

SOP: End-of-Run cNIST mAb Process: Harvest, Centrifugation, TFF Concentration

Approvals:

Preparer: Hetal Doshi

Date: 10JAN24

Reviewer: Dr. Maggie Bryans

Date: 17JAN24

1. Purpose:

- 1.1. To harvest anti cNIST mAb containing conditioned medium, concentrate and prepare for chromatography; intermediate steps include centrifugation and sterile filtration to remove cells and cell debris prior to concentrating and buffer exchange by tangential flow filtration.

2. Scope and Applicability:

- 2.1. A biomanufacturing environment requires proper steps to recover and purify active pharmaceutical ingredients from a bioreactor or fermenter. This SOP provides bench-scale procedures to accomplish that goal using a conditioned medium from cells expressing recombinant cNIST mAb. The method demonstrates the principles of tangential flow filtration, centrifugation, and sterile filtration in preparation for downstream processing by column chromatography as may be employed in a typical process development, for later scale up to manufacturing.

3. Summary of Method:

- 3.1. Preparation of solutions:
 - 3.1.1. PBS/Tween 80 for preconditioning of the Pellicon cassette (for TFF)
 - 3.1.2. 0.1 N NaOH for cleaning the Pellicon cassette following use
 - 3.1.3. 0.05 N NaOH for storage of the Pellicon cassette
- 3.2. Flushing and preconditioning of TFF/Pellicon.
- 3.3. Transfer of culture from bioreactor to centrifuge bottles.
- 3.4. Centrifugation to pellet cells.
- 3.5. Sterile filtration of the conditioned medium(CM) using 0.22 micron vacuum filter units with storage bottle.
- 3.6. Addition of protease inhibitors and Tween 80.
- 3.7. Concentration of supplemented CM by tangential flow filtration.

4. References:

- 4.1 Millipore Tangential Flow and Diafiltration Using Pellicon XL Device of tPA SOP
- 4.2 Oakton PC 700 Bench Series pH/Conductivity/°C/°F Meter SOP (Doc # 1.0).

5. Definitions:

- 5.1. Permeate- the material that passes through the membrane.
- 5.2. Retentate- the material that does not pass through the membrane.
- 5.3. TFF – tangential flow filtration
- 5.4. CM – conditioned medium, which contains the API of interest

SOP: End-of-Run cNIST mAb Process: Harvest, Centrifugation, TFF Concentration

6. Precautions:

- 6.1. 0.1N NaOH is very corrosive. It is extremely damaging to the eyes and mucous membranes. It causes burns. Avoid contact with skin. It is harmful if swallowed or inhaled. The Millipore Pellicon XL Device is stored flat at 4-25°C with 10 mL of 0.1N NaOH.
- 6.2. NEVER tighten the clamp enough to completely restrict the flow in the Retentate tube. This could damage the filter and cause the tubing to disconnect.
- 6.3. Luer Lock fittings on the TFF device should be tightened with care not to exert too much force, to avoid stripping threads or damaging the fitting.

7. Responsibilities:

- 7.1 It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
- 7.2 It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

8. Equipment and Materials:

- 8.1. 250 mL Nalgene centrifuge bottles (3)
- 8.2. 250 mL Corning bottles (3)
- 8.3. 0.1N NaOH (sodium hydroxide)
- 8.4. 0.05N NaOH (sodium hydroxide)
- 8.5. 10% (w/v) Tween 80
- 8.6. Preconditioning buffer (PBS containing 0.1% Tween 80)- 50 mL
- 8.7. Stock solutions of 100X protease inhibitors cocktail
- 8.8. Millipore Tangential Flow Filtration System and Pellicon XL Device and Accessories
- 8.9. MilliQ Water
- 8.10. 10 mL graduated cylinder
- 8.11. 0.22-micron Nalagene vacuum sterile filtration unit with storage bottle (3)

9. Procedure:

- 9.1. Preparation of solutions (provided).
 - 9.1.1. Prepare 0.1N NaOH for cleaning.
 - 9.1.1.1. Using a 1L graduated cylinder, measure 650ml of MilliQ water.
 - 9.1.1.2. Transfer the MilliQ water into 800ml beaker
 - 9.1.1.3. Weigh 3.2g \pm 0.05g of NaOH.
 - 9.1.1.4. Transfer NaOH to the beaker containing MilliQ water.
 - 9.1.1.5. Add magnetic stir bar and stir to dissolve.
 - 9.1.1.6. Bring the solution to a final volume of 700ml using MilliQ water.
 - 9.1.1.7. Sterile filter the solution and label container: 0.1N NaOH, [date], [initials], [company name].
 - 9.1.2. Prepare 0.05N NaOH for Pellicon XL Device Storage
 - 9.1.2.1 Transfer 5ml of 0.1N NaOH from step 9.1.1. in a 15ml conical tube
ure 5 mL of MilliQ water
 - 9.1.2.2 Transfer 5ml of milliQ water to a 15ml conical tube prepared in step 9.1.2.1.

