE**	**START** (h)	**END** (h)	**SIGN**	**CHECK**
Transfer 5.29 kg (5.10 L) of material out of FT-101 into Target Equipment at a rate of 600.00 kg/h (578.50 L/h). Make sure that the final working vessel volume does not exceed the maximum allowable working to vessel volume ratio (99.0 %). **Comments**		537.41	537.67		

Key Equations

Material and Energy Balance

$$In - Out + Generation = Accumulation$$

$$Q = mC_p\Delta T$$

$$Q = m\lambda$$

Tanks Sizing

$$V = \frac{\pi}{4}d^2l$$

Heat Transfer

$$Q = \frac{\Delta T}{\sum R}$$

$$R = \frac{\ln(^{R_2}/_{R_1})}{2\pi R_1 kl}$$

Costing

$$C_p^o = 10^{K_1 + K_2\log(A) + K_3(\log(A))^2}$$

$$C_{Bm} = C_p^o(B_1 + B_2F_mF_p)$$

$$F_p = \frac{\frac{(P+1)D}{2[850 - 0.6(P+1)]} + 0.00315}{0.0063}$$

$$F_T = 1 + 0.00184\Delta T - 0.00000335(\Delta T)^2$$

$$C_{Tm} = 1.18C_{Bm}$$

Boiler

$$Q = mC_p\Delta T + m\lambda$$

$$\dot{m} = \rho\dot{Q}$$

Inactivation Chamber:

$$A_o = 22.3(\frac{V}{.61})\sqrt{\frac{\rho}{\Delta P}}$$

Where:

A_o = Total Area of Holes (m^2)

ΔP = Pressure Drop (Pa)

V = Velocity of the Fluid (m/s)

ρ = Density of Fluid (kg/m^3)

$$\Delta P = (\frac{4000f(.15)(.35)}{1.1d} - 1)E_k$$

Where:

f = Friction Factor

D = Diameter (m)

E_k = Kinetic Energy (J)

ΔP = Pressure Drop (Pa)

$$E_k = \frac{810(1.1)\rho V^2}{d^4}$$

Where:

ρ = Density of Fluid (kg/m^3)

V = Velocity of the Fluid (m/s)

D = Diameter (m)

E_k = Kinetic Energy (J)

$$R_e = \frac{\rho V D}{\mu}$$

Where:

ρ = Density of Fluid (kg/m^3)

V = Velocity of the Fluid (m/s)

D = Diameter of the Pipe (m)

μ = Dynamic Viscosity of the Fluid (kg/s/m)

Re = Reynolds Number

Biological Growth

$$N = N_o e^{0.0289t}$$

Where:

N = Number of Cells

N_o = Initial Number of Cells

t = Residence time (hours)

Material Safety Data Sheet

1. PRODUCT AND COMPANY IDENTIFICATION

Product Name Formaldehyde 37% Solution

Cat No. 9300-1, 9300-5, 9300-55, 9311, 9315, C4320, C4320-5

Synonyms No information available.

Recommended Use Laboratory chemicals

2. HAZARDS IDENTIFICATION

Target Organs Gastrointestinal tract (GI), Central nervous system (CNS), Eyes, Respiratory system, Skin, Optic nerve, Liver, Kidney, spleen, Blood

Potential Health Effects

Acute Effects

Principle Routes of Exposure

Eyes Causes burns.

Skin Toxic in contact with skin. Causes burns. May produce an allergic reaction.

Inhalation Vapor harmful. Toxic by inhalation. Causes burns.

Ingestion May be fatal or cause blindness if swallowed. Ingestion may cause gastrointestinal irritation, nausea, vomiting and diarrhea. May cause burns to the digestive tract.

Chronic Effects May cause cancer. Tumorigenic effects have been reported in experimental animals.. Experiments have shown reproductive toxicity effects on laboratory animals. May cause adverse liver effects. May cause adverse kidney effects. Repeated contact may cause allergic reactions in very susceptible persons. Danger of very serious irreversible effects.

See Section 11 for additional Toxicological information.

Emergency Telephone Number

Chemtrec US: (800) 424-9300

Chemtrec EU: (202) 483-7616

Emergency Overview

Revision Number 1

Flammable liquid and vapor. Cancer hazard. Poison, may be fatal or cause blindness if swallowed. Cannot be made nonpoisonous.

Vapor harmful. Toxic by inhalation, in contact with skin and if swallowed. Causes burns by all exposure routes. May cause an allergic skin reaction. Danger of very serious irreversible effects.

Creation Date 17-Mar-2010 **Revision Date** 17-Mar-2010

Company

Richard Allan Scientific
A Subsidiary of Thermo Fisher Scientific
4481 Campus Drive
Kalamazoo, MI 49008
Tel: (800) 522-7270

DANGER!

Appearance Colorless **Physical State** Liquid **odor** pungent

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Aggravated Medical Conditions Central nervous system disorders. Gastrointestinal tract. Preexisting eye disorders. Skin disorders.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Haz/Non-haz

Component	CAS-No	Weight %
Water	7732-18-5	48 - 53
Buffers	NA	-
Formaldehyde	50-00-0	37 - 38
Methyl alcohol	67-56-1	10 - 15

4. FIRST AID MEASURES

Eye Contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Immediate medical attention is required.
Skin Contact Wash off immediately with plenty of water for at least 15 minutes. Immediate medical attention is required.
Inhalation Move to fresh air. If breathing is difficult, give oxygen. Do not use mouth-to-mouth resuscitation if victim ingested or inhaled the substance; induce artificial respiration with a respiratory medical device. Immediate medical attention is required.
Ingestion Do not induce vomiting. Call a physician or Poison Control Center immediately.
Notes to Physician Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Flash Point 60°C / 140°F
Method No information available.
Autoignition Temperature 430°C / 806°F
Explosion Limits
Upper 73 vol %
Lower 7 vol %
Suitable Extinguishing Media Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.
Unsuitable Extinguishing Media No information available.
Hazardous Combustion Products No information available.
Sensitivity to mechanical impact No information available.
Sensitivity to static discharge No information available.
Specific Hazards Arising from the Chemical
Flammable. Risk of ignition. Vapors may form explosive mixtures with air. Vapors may travel to source of ignition and flash back.
Containers may explode when heated.

Protective Equipment and Precautions for Firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective
gear. Thermal decomposition can lead to release of irritating gases and vapors.

6. ACCIDENTAL RELEASE MEASURES

Personal Precautions Use personal protective equipment. Remove all sources of ignition. Take precautionary measures against static discharges. Do not get in eyes, on skin, or on clothing.
Environmental Precautions Should not be released into the environment.
Methods for Containment and Clean Up
Remove all sources of ignition. Soak up with inert absorbent material. Take precautionary measures against static discharges. Keep in suitable and closed containers for disposal.

7. HANDLING AND STORAGE

Handling Use only under a chemical fume hood. Use explosion-proof equipment. Wear personal protective equipment. Keep away from open flames, hot surfaces and sources of ignition. Take precautionary measures against static discharges. Do not breathe vapors or spray mist. Do not get in eyes, on skin, or on clothing.
Storage Keep containers tightly closed in a dry, cool and well-ventilated place. Keep away from heat and sources of ignition. Flammables area.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Engineering Measures Use only under a chemical fume hood. Use explosion-proof electrical/ventilating/lighting/equipment. Ensure that eyewash stations and safety showers are close to the workstation location.
Exposure Guidelines

Component	ACGIH TLV	OSHA PEL	NIOSH IDLH
Formaldehyde	Ceiling: 0.3 ppm (Vacated)	TWA: 3 ppm	

(Vacated) STEL: 10 ppm
(Vacated) Ceiling: 5 ppm
TWA: 0.75 ppm
STEL: 2 ppm
IDLH: 20 ppm
TWA: 0.016 ppm
Ceiling: 0.1 ppm
Methyl alcohol TWA: 200 ppm
STEL: 250 ppm
Skin
(Vacated) TWA: 200 ppm
(Vacated) TWA: 260 mg/m3
(Vacated) STEL: 325 mg/m3
(Vacated) STEL: 250 ppm
Skin
TWA: 200 ppm
TWA: 260 mg/m3
IDLH: 6000 ppm
TWA: 200 ppm
TWA: 260 mg/m3
STEL: 250 ppm
STEL: 325 mg/m3