SOP: End-of-Run cNIST mAb Process: Harvest, Centrifugation, TFF Concentration

- 9.1.2.3 Sterile filter the solution with 0.2 micron syringe filter in a sterile 15 ml conical tube and label container: 0.05N NaOH, [date], [initials], [team name]
- 9.1.3. 10% w/v Tween 80
- 9.1.3.1 Measure 80ml of MilliQ water and add to a 200ml beaker with magnetic stir bar
- 9.1.3.2 Place the beaker on a balance and tare the balance when stable
- 9.1.3.3 Pipette 10g Tween 80(polyoxyethylene soebitam monooleate) into the beaker with water.
- 9.1.3.4 Stir until all of the Tween 80 is dissolved:this can take 30 minutes or more to complete. Carefully adjust the stir plate rpm to provide adequate mixing vigor without introducing air bubbles or frothing.
- 9.1.4. 1X PBS with 0.1% Tween 80
- 9.1.4.1. Measure 79.2 ml 1X PBS with 100 ml graduated cylinder and transfer to 100 ml beaker with a magnetic stirrer.
- 9.1.4.2. Measure 0.8 ml of 10% Tween 80 with 1ml serological pipette and add to the 79.2 ml 1X PBS
- 9.1.4.3. Mix the solution by placing the beaker on the magnetic stir plate set at . low stirring speed till Tween 80 is completely dissolved.
- 9.1.4.3. Sterile filter the solution with the 0.2 μ m filter. Store the prepared solution in a sterile bottle labeled [date],[solution name],[team name] and [Intials]
- 9.2. Labscale 500mL Reservoir Set Up
- 9.2.1. Install Retenate tubing
- Note: All tubing lengths are recommended to minimize recirculation volume. Longer lengths may be used. After prolonged storage, the tubing may absorb a small volume of water. As a result, the tubing color may change from translucent to opaque, which is normal. Air or oven drying will return the color to translucent.
- 9.2.1.1.Cut silicone (translucent) tubing and install fittings as shown in figure 10.
- 9.2.1.2.Remove plugs from retentate outlet (RET OUT) and retentate inlet (RET IN) ports.
- 9.2.1.3.Insert the male Luer end of the retentate tubing into the RET OUT port and the female Luer end of the retentate tubing into the RET IN port. Turn fittings until snug.
- 9.2.2. Install Permeate tubing
- 9.2.2.1.Cut silicone (translucent) tubing and install fittings as shown in figure 12.
- 9.2.2.2.Remove the plug from the permeate outlet port (PERM 2) and insert the male luer end of the permeate silicone (translucent) tubing into the PERM2 port. Turn fittings until snug.
- 9.2.3. Install Tank Outlet Valve
- 9.2.4. Remove plug from the tank outlet port (TANK OUT) and insert the female luer end of the tank outlet valve over the TANK OUT port. Turn the lock nut until snug.
- 9.2.5. Install Vent Filter (If required)

SOP: End-of-Run cNIST mAb Process: Harvest, Centrifugation, TFF Concentration

- 9.2.6. If a sterile vent is required, remove plug from the vent (VENT) port and insert the male luer end of MILLEX filter into the vent port.
- 9.2.4. Install Stir Bar
 - 9.2.4.1. If mixing is required, open reservoir cover and drop stir bar to the bottom of the reservoir.
- 9.3. Lab scale Stir Base Set Up
 - 9.3.1. Power Connection
 - 9.3.1.1. Turn Stirrer and pump speed controls to the off position.
 - 9.3.1.2. Connect power cord to the power cord receptacle located at the rear of the system base.
 - 9.3.1.3. Align detent on connector with receptacle.
 - 9.3.1.4. Press connector into receptacle and turn lock ring to secure.
 - 9.3.2. Check Operation
 - 9.3.2.1. Remove the plugs from the pump inlet and pump outlet ports.
 - 9.3.2.2. Turn on the pump speed control, set to 2, and listen for pump motor.
 - 9.3.2.3. Turn off the pump speed control.
 - 9.3.2.4. Turn on the stirrer speed control and listen for the stirrer motor.
 - 9.3.2.5. Turn off the stirrer speed control.
- 9.4. Install Pellicon XL Cassette
 - 9.4.1. Remove the plugs from FEED, RET, PERM 1, and PERM 2 ports on the Pellicon XL cassette.
 - 9.4.2. Align the Pellicon XL cassette ports with Labscale 500 ml Reservoir ports being sure the PERM and RET DEVICE ports of the Pellicon XL cassette and reservoir match. Press the cassette firmly onto the reservoir ports. Turn the lock nuts until snug.
- 9.5. Flushing of TFF and cassette.

One should become familiar with the location of ports and tubing connection points as shown in the attachments at the end of this SOP prior to beginning setup.

 - 9.5.1. Set up the apparatus and confirm that all tubing connections are secure, according to the SOP (Millipore Tangential Flow and Diafiltration Using Pellicon XL Device SOP).
 - 9.5.2. Remove the 4 plugs on the Pellicon cassette and attach the Pellicon cassette to the Labscale apparatus.
 - 9.5.3. Add 500 mL MilliQ water to the reservoir and flush the cassette as described in section 9.4.4.
 - 9.5.4. Flushing the Pellicon cassette.
 - 9.5.4.1. Disconnect retentate silicone (translucent) tubing from RET IN port and place end of retentate tubing in waste collection vessel.
 - 9.5.4.2. Place end of permeate silicone (translucent) tubing into waste collection vessel. Open retentate valve by turning the counterclockwise.
 - 9.5.4.3. Remove the reservoir cover and fill reservoir with 500 mL of MilliQ water. Remove the plug from VENT port and open tank outlet valve.
 - 9.5.4.4. Turn the pump on and increase the speed until the feed pressure gauge reads 1.38 Bar (20 psi).

SOP: End-of-Run cNIST mAb Process: Harvest, Centrifugation, TFF Concentration

- 9.5.4.5. Continue pumping to the waste collection vessel until the level in the reservoir drops to 350 mL and then turn the pump off.
 - 9.5.4.6. Reconnect the retentate silicone (translucent) tubing to the RET IN port and turn the pump on. Slowly increase the pump speed until feed pressure gauge reads 1.38 Bar (20 psi). Check the system for leaks and tighten connections if leaks are found.
 - 9.5.4.7. Adjust retentate valve restriction by slowly turning retentate valve clockwise until the retentate pressure gauge reads 0.69 Bar (10 psi).
 - 9.5.4.8. Adjust pump speed and retentate valve restriction to achieve 2.07 Bar (30 psi) feed pressure and 0.69 Bar (10 psi) retentate pressure.
 - 9.5.4.9. Allow to run until 50 mL remains in the chamber.
 - 9.5.4.10. Disconnect the pump outlet (Sta-pure, white) tubing from the pump outlet port and place in waste collection vessel.
 - 9.5.4.11. Disconnect the retentate silicone (translucent) tubing from the RET IN port. Fluid should now drain by gravity. If additional drainage is required, a syringe can be placed on the end of the retentate tube and fluid can be blown down.
 - 9.5.4.12. Remove the remainder of water in the chamber as follows: Replace retentate tubing (silicone, translucent) in retentate port. Reconnect pump outlet tubing (Sta-Pure, white).
 - 9.5.4.13. Disconnect FEED IN tubing and place in collection vessel. Open tank outlet valve, turn pump speed up to drain reservoir.
 - 9.5.4.14. Reconnect the pump outlet tubing (Sta-Pure, white) to the Feed In port.
- 9.6. Pre-conditioning
- 9.6.1. Place end of permeate tubing silicone (translucent) in the waste collection vessel.
 - 9.6.2. Remove reservoir cover and fill the reservoir with 50 mL of PBS containing 0.1% Tween 80 (or other appropriate buffer) and then remove the Vent port plug.
 - 9.6.3. Open the tank outlet valve. Turn the pump on and increase the pump speed until the feed pressure gauge reads 1.38 Bar (20 psi at its maximum; the needle will pulse as the pump turns). Check all system connections for leaks and tighten any connections as necessary.
 - 9.6.4. Continue pumping to the waste collection vessel until the level in the reservoir drops to the bottom of the reservoir stir bar well making sure to stop the pump before air is pumped into the system. Turn the pump off.
- 9.7. Transfer of culture from bioreactor to centrifuge bottles.
- 9.7.1. Refer to the SOP: Applikon Bioreactor Controller Operation for instructions on removing the headplate of the bioreactor, providing access to the cells and conditioned medium.
 - 9.7.2. Transfer the culture to three 250 mL centrifuge bottles using a 100 mL pipet and PipetAid. Residual culture can be transferred to an Ehrlenmeyer flask for temporary storage.
 - 9.7.3. Centrifuge cells in pre-chilled Sorvall centrifuge, fitted with a SLA1500 rotor, at 2500 x g for 10 min at 4° C.
 - 9.7.4. Transfer conditioned medium (CM) from centrifuge bottle to storage vessel/bottle by carefully decanting the supernatant to appropriately labeled 250 mL Corning bottles.