Component Quebec Mexico OEL (TWA) Ontario TWAEV
Formaldehyde Ceiling: 3 mg/m3
Ceiling: 2 ppm
Peak: 3 mg/m3
Peak: 2 ppm
STEL: 1.0 ppm
CEV: 1.5 ppm

Component Quebec Mexico OEL (TWA) Ontario TWAEV
Methyl alcohol TWA: 200 ppm
TWA: 262 mg/m3
STEL: 328 mg/m3
STEL: 250 ppm
Skin
TWA: 200 ppm
TWA: 260 mg/m3
STEL: 250 ppm
STEL: 310 mg/m3
TWA: 200 ppm
TWA: 260 mg/m3
STEL: 325 mg/m3
STEL: 250 ppm
Skin

NIOSH IDLH: *Immediately Dangerous to Life or Health*

Personal Protective Equipment

Eye/face Protection Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166

Skin and body protection Wear appropriate protective gloves and clothing to prevent skin exposure

Respiratory Protection Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN
149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State Liquid

Appearance Colorless
odor pungent
Odor Threshold No information available.
pH 2.8 - 4.0
Vapor Pressure 6768 mmHg @ 20 °C
Vapor Density 1.01
Viscosity No information available.
Boiling Point/Range 96.1 - 101°C / 205 - 213.8°F
Melting Point/Range 0°C / 32°F
Decomposition temperature °C No information available.
Flash Point 60°C / 140°F
Evaporation Rate No information available.
Specific Gravity 1.0749 - 1.2020
Solubility Soluble in water
log Pow No data available

10. STABILITY AND REACTIVITY

Stability Stable under normal conditions.
Conditions to Avoid Incompatible products. Heat, flames and sparks.
Incompatible Materials Strong oxidizing agents, Strong bases, Acids, Acid anhydrides, Acid chlorides, Metals, Peroxides
Hazardous Decomposition Products Carbon monoxide (CO), Formaldehyde, Methanol
Hazardous Polymerization Hazardous polymerization does not occur
Hazardous Reactions . None under normal processing.

11. TOXICOLOGICAL INFORMATION

Acute Toxicity

Product Information No acute toxicity information is available for this product
Component Information

Component	LD50 Oral	LD50 Dermal	LC50 Inhalation
Water	90 mL/kg (Rat)	Not listed	Not listed
Formaldehyde	500 mg/kg (Rat)	Not listed	0.578 mg/L (Rat) 4 h
Methyl alcohol	5628 mg/kg (Rat)	15800 mg/kg (Rabbit)	64000 ppm (Rat) 4 h 83.2 mg/L (Rat) 4 h

Irritation Causes burns by all exposure routes
Toxicologically Synergistic Products
No information available.
Chronic Toxicity
Carcinogenicity The table below indicates whether each agency has listed any ingredient as a carcinogen.

Component	ACGIH	IARC	NTP	OSHA	Mexico
Formaldehyde	A2	Group 1	Reasonably Anticipated	X	Not listed

ACGIH: (American Conference of Governmental Industrial Hygienists)
A1 - Known Human Carcinogen
A2 - Suspected Human Carcinogen
A3 - Animal Carcinogen
ACGIH: (American Conference of Governmental Industrial Hygienists)
IARC: (International Agency for Research on Cancer)
IARC: (International Agency for Research on Cancer)
Group 1 - Carcinogenic to Humans
Group 2A - Probably Carcinogenic to Humans
Group 2B - Possibly Carcinogenic to Humans
NTP: (National Toxicity Program)
NTP: (National Toxicity Program)
Known - Known Carcinogen
Reasonably Anticipated - Reasonably Anticipated to be a Human Carcinogen
Sensitization May cause sensitization by skin contact

Mutagenic Effects Mutagenic effects have occurred in humans.
Reproductive Effects Experiments have shown reproductive toxicity effects on laboratory animals.
Developmental Effects Developmental effects have occurred in experimental animals.
Teratogenicity Teratogenic effects have occurred in experimental animals..
Other Adverse Effects Tumorigenic effects have been reported in experimental animals.. See actual entry in RTECS
for complete information.
Endocrine Disruptor Information No information available

12. ECOLOGICAL INFORMATION

12. ECOLOGICAL INFORMATION

Ecotoxicity

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Component	Freshwater Algae	Freshwater Fish	Microtox	Water Flea
Formaldehyde	Not listed	Leuciscus idus: LC50 = 15 mg/L 96h Not listed	EC50 = 20 mg/L 96h	EC50 = 2 mg/L 48h
Methyl alcohol	Not listed	Pimephales promelas: LC50 > 10000 mg/L 96h	EC50 = 39000 mg/L 25 min EC50 = 40000 mg/L 15 min EC50 = 43000 mg/L 5 min	EC50 > 10000 mg/L 24h

Persistence and Degradability No information available
Bioaccumulation/ Accumulation No information available
Mobility .

Component	log Pow
Water	-1.87
Formaldehyde	0.35
Methyl alcohol	-0.74

13. DISPOSAL CONSIDERATIONS

Waste Disposal Methods Chemical waste generators must determine whether a discarded chemical is classified as a
hazardous waste. Chemical waste generators must also consult local, regional, and national
hazardous waste regulations to ensure complete and accurate classification

Component	RCRA - U Series Wastes	RCRA - P Series Wastes
Formaldehyde - 50-00-0	U122	-
Methyl alcohol - 67-56-1	U154	-

14. TRANSPORT INFORMATION

DOT
UN-No UN1198
Proper Shipping Name FORMALDEHYDE, SOLUTIONS, FLAMMABLE
Hazard Class 3
Subsidiary Hazard Class 8
Packing Group III
TDG
UN-No UN1198
Proper Shipping Name FORMALDEHYDE, SOLUTIONS, FLAMMABLE
Hazard Class 3
Subsidiary Hazard Class 8
Packing Group III

14. TRANSPORT INFORMATION

IATA
UN-No UN1198
Proper Shipping Name FORMALDEHYDE, SOLUTIONS, FLAMMABLE
Hazard Class 3
Subsidiary Hazard Class 8
Packing Group III
IMDG/IMO
UN-No UN1198
Proper Shipping Name FORMALDEHYDE, SOLUTIONS, FLAMMABLE
Hazard Class 3
Subsidiary Hazard Class 8
Packing Group III

15. REGULATORY INFORMATION

International Inventories

Component	TSCA	DSL	NDSL	EINECS	ELINCS	NLP	PICCS	ENCS	AICS	CHINA	KECL
Water	X	X	-	231-791-2	-	X	-	X	X	KE-35400	X
Formaldehyde	X	X	-	200-001-8	-	X	X	X	X	KE-17074	X
Methyl alcohol	X	X	-	200-659-6	-	X	X	X	X	KE-23193	X

Legend:
X - Listed
E - Indicates a substance that is the subject of a Section 5(e) Consent order under TSCA.
F - Indicates a substance that is the subject of a Section 5(f) Rule under TSCA.
N - Indicates a polymeric substance containing no free-radical initiator in its inventory name but is considered to cover the designated
polymer made with any free-radical initiator regardless of the amount used.
P - Indicates a commenced PMN substance
R - Indicates a substance that is the subject of a Section 6 risk management rule under TSCA.
S - Indicates a substance that is identified in a proposed or final Significant New Use Rule
T - Indicates a substance that is the subject of a Section 4 test rule under TSCA.
XU - Indicates a substance exempt from reporting under the Inventory Update Rule, i.e. Partial Updating of the TSCA Inventory Data Base
Production and Site Reports (40 CFR 710(B).
Y1 - Indicates an exempt polymer that has a number-average molecular weight of 1,000 or greater.
Y2 - Indicates an exempt polymer that is a polyester and is made only from reactants included in a specified list of low concern reactants that
comprises one of the eligibility criteria for the exemption rule.

U.S. Federal Regulations
TSCA 12(b) Not applicable
SARA 313

Component CAS-No Weight % SARA 313 - Threshold Values %
Formaldehyde 50-00-0 37 - 38 0.1
Methyl alcohol 67-56-1 10 - 15 1.0
SARA 311/312 Hazardous Categorization
Acute Health Hazard No
Chronic Health Hazard No
Fire Hazard Yes
Sudden Release of Pressure Hazard No
Reactive Hazard No
Clean Water Act
Component CWA - Hazardous Substances CWA - Reportable Quantities CWA - Toxic Pollutants CWA - Priority Pollutants
Formaldehyde X 100 lb - -
Clean Air Act
Component HAPS Data Class 1 Ozone Depletors Class 2 Ozone Depletors
Formaldehyde X -
Methyl alcohol X -
OSHA
Component Specifically Regulated Chemicals Highly Hazardous Chemicals
Formaldehyde 0.5 ppm Action Level
0.75 ppm TWA
2 ppm STEL
TQ: 1000 lb
CERCLA
This material, as supplied, contains one or more substances regulated as a hazardous substance under the Comprehensive
Environmental Response Compensation and Liability Act (CERCLA) (40 CFR 302)
Component Hazardous Substances RQs CERCLA EHS RQs
Formaldehyde 100 lb 100 lb
Methyl alcohol 5000 lb -
California Proposition 65
This product contains the following Proposition 65 chemicals:
Component CAS-No California Prop. 65 Prop 65 NSRL
Formaldehyde 50-00-0 Carcinogen 40 µg/day
State Right-to-Know
Component Massachusetts New Jersey Pennsylvania Illinois Rhode Island
Formaldehyde X X X X X

Component Massachusetts New Jersey Pennsylvania Illinois Rhode Island
Methyl alcohol X X X X X
U.S. Department of Transportation
Reportable Quantity (RQ): Y
DOT Marine Pollutant N
DOT Severe Marine Pollutant N
U.S. Department of Homeland Security
This product contains the following DHS chemicals:
Component DHS Chemical Facility Anti-Terrorism Standard
Formaldehyde 11250 lb STQ (solution)
Other International Regulations
Mexico - Grade Moderate risk, Grade 2

Canada
This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the
MSDS contains all the information required by the CPR.
WHMIS Hazard Class
B3 Combustible liquid
D1B Toxic materials
D2A Very toxic materials
D2B Toxic materials
E Corrosive material

16. OTHER INFORMATION

Prepared By Regulatory Affairs
Thermo Fisher Scientific
Tel: (412) 490-8929
Creation Date 17-Mar-2010
Print Date 17-Mar-2010
Revision Summary "***", and red text indicates revision

Disclaimer
The information provided on this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guide for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered as a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other material or in any process, unless specified in the text.
End of MSDS

Safety Data Sheet

GHS - Classification

Precautionary Statements

P210 - Keep away from heat/sparks/open flames/hot surfaces. - No smoking

P280 - Wear protective gloves/protective clothing/eye protection/face protection

P370 + P378 - In case of fire, use water/water spray/water jet/carbon dioxide/sand/foam/alcohol resistant foam/chemical powder for extinction

Health hazards

Not Hazardous

Signal Word

WARNING

Hazard Statements

H227 - Combustible liquid

SECTION 2: Hazards identification

Product code D12345

Company/undertaking identification

Physical hazards

Country specific Emergency Number (if available):

SECTION 1: Identification of the substance/mixture and of the company/undertaking

GHS Physical Hazard 1 Flammable liquids

Product name DMSO (dimethylsulfoxide), anhydrous

GHS Physical Hazard Category Number Category 4

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

D12345

Identification of the substance or mixture

Revision date

Product name DMSO (dimethylsulfoxide), anhydrous

Page 1 / 6

24 hour Emergency Response: 866-536-0631

301-431-8585

Outside of the U.S. +1-301-431-8585

31-Jul-2015

+(55)-2139581449 (português)

www.lifetechnologies.com

Life Technologies

5250 Mainway Drive

Burlington, ONT

CANADA L7L 6A4

800/263-6236

Product code

Life Technologies

5791 Van Allen Way

PO Box 6482
Carlsbad, CA 92008
+1 760 603 7200
CHEMTREC Brazil (Rio De Janeiro)
inhalation Remove to fresh air. If symptoms persist, call a physician. If not breathing, give artificial respiration.
Notes to Physician Treat symptomatically.
eyes

SECTION 5: Firefighting measures

Target Organ Effects No known effects under normal use conditions.
Suitable extinguishing media Water spray. Carbon dioxide (CO2). Foam. Dry chemical.
Mild eye irritation.
Special protective equipment for firefighters Wear self-contained breathing apparatus and protective suit.
Carcinogenic effects None.

SECTION 6: Accidental release measures

Principle Routes of Exposure
Potential Health Effects
HMIS
Personal precautions ELIMINATE all ignition sources (no smoking, flares, sparks or flames in immediate area). Use personal protection equipment.
Methods for cleaning up Soak up with inert absorbent material. Sweep up and shovel into suitable containers for disposal. After cleaning, flush away traces with water.
Mutagenic effects None.
Skin
Reproductive toxicity

SECTION 4: First aid measures

None.
Mild skin irritation. Components of the product may be absorbed into the body through the skin.
Skin contact Wash off immediately with plenty of water. If symptoms occur, obtain medical advice.
Sensitization
Eye contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Consult a physician if necessary.
No sensitization responses were observed.
Ingestion Never give anything by mouth to an unconscious person. If symptoms persist, call a physician. Do not induce vomiting without medical advice.
Specific effects

Health 1
Chemical Name CAS-No EINECS-No Weight %
Reactivity 0
dimethylsulfoxide 67-68-5
D12345
200-664-3 95-100
Revision date
Product name DMSO (dimethylsulfoxide), anhydrous
Page 2 / 6
We recommend handling all chemicals with caution.
31-Jul-2015

SECTION 3: Composition/information on ingredients

www.lifetechnologies.com
Product code
Flammability 2

Environmental precautions
Odor No data available
Odor Threshold No data available
Storage
Respiratory protection In case of insufficient ventilation, wear suitable respiratory equipment.
Keep away from heat, sparks, flame and other sources of ignition (i.e., pilot lights,
electric motors and static electricity). Do not store near combustible materials.
Keep in properly labeled containers.
Chemical Name OSHA PEL OSHA PEL (Ceiling) ACGIH OEL (TWA) ACGIH OEL (STEL)
Hand protection Impervious butyl rubber gloves. Nitrile gloves are not recommended. Some brands of Nitrile gloves have breakthrough times of five minutes.

SECTION 7: Handling and storage

See Section 12 for more information.
Eye protection Wear safety glasses with side shields (or goggles).
dimethylsulfoxide None
Skin and Body Protection Lightweight protective clothing.
None None
Hygiene measures Handle in accordance with good industrial hygiene and safety practice.
None
Oxidizing properties No information available
Water solubility miscible
Environmental exposure
controls
Upper explosion limit 61% - 64%
Prevent product from entering drains.
Lower explosion limit 2.4% - 2.8%

SECTION 8: Exposure controls/personal protection

Engineering measures Ensure adequate ventilation, especially in confined areas.

SECTION 9: Physical and chemical properties

Handling
General information

Personal Protective Equipment
Avoid contact with skin and eyes. Always wear reccommended Personal
Protective Equipment.
Exposure Limits
Form Liquid
Personal Protective Equipment requirements are dependent on the user institution's risk assessment and are specific
to the risk assessment for each laboratory where this material may be used.
Prevent further leakage or spillage if safe to do so.
D12345
Appearance No information available
Revision date
°C 188 - 190
Product name DMSO (dimethylsulfoxide), anhydrous
°F 64.4 - 66.2
Page 3 / 6
°F 417.2 - 420.8
Melting point / melting range °C 18 - 19
Autoignition Temperature
31-Jul-2015
°C 87 - 89
Boiling point / boiling range
www.lifetechnologies.com

°F 370 - 374
flash point
Product code
°C 214 - 216
°F 188.6 - 192.2
Hazardous decomposition products formed under fire conditions. Sulphur oxides. Carbon oxides.
Sensitization No sensitization responses were observed.