SOP: End-of-Run cNIST mAb Process: Harvest, Centrifugation, TFF Concentration

- 9.7.5. Sterile filter the conditioned media using the vacuum sterile filter unit with storage bottle in the BSC. Store the Conditioned Media at 4°C for further processing.
- 9.7.6. Add 100X protease inhibitors cocktail and Tween 80 as follows. To each 250 mL bottle of CM supernatant add 100X protease inhibitor cocktail to 1X working concentration (e.g. add 1ml of cocktail to 100ml of conditioned media). Also add 2.5 mL 10% Tween 80 (final concentration will be 0.1%).
- 9.8. Concentrate the Sample
 - 9.8.1. Remove the reservoir cover and fill the reservoir with the sterile filtered conditioned media (up to 500 mL) to be concentrated.
 - 9.8.2. Ensure the vent port is open by removing the plug from the VENT port and leaving it open or installing a Millex Filter if required. Open the tank outlet valve.
 - 9.8.3. Turn the pump on and increase the pump speed until the feed pressure gauge reads 1.38 Bar (20 psi). Check all system connections for leaks and tighten any connections as necessary.
 - 9.8.4. Adjust the retentate valve restriction by slowly turning the retentate valve clockwise until the retentate pressure gauge reads 0.69 Bar (10 psi).
 - 9.8.5. Adjust the pump speed and retentate valve restriction to achieve desired feed retentate pressures [2.07 Bar (30 psi) feed / 0.69 Bar (10 psi) retentate]. Do not exceed 4.14 Bar (60 psi) feed pressure.
 - 9.8.6. Concentrate the solution until the desired volume is reduced 10-fold or greater, but ideally down to about 20 mL.
 - 9.8.7. Turn off the pump and empty the permeate container into a large bottle with a cap and label as: Permeate Waste; bleach then dispose.
- 9.9. Retrieve the Sample
 - 9.9.1. Disconnect the pump outlet tubing (Sta-Pure, white) from pump outlet port and place in product recovery collection vessel (50 mL conical tube).
 - 9.9.2. Disconnect the retentate tubing (silicone, translucent) from the retentate in port and open back pressure regulation valve (turn counterclockwise). Fluid should now drain by gravity.
 - 9.9.3. When drainage ceases, rinse the Pellicon innards by injection of 5 mL of 1X PBS with 0.1% Tween 80 from the retentate tube using a 10 mL syringe. If additional drainage is required; a syringe can be placed on the end of the retentate tube and fluid can be blown down.
 - 9.9.4. Replace retentate tubing (silicone, translucent) in retentate port. Reconnect pump outlet tubing (Sta-Pure, white).
 - 9.9.5. Disconnect FEED IN tubing and place in collection vessel. Open tank outlet valve, turn pump speed up to drain reservoir.
 - 9.9.6. Stop the pump. Reconnect the pump outlet tubing (Sta-Pure, white) to the Feed In port. Close the tank outlet valve.
 - 9.9.7. Add 10 ml 1X PBS/0.1% Tween 80 to the reservoir. Open the tank outlet valve.
 - 9.9.8. Connect the male luer end of the permeate tubing to the recirculation (DIA / RECIRC) port. Turn the pump on and increase the pump speed until the feed pressure