SECTION 10: Stability and reactivity

polymerization
Chemical Name LD50 (oral,rat/mouse) LD50 (dermal,rat/rabbit)

SECTION 12: Ecological information

LC50 (inhalation,rat/mouse)
Hazardous polymerization does not occur.
dimethylsulfoxide
Ecotoxicity Contains no substances known to be hazardous to the environment or not degradable in waste water treatment plants.
14500 mg/kg Oral LD50 >40000 mg/kg bw
Mobility completely soluble.
>5000 mg/l
Biodegradation Inherently biodegradable.
Bioaccumulation Material does not bioaccumulate.
Principle Routes of Exposure
Potential Health Effects
Chemical Name Freshwater Algae
Data
Water Flea Data Freshwater Fish
Species Data
Microtox Data log Pow

SECTION 11: Toxicological information

dimethylsulfoxide logPow-2.03
Viscosity
eyes Mild eye irritation.
Stability

SECTION 13: Disposal considerations

Skin Mild skin irritation. Components of the product may be absorbed into the body through the skin.
Dispose of contents/containers in accordance with local regulations.
Stable under normal conditions.
inhalation

No information available.
PH Range

Acute Toxicity

Ingestion No information available.
Materials to avoid
D12345
Strong acids. Strong oxidizing agents.
Revision date
Carcinogenic effects
Product name DMSO (dimethylsulfoxide), anhydrous
None.
Page 4 / 6
6-8
Mutagenic effects
31-Jul-2015

None.
No data available
www.lifetechnologies.com
Hazardous decomposition products
Product code
Reproductive toxicity None.
IATA
For research use only. Not intended for human or animal diagnostic or therapeutic uses.
Packing group None

SECTION 14: Transport information

UN-No none
Proper Shipping Name No dangerous good in sense of these transport regulations

SECTION 16: Other information

Hazard Class None
Subsidiary class
Reason for revision SDS sections updated.
None
-
TSCA
Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product does not contains HAPs.
California Proposition 65
This product does not contain any Proposition 65 chemicals.

US Federal Regulations

WHMIS Hazard Class

B3 - Combustible liquid

SECTION 15: Regulatory information

_____
dimethylsulfoxide
67-68-5 (95-100)
This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the
MSDS contains all the information required by the CPR.

US State Regulations

Listed

SARA 313

This product is not regulated by SARA.
D12345
Component
Chemical Name
Revision date
Massachusetts - RTK
Product name DMSO (dimethylsulfoxide), anhydrous
New Jersey - RTK
Page 5 / 6
Pennsylvania - RTK Illinois - RTK Rhode Island - RTK
31-Jul-2015
dimethylsulfoxide
www.lifetechnologies.com
- -
Product code
- -

_____
"The above information was acquired by diligent search and/or investigation and the recommendations are based on
prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be

used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution.
Since the Company cannot control the actual methods, volumes, or conditions of use, the Company shall not be held
liable for any damages or losses resulting from the handling or from contact with the product as described herein.
THE INFORMATION IN THIS SDS DOES NOT CONSTITUTE A WARRENTY, EXPRESSED OR IMPLIED,
INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PUPOSE"
Revision date 31-Jul-2015
Product code Product name DMSO (dimethylsulfoxide), anhydrous
www.lifetechnologies.com
End of Safety Data Sheet
D12345

Ethylenediamine Tetraacetic Acid, Disodium Salt
Glycine, N,N'-1,2-ethanediylbis[N-(carboxymethyl)-, disodium salt, dihydrate; Ethylenediaminetetraacetic acid disodium salt dihydrate; Ethylenediamine tetraacetic acid disodiumsalt dihydrate; Disodium edetate dihydrate; Glycine, N,N'-1,2-ethanedlylbis [N-(carboxymethyl), disodium salt, dihydrate; Disodium dihydrogen ethylenediamine-N,N, N',N'-tetraacetate, dihydrate; EDTA, disodium, dihydrate; Ethylene diamine tetraacetic acid, disodium salt; Disodium EDTA, dihydrate; Acetic acid, (ethylenedinitrilo)tetra-, disodium salt, dihydrate
Powder.
CHEMTREC: 800.424.9300
Outside US: 703.527.3887

SAFETY DATA SHEET

GHS product identifier
Other means of identification
Product type
Emergency telephone number (with hours of operation)

Section 1. Identification

:
:
:
Chemical name : Glycine, N,N'-1,2-ethanediylbis[N-(carboxymethyl)-, sodium salt, hydrate (1:2:2)
Supplier's details : Thermo Fisher Scientific
Pierce Biotechnology
P.O. Box 117
Rockford, IL 61105
United States
815.968.0747 or
800.874.3723
7 AM - 5 PM Central Time (GMT -06:00)
Ethylenediamine Tetraacetic Acid, Disodium Salt
Relevant identified uses of the substance or mixture and uses advised against
Not applicable.
Product code
SDS #
CAS #
Chemical formula
:

:
:
:
:
0017892 1879480 1890270 1896264 1896649
1336
C10-H14-N2-O8.2Na.2H2-O
6381-92-6

Section 2. Hazards identification

Classification of the substance or mixture Not classified.

Signal word : No signal word.

Hazard statements : No known significant effects or critical hazards.

Precautionary statements

Prevention : Not applicable.

Response : Not applicable.

Storage : Not applicable.

Disposal : Not applicable.

GHS label elements

OSHA/HCS status : While this material is not considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200), this MSDS contains valuable information critical to the safe handling and proper use of the product. This MSDS should be retained and available for employees and other users of this product.

Hazards not otherwise classified

: Handling and/or processing of this material may generate a dust which can cause mechanical irritation of the eyes, skin, nose and throat.

Section 3. Composition/information on ingredients

Glycine, N,N'-1,2-ethanediylbis[N-(carboxymethyl)-, sodium salt, hydrate (1:2:2)
98 - 100 6381-92-6

Ingredient name % CAS number

There are no additional ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.

Chemical name : Glycine, N,N'-1,2-ethanediylbis[N-(carboxymethyl)-, sodium salt, hydrate (1:2:2)

Other means of identification

: Glycine, N,N'-1,2-ethanediylbis[N-(carboxymethyl)-, disodium salt, dihydrate; Ethylenediaminetetraacetic acid disodium salt dihydrate; Ethylenediamine tetraacetic acid disodiumsalt dihydrate; Disodium edetate dihydrate; Glycine, N,N'-1,2-ethanedlylbis [N-(carboxymethyl), disodium salt, dihydrate; Disodium dihydrogen ethylenediamine-N,N, N',N'-tetraacetate, dihydrate; EDTA, disodium, dihydrate; Ethylene diamine tetraacetic acid, disodium salt; Disodium EDTA, dihydrate; Acetic acid, (ethylenedinitrilo)tetra-, disodium salt, dihydrate

CAS number : 6381-92-6

Substance/mixture

CAS number/other identifiers

:

Occupational exposure limits, if available, are listed in Section 8.

Substance

Any concentration shown as a range is to protect confidentiality or is due to batch variation.

Wash out mouth with water. Remove victim to fresh air and keep at rest in a position comfortable for breathing. If material has been swallowed and the exposed person is conscious, give small quantities of water to drink. Do not induce vomiting unless directed to do so by medical personnel. Get medical attention if symptoms occur.
Immediately flush eyes with plenty of water, occasionally lifting the upper and lower eyelids. Check for and remove any contact lenses. Get medical attention if irritation occurs.
Flush contaminated skin with plenty of water. Remove contaminated clothing and shoes. Get medical attention if symptoms occur.
Remove victim to fresh air and keep at rest in a position comfortable for breathing. Get medical attention if symptoms occur. In case of inhalation of decomposition products in a fire, symptoms may be delayed. The exposed person may need to be kept under medical surveillance for 48 hours.

Section 4. First aid measures

Eye contact
Skin contact
Inhalation
Ingestion :
:
:
:
Description of necessary first aid measures
Most important symptoms/effects, acute and delayed
Inhalation : Exposure to airborne concentrations above statutory or recommended exposure limits may cause irritation of the nose, throat and lungs. Exposure to decomposition products may cause a health hazard. Serious effects may be delayed following exposure.
Ingestion : No known significant effects or critical hazards.
Skin contact : No known significant effects or critical hazards.
Exposure to airborne concentrations above statutory or recommended exposure limits may cause irritation of the eyes.
Eye contact :
Over-exposure signs/symptoms
Skin contact
Inhalation Adverse symptoms may include the following:
respiratory tract irritation
coughing
No specific data.
:
:
Eye contact : Adverse symptoms may include the following:
irritation
redness
Potential acute health effects

Section 4. First aid measures

Protection of first-aiders : No action shall be taken involving any personal risk or without suitable training.
Notes to physician : In case of inhalation of decomposition products in a fire, symptoms may be delayed. The exposed person may need to be kept under medical surveillance for 48 hours.
Specific treatments : No specific treatment.
Ingestion : No specific data.
See toxicological information (Section 11)
Indication of immediate medical attention and special treatment needed, if necessary

Section 5. Fire-fighting measures

Promptly isolate the scene by removing all persons from the vicinity of the incident if there is a fire. No action shall be taken involving any personal risk or without suitable

training.

Hazardous thermal decomposition products

Specific hazards arising from the chemical

Decomposition products may include the following materials:
carbon dioxide
carbon monoxide
nitrogen oxides
metal oxide/oxides
No specific fire or explosion hazard.
Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.

Special protective equipment for fire-fighters

Use an extinguishing agent suitable for the surrounding fire.

Extinguishing media

:
:
:

Remark : May be combustible at high temperature.
None known.

Suitable extinguishing media

:

Unsuitable extinguishing media

:

Special protective actions for fire-fighters

:

Section 6. Accidental release measures

Environmental precautions

Personal precautions, protective equipment and emergency procedures

Move containers from spill area. Approach release from upwind. Prevent entry into sewers, water courses, basements or confined areas. Vacuum or sweep up material and place in a designated, labeled waste container. Avoid creating dusty conditions and prevent wind dispersal. Dispose of via a licensed waste disposal contractor. Note: see Section 1 for emergency contact information and Section 13 for waste disposal.

:

: No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing dust. Put on appropriate personal protective equipment.
Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).

Large spill :

Move containers from spill area. Vacuum or sweep up material and place in a designated, labeled waste container. Dispose of via a licensed waste disposal contractor.

Small spill :

Methods and materials for containment and cleaning up

For non-emergency personnel

For emergency responders : If specialised clothing is required to deal with the spillage, take note of any information in
Section 8 on suitable and unsuitable materials. See also the information in "For nonemergency

personnel".

Section 7. Handling and storage

Advice on general occupational hygiene

Conditions for safe storage, including any incompatibilities

Eating, drinking and smoking should be prohibited in areas where this material is handled, stored and processed. Workers should wash hands and face before eating, drinking and smoking. Remove contaminated clothing and protective equipment before entering eating areas. See also Section 8 for additional information on hygiene measures.
Store in accordance with local regulations. Store in original container protected from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see Section 10) and food and drink. Keep container tightly closed and sealed until ready for use. Containers that have been opened must be carefully resealed and kept upright to prevent leakage. Do not store in unlabeled containers. Use appropriate containment to avoid environmental contamination.

:

:

Protective measures : Put on appropriate personal protective equipment (see Section 8). Avoid breathing dust.

Precautions for safe handling

None.

Section 8. Exposure controls/personal protection

Hand protection

Use a properly fitted, particulate filter respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.
Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary.
Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists, gases or dusts. If contact is possible, the following protection should be worn, unless the assessment indicates a higher degree of protection: safety glasses with side-shields. If operating conditions cause high dust concentrations to be produced, use dust goggles.

Eye/face protection

Respiratory protection :

:

:

Body protection Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.

:

Environmental exposure controls

: Emissions from ventilation or work process equipment should be checked to ensure they comply with the requirements of environmental protection legislation. In some cases, fume scrubbers, filters or engineering modifications to the process equipment will be necessary to reduce emissions to acceptable levels.

Appropriate engineering controls

: Use only with adequate ventilation. If user operations generate dust, fumes, gas, vapor or mist, use process enclosures, local exhaust ventilation or other engineering controls to

keep worker exposure to airborne contaminants below any recommended or statutory limits.
Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period. Appropriate techniques should be used to remove potentially contaminated clothing. Wash contaminated clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location.

Hygiene measures :

Control parameters

Individual protection measures

Occupational exposure limits

Skin protection

Other skin protection : Appropriate footwear and any additional skin protection measures should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.

Section 9. Physical and chemical properties

Physical state

Solid. [Crystalline powder.]

Melting point

248°C (478.4°F)

Vapor pressure

Not available.

Relative density

Not available.

Vapor density

Not available.

Solubility

Soluble in the following materials: cold water and hot water.

Odor Odorless.

pH

Color White.

Evaporation rate Not available.

Auto-ignition temperature

Flash point

Not available.

Closed cup: >100°C (>212°F)

Not available.

4 to 6 [Conc. (% w/w): 5%]

Viscosity Not available.

Odor threshold Not available.

Partition coefficient: noctanol/ water

:
:
:
:
:
:
:
:
:
:
:
:
:

:
:
Appearance
Boiling point : Not available.
Flammability (solid, gas) : Slightly flammable in the presence of the following materials or conditions: heat.
Non-flammable in the presence of the following materials or conditions: shocks and mechanical impacts.
May be combustible at high temperature.
Lower and upper explosive (flammable) limits
: Not available.
Burning rate : Not available.
Burning time : Not available.
SADT : Not available.
Decomposition temperature : >240°C (>464°F)
Solubility in water : 37.22 g/l

Section 10. Stability and reactivity

Hazardous decomposition products
Conditions to avoid No specific data.
Under normal conditions of storage and use, hazardous decomposition products should not be produced.
Chemical stability The product is stable.
No specific data.
:
:
:
Incompatible materials :
Possibility of hazardous reactions
: Under normal conditions of storage and use, hazardous reactions will not occur.
Reactivity : No specific test data related to reactivity available for this product or its ingredients.

Section 11. Toxicological information

Acute toxicity
Information on toxicological effects

Section 11. Toxicological information

Glycine, N,N'-1, 2-ethanediylbis[N-(carboxymethyl)-, sodium salt, hydrate (1:2:2)
LD50 Oral Rat >2000 mg/kg -
Product/ingredient name Result Species Dose Exposure
Carcinogenicity
Not available.
Mutagenicity
Not available.
Teratogenicity
Not available.
Reproductive toxicity
Not available.
Irritation/Corrosion
Not available.
Sensitization
Not available.

Information on the likely
routes of exposure
Inhalation : Exposure to airborne concentrations above statutory or recommended exposure limits may cause irritation of the nose, throat and lungs. Exposure to decomposition products may cause a health hazard. Serious effects may be delayed following exposure.
Ingestion : No known significant effects or critical hazards.
Skin contact : No known significant effects or critical hazards.
Exposure to airborne concentrations above statutory or recommended exposure limits may cause irritation of the eyes.
Eye contact :
Symptoms related to the physical, chemical and toxicological characteristics
Skin contact
Ingestion
Inhalation Adverse symptoms may include the following:
respiratory tract irritation
coughing
No specific data.
No specific data.
:
:
:
Eye contact : Adverse symptoms may include the following:
irritation
redness
Specific target organ toxicity (single exposure)
Specific target organ toxicity (repeated exposure)
Not available.
Not available.
Aspiration hazard
Not available.
: Routes of entry anticipated: Oral, Inhalation.
Potential acute health effects
Classification
Glycine, N,N'-1,
2-ethanediylbis[N-
(carboxymethyl)-, sodium salt,
hydrate (1:2:2)
None. - -
Product/ingredient name OSHA IARC NTP

Section 11. Toxicological information

Not available.
Conclusion/Summary : Exposure can cause stomach pains, vomiting and diarrhea.
General : Repeated or prolonged inhalation of dust may lead to chronic respiratory irritation.
Carcinogenicity : No known significant effects or critical hazards.
Mutagenicity : No known significant effects or critical hazards.
Teratogenicity : No known significant effects or critical hazards.
Developmental effects : No known significant effects or critical hazards.
Fertility effects : No known significant effects or critical hazards.
Potential chronic health effects
Delayed and immediate effects and also chronic effects from short and long term exposure
Numerical measures of toxicity
Not available.
Acute toxicity estimates
Potential immediate
effects

: Not available.
Short term exposure
Potential delayed effects : Not available.
Potential immediate effects
: Not available.
Long term exposure
Potential delayed effects : Not available.

Section 12. Ecological information

Bioaccumulative potential
Other adverse effects : No known significant effects or critical hazards.
Not available.
Toxicity
Not available.
Persistence and degradability
Soil/water partition coefficient (K_{OC})
: Not available.
Mobility in soil
Not available.

Section 13. Disposal considerations

The generation of waste should be avoided or minimized wherever possible. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Waste should not be disposed of untreated to the sewer unless fully compliant with the requirements of all authorities with jurisdiction. Waste packaging should be recycled. Incineration or landfill should only be considered when recycling is not feasible. This material and its container must be disposed of in a safe way. Empty containers or liners may retain some product residues. Avoid dispersal
Disposal methods :

Section 13. Disposal considerations

of spilled material and runoff and contact with soil, waterways, drains and sewers.