SOP: End-of-Run cNIST mAb Process: Harvest, Centrifugation, TFF Concentration

- gauge reads 1.38 Bar (20 psi). Check all system connections for leaks and tighten any connections as necessary.
- 9.9.9. Adjust the retentate valve restriction by slowly turning the retentate valve clockwise until the retentate pressure gauge reads 0.69 Bar (10 psi). Adjust the pump speed and retentate valve restriction to achieve 2.07 Bar (30 psi) feed pressure and 0.69 Bar (10 psi) retentate pressure.
 - 9.9.10. Recirculate the solution for 10 minutes and then turn the pump off
 - 9.9.11. Disconnect the male luer end of permeate tubing from the recirculation port.
 - 9.9.12. Disconnect the pump outlet tubing (Sta-Pure, white) from pump outlet port and place in product recovery collection vessel (50 mL conical tube).
 - 9.9.13. Disconnect the retentate tubing (silicone, translucent) from the retentate in port and open back pressure regulation valve (turn counterclockwise). Fluid should now drain by gravity.
 - 9.9.14. When drainage ceases, if additional drainage is required; a syringe can be placed on the end of the retentate tube and fluid can be blown down.
 - 9.9.15. Replace retentate tubing (silicone, translucent) in retentate port. Reconnect pump outlet tubing (Sta-Pure, white).
 - 9.9.16. Disconnect FEED IN tubing and place in collection vessel. Open tank outlet valve, turn pump speed up to drain reservoir. Stop the pump
 - 9.9.17. Reconnect the pump outlet tubing (Sta-Pure, white) to the Feed In port. Close the tank outlet valve.
 - 9.9.18. Stop the pump, close the outlet valve, and add 5 mL 1X PBS/Tween80 to the chamber. Pipet the solution along the walls repeatedly to rinse, then collect and transfer to the collection vessel.
 - 9.9.19. Label the recovery collection vessel Concentrated ant-IL8, [date], [initials] [Volume of mAb retrieved].
- 9.10. Flushing
- 9.10.1. To begin cleaning the Millipore TFF apparatus and Pellicon filter, repeat Flushing as described in 9.8.4
- 9.11. Cleaning the Labscale TFF/Pellicon cassette.
- 9.11.1. Disconnect the retentate tubing (silicone, translucent) from RET IN port and place in waste collection vessel. Place the end of the permeate tubing in the waste collection vessel.
 - 9.11.2. Open the retentate valve by turning it counterclockwise.
 - 9.11.3. Remove the reservoir cover and fill with 500 mL of 0.1N NaOH. Ensure the vent port is open by removing the plug from the VENT port and either leave open or install a Millex Filter.
 - 9.11.4. Open the tank outlet valve.
 - 9.11.5. Turn the pump on and increase the pump speed until the feed pressure gauge reads 1.38 Bar (20 psi). Check all system connections for leaks and tighten any connections as necessary.

SOP: End-of-Run cNIST mAb Process: Harvest, Centrifugation, TFF Concentration

- 9.11.6. Continue pumping to the waste collection vessel until the level in the reservoir drops to 250 mL and then turn the pump off. Reconnect the retentate (silicone, translucent) tubing to the RET IN port.
- 9.11.7. Recirculate the cleaning solution for 30-60 minutes and then turn the pump off.
- 9.12. Drain the System
 - 9.12.1. Disconnect the pump outlet (Sta-pure, white) tubing from the pump outlet port and place in waste collection vessel.
 - 9.12.2. Disconnect the retentate silicone (translucent) tubing from the RET IN port. Fluid should now drain by gravity. If additional drainage is required, a syringe can be placed on the end of the retentate tube and fluid can be blown down.
- 9.13. Flushing
 - 9.13.1. Repeat Flushing as described in 9.4.4.
- 9.14. Pellicon XL Device Storage
 - 9.14.1. Turn all of the lock nuts until you are able to remove the Pellicon XL Device.
 - 9.14.2. Fill a 10 mL syringe with 0.05N NaOH Storage solution.
 - 9.14.3. Place the cassette in sink or tray that can contain any overflow. Attach the syringe to the retentate port and slowly push the solution into the device. Remove the syringe and replace all of the plugs on the ports and store flat at 4°C-25°C.
- 9.15. Clean Base
 - 9.15.1. Disconnect the power cord.
 - 9.15.2. Clean exterior surfaces, reservoir, and Labscale System Base with a mild soap and water solution.