Section 14. Transport information

-
-
-
-
Not regulated.
-
-
Not regulated.
- -
DOT Classification IATA
UN number
UN proper shipping name
Transport hazard class(es)
Packing group
Additional information
Environmental

hazards
Special precautions for user
Transport in bulk according to Annex II of MARPOL 73/78 and the IBC Code
No. No.
Transport within user's premises: always transport in closed containers that are upright and secure. Ensure that persons transporting the product know what to do in the event of an accident or spillage.
: Not available.
:

Section 15. Regulatory information

U.S. Federal regulations :
Clean Air Act Section 112 (b) Hazardous Air Pollutants (HAPs)
: Not listed
Clean Air Act Section 602 Class I Substances
: Not listed
Clean Air Act Section 602 Class II Substances
: Not listed
DEA List I Chemicals (Precursor Chemicals)
: Not listed
DEA List II Chemicals (Essential Chemicals)
: Not listed
State regulations
TSCA 8(a) CDR Exempt/Partial exemption: Not determined
United States inventory (TSCA 8b): This material is listed or exempted.
SARA 302/304
SARA 304 RQ : Not applicable.
No products were found.
Composition/information on ingredients
SARA 311/312
Classification : Not applicable.
No products were found.
Composition/information on ingredients
Date of issue/Date of revision **:** *8/23/2013.* ***Date of previous issue :*** *No previous validation.* ***Version :*** *1 8/10*
Ethylenediamine Tetraacetic Acid, Disodium Salt

Section 15. Regulatory information

Massachusetts : This material is not listed.
New York : This material is not listed.
New Jersey : This material is not listed.
Pennsylvania : This material is not listed.
Canada inventory : This material is listed or exempted.
Australia inventory (AICS): This material is listed or exempted.
China inventory (IECSC): This material is listed or exempted.
Japan inventory: This material is listed or exempted.
Korea inventory: This material is listed or exempted.
Malaysia Inventory (EHS Register): Not determined.
New Zealand Inventory of Chemicals (NZIoC): This material is listed or exempted.
Philippines inventory (PICCS): This material is listed or exempted.
Taiwan inventory (CSNN): Not determined.
International regulations

International lists :
Chemical Weapons
Convention List Schedule I
Chemicals
: Not listed
Chemical Weapons
Convention List Schedule
II Chemicals
: Not listed
Chemical Weapons
Convention List Schedule
III Chemicals
: Not listed

Section 16. Other information

12/12/2013.
History
Date of printing :
: 8/23/2013.
Hazardous Material Information System (U.S.A.)
0
0
0
0
0
0
National Fire Protection Association (U.S.A.)
Health
Special
Instability/Reactivity
Flammability
Health
Flammability
Caution: HMIS® ratings are based on a 0-4 rating scale, with 0 representing minimal hazards or risks, and 4
representing significant hazards or risks Although HMIS® ratings are not required on SDSs under 29 CFR 1910.
1200, the preparer may choose to provide them. HMIS® ratings are to be used with a fully implemented HMIS®
program. HMIS® is a registered mark of the National Paint & Coatings Association (NPCA). HMIS® materials may
be purchased exclusively from J. J. Keller (800) 327-6868.
The customer is responsible for determining the PPE code for this material.
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Response Copyright ©1997, National Fire Protection Association, Quincy, MA 02269. This reprinted material is
not the complete and official position of the National Fire Protection Association, on the referenced subject
which is represented only by the standard in its entirety.
Copyright ©2001, National Fire Protection Association, Quincy, MA 02269. This warning system is intended to be
interpreted and applied only by properly trained individuals to identify fire, health and reactivity hazards of
chemicals. The user is referred to certain limited number of chemicals with recommended classifications in NFPA
49 and NFPA 325, which would be used as a guideline only. Whether the chemicals are classified by NFPA or not,
anyone using the 704 systems to classify chemicals does so at their own risk.

Section 16. Other information

Date of issue/Date of revision

Version

To the best of our knowledge, the information contained herein is accurate. However, neither the above-named supplier, nor any of its subsidiaries, assumes any liability whatsoever for the accuracy or completeness of the information contained herein.

Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

Notice to reader

Date of previous issue

:

:

Indicates information that has changed from previously issued version.

References : Not available.

Key to abbreviations : ATE = Acute Toxicity Estimate
BCF = Bioconcentration Factor
GHS = Globally Harmonized System of Classification and Labelling of Chemicals
IATA = International Air Transport Association
IBC = Intermediate Bulk Container
IMDG = International Maritime Dangerous Goods
LogPow = logarithm of the octanol/water partition coefficient
MARPOL 73/78 = International Convention for the Prevention of Pollution From Ships, 1973 as modified by the Protocol of 1978. ("Marpol" = marine pollution)
UN = United Nations
No previous validation.
1

Prepared by : SDS Specialist

1. PRODUCT AND COMPANY IDENTIFICATION

PRODUCT NAME: Nitrogen, refrigerated liquid
CHEMICAL NAME: Nitrogen
CHEMICAL FAMILY: Inert gas
SYNONYMS: Cryogenic Liquid Nitrogen, Liquid Nitrogen, LIN
CHEMICAL FORMULA: N_2
USE: Medical purposes, Inerting, Safe storage of Food, Concrete strengthening, etc.,
NAME AND ADDRESS: **Refrigeration & Oxygen Co.**

Corporate Office
Area No 1, Block 21 C,
Central Slaughter House Street
Shuwaikh Industrial Area
Kuwait.
WEB ADDRESS: www.rockuwait.com; E-mail: info@rocq8.com
TELEPHONE: (+965) 844 844

2. HAZARDS IDENTIFICATION

EMERGENCY OVERVIEW:
WARNING! Extremely cold liquid and gas under pressure.
Can cause rapid suffocation.

Can cause severe frostbite.
POTENTIAL HEALTH EFFECTS INFORMATION:
ROUTES OF EXPOSURE:
INHALATION: Simple Asphyxiant.
Nontoxic, but may cause suffocation by displacing the oxygen in air. Exposure to oxygen-deficient
atmosphere (<19.5%) may cause dizziness, drowsiness, nausea, vomiting, excess salivation, diminished mental alertness, loss of consciousness and death. Exposure to atmospheres containing
8% to 10% or less oxygen will bring about unconsciousness without warning and so quickly that the individuals cannot help or protect themselves. Lack of sufficient oxygen may cause serious injury or death.
EYE CONTACT: Tissue freezing and severe cryogenic burns of eyes
SKIN CONTACT: Tissue freezing and severe cryogenic burns of skin
SKIN ABSORPTION: Not applicable
INGESTION: Not applicable
CHRONIC EFFECTS: None established
MEDICAL CONDITIONS AGGRAVATED BY OVEREXPOSURE: None
OTHER EFFECTS OF OVEREXPOSURE: None
CARCINOGENICITY: Not listed by NTP, OSHA, or IARC.
POTENTIAL ENVIRONMENTAL EFFECTS: No adverse ecological effects are expected.

3. COMPOSITION/INFORMATION ON INGREDIENTS

INGREDIENT NAME: Nitrogen
PERCENTAGE >99%
CAS NUMBER 7727-37-9

4. FIRST AID MEASURES

FIRST AID PROCEDURES:
INHALATION: Persons suffering from lack of oxygen should be removed to fresh air. If victim is
not breathing, give artificial respiration. If breathing is difficult, give oxygen. Obtain prompt medical attention.
EYE CONTACT: In case of splash contamination, immediately flush eyes with water for at least 15 minutes. See a physician, preferably an ophthalmologist.
SKIN CONTACT: Remove any clothing that may restrict circulation to frozen area. Do not rub frozen parts as tissue damage may result. As soon as practical, place the affected area in a warm water bath which has a temperature not exceeding 105 °F (40°C). Never use dry heat. In case of massive exposure, remove clothing while showering with warm water. Call a physician as soon as
possible.
Frozen tissue is painless and appears waxy with a possible yellow color. It will become swollen, painful, and prone to infection when thawed. If the frozen part of the body has been thawed by the
time medical attention has been obtained, cover the area with dry sterile dressing with a large

bulky protective covering.
INGESTION: Not applicable
NOTES TO PHYSICIAN: None

5. FIREFIGHTING MEASURES

FLAMMABLE PROPERTIES: Nonflammable and does not support combustion.
EXTINGUISHING MEDIA: Use extinguishing media appropriate for the surrounding fire.

PROTECTION OF FIREFIGHTERS:

SPECIFIC HAZARDS ARISING FROM THE CHEMICAL: When spilled the liquid will vaporize
rapidly forming an oxygen-deficient vapor cloud. Evacuate this vapor cloud area. Visibility may be
obscured in the vapor cloud. Pressure in a container can build up due to heat and it may rupture if pressure relief devices should fail to function. Contact with cold liquid or gas may cause frostbite.
PROTECTIVE EQUIPMENT AND PRECAUTIONS FOR FIREFIGHTERS: Simple asphyxiant.
If possible, remove containers from fire area or cool with water. Do not direct water spray at the container vent. Self contained breathing apparatus may be required for rescue workers. Evacuate this area.
SENSITIVITY TO STATIC DISCHARGE: Not Applicable
SENSITIVITY TO MECHANICAL IMPACT: None

6. ACCIDENTAL RELEASE MEASURES

PERSONAL PRECAUTIONS: Use personal protection recommended in Section 8. Evacuate all personnel from the affected area. Ventilate area or remove containers to a well ventilated location.
To increase rate of vaporization, spray large amounts of water onto the spill from an upwind position. If leaking from container or its valve, contact your supplier.
ENVIRONMENTAL PRECAUTIONS: Not applicable.
METHODS FOR CONTAINMENT: Shut off source if possible without risk.
METHODS FOR CLEAN-UP: Not applicable.
OTHER INFORMATION: None.

7. HANDLING AND STORAGE

HANDLING: Never allow any unprotected part of the body to touch un-insulated pipes or vessels
that contain cryogenic fluids. The extremely cold metal will cause the flesh to stick fast and tear when one attempts to withdraw from it.
Use a suitable four-wheel hand truck for container movement. Cryogenic containers shall be handled and stored in an upright position. Do not drop or roll containers on their sides. If user experiences any difficulty operating container valve discontinue use and contact supplier. For additional precautions see Section 16, Other Information.
STORAGE: Store and use with adequate ventilation. Compressed gas cylinders shall be separated

from materials and conditions that present exposure hazards to or from each other. Do not store in
a confined space. Cryogenic containers are equipped with pressure relief devices to control internal
pressure. Under normal conditions these containers will periodically vent product. Some metals such as carbon steel may become brittle at low temperatures and will easily fracture. Prevent entrapment of liquid in closed systems or piping without pressure relief.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

EXPOSURE GUIDELINES:
OSHA PEL-TWA: None NIOSH IDLH: None
ACGIH TLV: Simple asphyxiant
ENGINEERING CONTROLS:
VENTILATION: Natural or mechanical to prevent oxygen-deficient atmospheres below 19.5% oxygen.
PERSONAL PROTECTIVE EQUIPMENT:
EYE/FACE PROTECTION: Full face shield and safety glasses are recommended.
SKIN PROTECTION: Loose fitting thermal insulated or leather gloves. Safety shoes are recommended when handling liquid containers. Long sleeve shirts and trousers without cuffs.
RESPIRATORY PROTECTION (SPECIFY TYPE):
General Use: None required
Emergency Use: Self-contained breathing apparatus (SCBA) or positive pressure airline with mask
are to be used in oxygen-deficient atmosphere. Air purifying respirators will not function.

9. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE: Colorless
ODOR: Odorless
ODOR THRESHOLD: Not applicable
PHYSICAL STATE: Cryogenic liquid
pH: Not applicable
MELTING POINT: -345.8 ºF (-209.9 ºC) @ 1 atm
BOILING POINT: -320.4 ºF (-195.8 ºC) @ 1 atm
FLASH POINT: Not applicable
EVAPORATION RATE (Butyl Acetate=1): Not applicable
FLAMMABILITY: Nonflammable
FLAMMABLE LIMITS IN AIR BY VOLUME:
LOWER: Not applicable; UPPER: Not applicable
VAPOR PRESSURE (AT 20 °C): Not applicable
GAS DENSITY: 0.072 lb/ft^3 (1.153 kg/m^3) @ 70 of (21.1 °C) and 1 atm
SPECIFIC GRAVITY (Air =1): 0.967 @ 70 °F (21.1 °C) and 1 atm
SOLUBILITY IN WATER: Vol/Vol at 32 °F (0 °C): 0.023
COEFFICIENT OF WATER/OIL DISTRIBUTION: Not available
AUTOIGNITION: Nonflammable
DECOMPOSITION TEMPERATURE: Not applicable

MOLECULAR WEIGHT: 28.01
EXPANSION RATIO: (for liquid to gas) 70 °F (21.1 °C): 1 to 696.5

10. STABILITY AND REACTIVITY

CHEMICAL STABILITY: Stable
CONDITIONS TO AVOID: None
INCOMPATIBLE MATERIALS: None
HAZARDOUS DECOMPOSITION PRODUCTS: None
POSSIBILITY OF HAZARDOUS REACTIONS: Will not occur

11. TOXICOLOGICAL INFORMATION

The product is simple asphyxiant.
ACUTE DOSE EFFECTS: LD_{50}: None LCso: None
REPEATED DOSE EFFECTS: None established
IRRITATION: None
SENSITIZATION: None
GENETIC EFFECTS: None
DEVELOPMENTAL EFFECTS: None
TERATOGENICITY: None
SYNERGISTIC MATERIALS: None
REPRODUCTIVE EFFECTS: None
TARGET ORGAN EFFECTS: None

MUTAGENICITY: None

12. ECOLOGICAL INFORMATION

ECOTOXICITY: No adverse ecological effects are expected. It does not contain any Class I or Class II ozone depleting chemicals (40 CFR Part 82). Not listed as a marine pollutant by DOT (49
CFR Part 171).

13. DISPOSAL CONSIDERATIONS

WASTE DISPOSAL METHOD: Do not attempt to dispose of residual or unused quantities. Contact your supplier.
For emergency disposal, discharge slowly to the atmosphere in a well ventilated area or outdoors.

14. TRANSPORT INFORMATION

Product Identification Number: 1977
BASIC SHIPPING DESCRIPTION:
PROPER SHIPPING NAME: Nitrogen, refrigerated liquid
HAZARD CLASS: 2.2 (Nonflammable Gas)
IDENTIFICATION NUMBER: UN 1977
ADDITIONAL INFORMATION:
PRODUCT RQ: Not applicable
SHIPPING LABEL(s): Nonflammable gas
PLACARD (When required): Nonflammable gas
SPECIAL SHIPPING INFORMATION: Containers should be transported in a secure position, in a

well ventilated vehicle. The transportation of compressed gas containers in automobiles or in closed-body vehicles can present serious safety hazards and should be discouraged. For air shipments, the "Cryogenic Liquid" handling label must be used in addition to the nonflammable gas (Division 2.2) hazard label on packages and over packs containing cryogenic liquids.

15. REGULATORY INFORMATION & OTHER INFORMATION

SPECIAL PRECAUTIONS: Use piping and equipment adequately designed to withstand pressures and temperatures to be encountered. Use a check valve or other protective apparatus in any line or piping from the container to prevent reverse flow. Cross contamination of gases, liquids, or both can also create a hazardous condition inside a cylinder, dewar, or vessel (e.g., flammable and oxidizing gases can create an explosive mixture), which may result in rupture. To prevent cryogenic liquids or cold gas from being trapped in piping between valves the piping shall
be equipped with pressure relief devices. Only transfer lines designed for cryogenic liquids shall be
used. It is recommended that all vents be piped to the exterior of the building.

Shipment of compressed gas containers that have not been filled with the owner's consent is a violation of Federal law (49 CFR Part 173.301.b)

HAZARD RATINGS AND RATING SYSTEMS:

NFPA RATINGS:

HEALTH: =3; FLAMMABILITY: =0; INSTABILITY: =0; SPECIAL: SA

STANDARD VALVE CONNECTIONS:

THREADED: CGA 295

PIN-INDEXED YOKE: Not applicable

ULTRA HIGH INTEGRITY: Not applicable

Use the proper connections; DO NOT USE ADAPTERS. DO NOT FORCE FIT CONNECTIONS.

The information and recommendations in this Material Safety Data Sheet relate only to the specific material mentioned herein and do
not relate to use otherwise ie., in combination with any other material or in any process.

The information and recommendations herein are taken from our extensive experiences and the data contained in recognized references and believed by us to be accurate. Refrigeration group
of companies make no warranties either expressed or implied with respect there to and assume no liability in connection with the use of such information and recommendation.