SOP: End-of-Run cNIST mAb Process: Harvest, Centrifugation, TFF Concentration

10. Attachments:

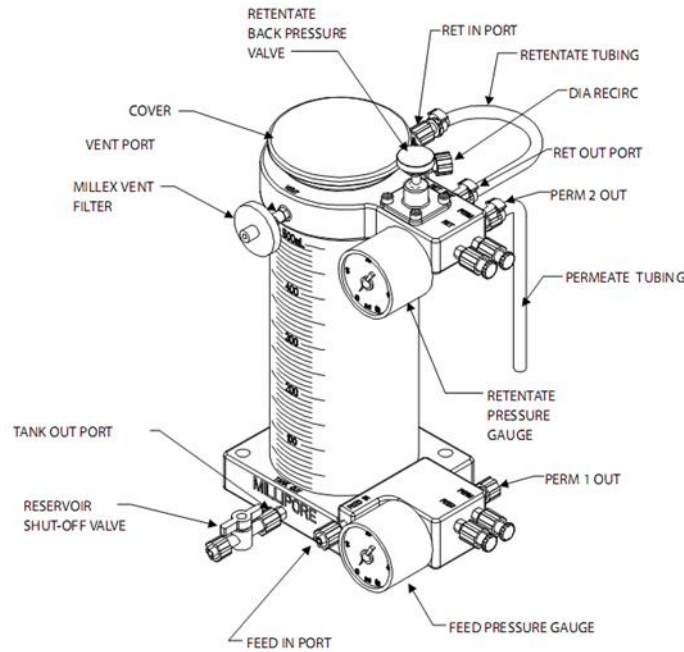


Figure 1: Reservoir Set Up

<http://www.millipore.com/userguides.nsf/docs/p60085>

SOP: End-of-Run cNIST mAb Process: Harvest, Centrifugation, TFF Concentration

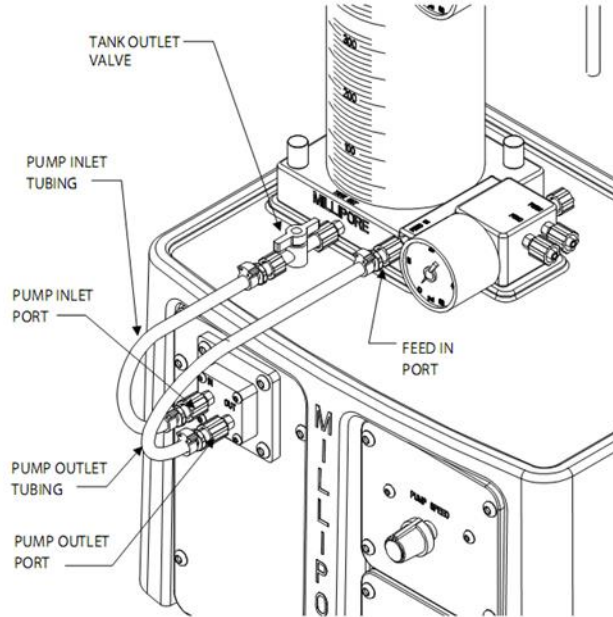


Figure 2: Pump Base Set Up
<http://www.millipore.com/userguides.nsf/docs/p60085>

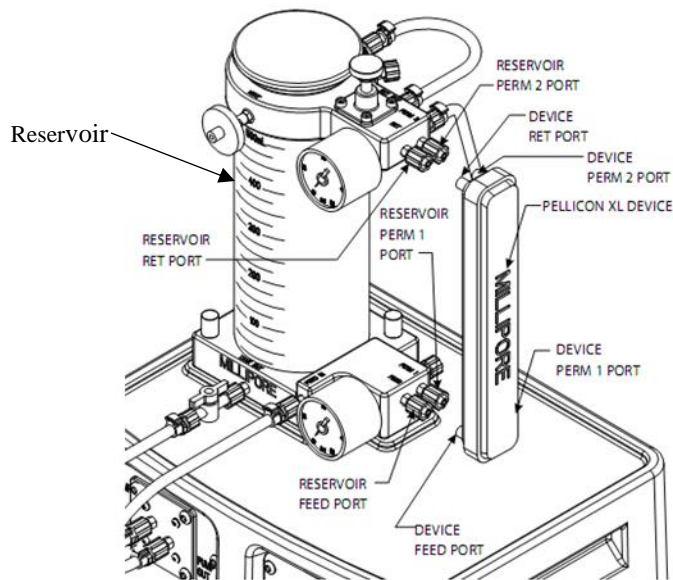


Figure 3: Installation of Pellicon XL Device
<http://www.millipore.com/userguides.nsf/docs/p60085>

**SOP: End-of-Run cNIST mAb Process: Harvest, Centrifugation, TFF
Concentration**

History

<i>Revision Number</i>	<i>Effective Date</i>	<i>Preparer</i>	<i>Description of Change</i>
0	17JAN24	Hetal Doshi	Initial release