Safety Data Sheet

SECTION 2: Hazards identification

Health hazards

GHS - Classification

Skin corrosion/irritation Category 2
Serious eye damage/eye irritation Category 2
Respiratory sensitization Category 1
Specific target organ systemic toxicity (single exposure) Category 3

Signal Word

DANGER

Identification of the substance or mixture

---

24 hour Emergency Response: 866-536-0631
301-431-8585
Outside of the U.S. +1-301-431-8585

Product code

Physical hazards

27250018
27250018

Company/undertaking identification

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

Revision date

Product name TRYPSIN, 1/250Trypsin (1:250) porcine parvovirus tested

Not Hazardous

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SECTION 1: Identification of the substance/mixture and of the company/undertaking

Product name

30-Jan-2015
TRYPSIN, 1/250Trypsin (1:250) porcine parvovirus tested

www.lifetechnologies.com

Product code

Life Technologies
5250 Mainway Drive
Burlington, ONT
CANADA L7L 6A4
800/263-6236
Life Technologies
5791 Van Allen Way
PO Box 6482
Carlsbad, CA 92008
+1 760 603 7200

Reproductive toxicity None

Skin

Sensitization R42 - May cause sensitization by inhalation
Irritating to skin.

Target Organ Effects None under normal use conditions

inhalation Irritating to respiratory system.

SECTION 4: First aid measures

Ingestion

Skin contact Wash off immediately with plenty of water for at least 15 minutes. Take off all contaminated clothing and wash it before reuse. Immediate medical attention is required.

HMIS
May be harmful if swallowed. Ingestion may cause gastrointestinal irritation,
nausea, vomiting and diarrhea.
Hazard Statements
H316 - Causes mild skin irritation
H320 - Causes eye irritation
H335 - May cause respiratory irritation
H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled
Precautionary Statements
P261 - Avoid breathing dust/fume/gas/mist/vapors/spray
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if
present and easy to do. Continue rinsing
P342 + P311 - If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician
P332 + P313 - If skin irritation occurs: Get medical advice/attention
P304 + P312 - IF INHALED: Call a POISON CENTER or doctor if you feel unwell
P312 - Call a POISON CENTER or doctor/physician if you feel unwell
Principle Routes of Exposure
Potential Health Effects
Specific effects
Carcinogenic effects None
eyes Irritating to eyes.
Mutagenic effects None

SECTION 3: Composition/information on ingredients

Flammability 0
Health 2 * Chronic Hazard
27250018
Chemical Name CAS-No
Revision date
EINECS-No
Product name TRYPSIN, 1/250Trypsin (1:250) porcine parvovirus
tested
Weight %
Page 2 / 6
Reactivity 0
Trypsin 9002-07-7
30-Jan-2015
232-650-8 60-100
www.lifetechnologies.com
Product code
We recommend handling all chemicals with caution.
Engineering measures Ensure adequate ventilation, especially in confined areas.
Ingestion Call a physician or poison control center immediately. Never give anything by
mouth to an unconscious person. Do not induce vomiting without medical advice.
See Section 12 for more information.

SECTION 6: Accidental release measures

Personal Protective Equipment
Personal Protective Equipment requirements are dependent on the user institution's risk assessment and are specific
to the risk assessment for each laboratory where this material may be used.
Notes to Physician

SECTION 7: Handling and storage

Treat symptomatically.
Respiratory protection In case of insufficient ventilation, wear suitable respiratory equipment.
Suitable extinguishing media

Personal precautions Ensure adequate ventilation. Avoid contact with skin, eyes or clothing. Use personal protection equipment.
Handling Always wear reccommended Personal Protective Equipment. No special handling advices are necessary.
Hand protection Impervious gloves.
Water spray. Carbon dioxide (CO2). Foam. Dry chemical.
Eye contact
Eye protection Wear safety glasses with side shields (or goggles).
Storage Keep in a dry, cool and well-ventilated place. Keep in properly labeled containers.
Skin and Body Protection Lightweight protective clothing.
Methods for cleaning up Take up mechanically, placing in appropriate containers for disposal.
Hygiene measures Handle in accordance with good industrial hygiene and safety practice.
Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Immediate medical attention is required.
Special protective equipment for firefighters

SECTION 8: Exposure controls/personal protection

Wear self-contained breathing apparatus and protective suit.
Exposure Limits
Environmental precautions
inhalation
27250018
Chemical Name OSHA PEL
Revision date
OSHA PEL (Ceiling)
Product name TRYPSIN, 1/250Trypsin (1:250) porcine parvovirus tested
ACGIH OEL (TWA)
Page 3 / 6
ACGIH OEL (STEL)
Remove to fresh air. If not breathing, give artificial respiration. Call a physician or poison control center immediately.
Trypsin
30-Jan-2015
None None
www.lifetechnologies.com
None None
Product code
Prevent further leakage or spillage if safe to do so. Prevent product from entering drains.

SECTION 5: Firefighting measures

Principle Routes of Exposure
Potential Health Effects
eyes Irritating to eyes

SECTION 9: Physical and chemical properties

Skin Irritating to skin

SECTION 10: Stability and reactivity

inhalation Irritating to respiratory system
Ingestion May be harmful if swallowed Ingestion may cause gastrointestinal irritation, nausea, vomiting and diarrhea
Environmental exposure controls
Carcinogenic effects None
Stability Stable under normal conditions.
Mutagenic effects None
Prevent product from entering drains. Do not allow material to contaminate ground

water system.
Reproductive toxicity None.
Materials to avoid Strong oxidizing agents.
Sensitization R42 - May cause sensitization by inhalation
Form
Hazardous decomposition
products
None under normal use conditions.
Target Organ Effects None under normal use conditions
solid
polymerization None under normal processing.

SECTION 12: Ecological information

Appearance

SECTION 11: Toxicological information

Ecotoxicity No information available
powder

Acute Toxicity

General information
Oxidizing properties No information available

Odor
Chemical Name LD50 (oral,rat/mouse) LD50 (dermal,rat/rabbit) LC50 (inhalation,rat/mouse)
Water solubility soluble
Trypsin No data available No data available
27250018
No data available
No information available
Revision date
°F No data available
Product name TRYPSIN, 1/250Trypsin (1:250) porcine parvovirus
tested
°C No data available

°F No data available
°F No data available
Melting point / melting range °C No data available
30-Jan-2015
Autoignition Temperature
°C No data available
www.lifetechnologies.com
Boiling point / boiling range °F No data available
Product code
flash point
°C No data available

SECTION 13: Disposal considerations

Mobility
IATA
No information available
Proper shipping name No dangerous good in sense of these transport regulations
Dispose of contents/containers in accordance with local regulations.
Hazard Class None
Bioaccumulation
Subsidiary class None
No information available
Packing group None

SECTION 14: Transport information

UN-No none

Biodegradation No information available

SECTION 15: Regulatory information

Trypsin

9002-07-7 (60-100)

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the

MSDS contains all the information required by the CPR.

US State Regulations

Listed

SARA 313

This product is not regulated by SARA.

Component

Chemical Name Massachusetts - RTK New Jersey - RTK Pennsylvania - RTK

Illinois - RTK Rhode Island - RTK

Trypsin - Listed - - -

27250018

Revision date

Product name TRYPSIN, 1/250Trypsin (1:250) porcine parvovirus tested

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TSCA

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)

This product does not contains HAPs.

California Proposition 65

This product does not contain any Proposition 65 chemicals.

30-Jan-2015

US Federal Regulations

www.lifetechnologies.com

Product code

WHMIS Hazard Class

D2A - Very toxic materials

D2B - Toxic materials

"The above information was acquired by diligent search and/or investigation and the recommendations are based on

prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be

used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution.

Since the Company cannot control the actual methods, volumes, or conditions of use, the Company shall not be held

liable for any damages or losses resulting from the handling or from contact with the product as described herein.

THE INFORMATION IN THIS SDS DOES NOT CONSTITUTE A WARRENTY, EXPRESSED OR IMPLIED,

INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PUPOSE"

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

Product name TRYPSIN, 1/250Trypsin (1:250) porcine parvovirus tested

End of Safety Data Sheet

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SECTION 16: Other information

Reason for revision

30-Jan-2015
Product code
www.lifetechnologies.com
SDS sections updated.
27250018
Revision date