SOP: End-of-Run Anti-IL8 mAb Process: Harvest, Centrifugation, TFF Concentration

Approvals:

Preparer: Hetal Doshi Date: 18DEC18
Reviewer: Dr. Maggie Bryans Date: 20DEC18

1. **Purpose:**
   1.1. To harvest anti-IL8 mAb containing conditioned medium, concentrate and prepare for chromatography; intermediate steps include centrifugation and sterile filteration 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 ingredient from a bioreactor or fermentor. This SOP provides bench scale procedures to accomplish that goal using conditioned medium from cells expressing recombinant anti-IL8 mAb. The method demonstrates the principles of tangential flow filtration, centrifugation, and sterile filteration 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. 0.1N NaOH for cleaning the Pellicon cassette following use
      3.1.3. 0.05N 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 filteration 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 Anti-IL8 mAb Process: Harvest, Centrifugation, TFF Concentration

6. Precautions:

6.1. 0.1N NaOH is very corrosive. It is extremely damaging to 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 10mL 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. 250mL Nalgene centrifuge bottles (3)
8.2. 250mL 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)- 50mL
8.7. NaH_2PO_4 (sodium phosphate monobasic, anhydrous)
8.8. Na_2HPO_4-$7H_2O$ (sodium phosphate dibasic, heptahydrate)
8.9. Stock solutions of protease inhibitors:
  8.9.1. 10mg/mL PMSF (phenylmethylsulfonylflouride); 250X
  8.9.2. Leupeptin, 2mg/mL; 4000x
  8.9.3. Aprotinin, 10mg/mL, 5000x
8.10. Millipore Tangential Flow Filtration System and Pellicon XL Device and Accessories
8.11. MilliQ Water
8.12. 10mL graduated cylinder
8.13. 0.22 micron Nalagene vaccum sterile filteration unit with storage bottle (3)

9. Procedure:

9.1. ***Preparation and Set Up***

9.2. Preparation of solutions (provided).
  9.2.1. Prepare 0.1N NaOH for cleaning.
    9.2.1.1. Using a 1L graduated cylinder, measure 1L of MilliQ water.
    9.2.1.2. Transfer water to a 1L flask.
    9.2.1.3. Weigh 4.0±0.05g of NaOH.
    9.2.1.4. Transfer NaOH to flask.
    9.2.1.5. Add magnetic stir bar and stir to dissolve.
    9.2.1.6. Sterile filter the solution and label container: 0.1N NaOH, [date], [initials], [group number], Storage: room temp, Disposal: adjust to pH 7 then drain.

SOP: End-of-Run Anti-IL8 mAb Process: Harvest, Centrifugation, TFF Concentration

9.2.2. Prepare 0.05N NaOH for Pellicon XL Device Storage
- 9.1.2.1 Using a 10mL graduated cylinder, measure 5mL of MilliQ water
- 9.1.2.2 Transfer MilliQ water to 25mL beaker
- 9.1.2.3 Using a 10mL graduated cylinder, measure 5mL of 0.1N NaOH
- 9.1.2.4 Transfer 5mL of 0.1N NaOH to 25mL beaker
- 9.1.2.5 Add magnetic stir bar and stir to dissolve.
- 9.1.2.6 Sterile filter the solution and label container: 0.05N NaOH, [date], [initials], [group number], Storage: room temp

9.2.3. 10% w/v Tween 80
- 9.2.3.1 Measure 80ml of MilliQ water and add to a 200ml beaker with magnetic stir bar
- 9.2.3.2 Place the beaker on a balance and tare the balance when stable
- 9.2.3.3 Pipette 10g Tween 80(polyoxyethylene soebitam monooleate) into the beaker with water.
- 9.2.3.4 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.

9.2.4. Diafiltration Buffer Preparation (20mM Phosphate Buffer pH 7.0 with 0.1% Tween 80)
- 9.2.4.1 Weigh out 0.80±0.02g NaH_2PO_4 and place into 1200ml beaker
- 9.2.4.2 Weigh out 3.60±0.2g Na_2HPO_4-$7H_2O$ and place into the 1200ml beaker containing NaH_2PO_4.
- 9.2.4.3 Using a 1L graduated cylinder, measure 980ml of MilliQ water.
- 9.2.4.4 Add the water to the 1200ml beaker containing the phosphates.
- 9.2.4.5 Add 10ml of 10% Tween 80 into the beaker with water and Phoshphates
- 9.2.4.6 Add a magnetic stir bar and stir to dissolve
- 9.2.4.7 Check the pH. If required adjust pH to 7.0±0.1. with 1N phosphoric acid. Bring to the Volume to 1L.
- 9.2.4.8 Sterile filter the solution and label container: 20mM Phosphate Buffer pH 7.1, [date], [initials], [group number], Storage: room temp, Disposal: drain

9.3. ***Labscale 500mL Reservoir Set Up***

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.

- 9.3.1.1. Cut silicone (translucent) tubing and install fittings as shown in figure 10.
- 9.3.1.2. Remove plugs from retenate outlet (RET OUT) and retenate inlet (RET IN) ports.
- 9.3.1.3. Insert the male luer end of the retenate tubing into the RET OUT port and the female luer end of the retenate tubing into the RET IN port. Turn fittings until snug.

9.3.2. **Install Permeate tubing**

SOP: End-of-Run Anti-IL8 mAb Process: Harvest, Centrifugation, TFF Concentration

9.3.2.1. Cut silicone (translucent) tubing and install fittings as shown in figure 12.
9.3.2.2. 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.

9.4. ***Install Tank Outlet Valve***

9.4.1. 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.
9.4.2. Install Vent Filter (If required)
9.4.3. 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.

9.5. ***Install StirBar***

9.5.1. If mixing is required, open reservoir cover and drop stir bar to the bottom of the reservoir.

9.6. ***Labscale Stir Base Set Up***

9.6.1. **Power Connection**
9.6.1.1. Turn Stirrer and pump speed controls to the off position.
9.6.1.2. Connect power cord to the power cord receptacle located at the rear of the system base.
9.6.1.3. Align detent on connector with receptacle.
9.6.1.4. Press connector into receptacle and turn lock ring to secure.
9.6.2. **Check Operation**
9.6.2.1. Remove the plugs from the pump inlet and pump outlet ports.
9.6.2.2. Turn on the pump speed control, set to 2, and listen for pump motor.
9.6.2.3. Turn off the pump speed control.
9.6.2.4. Turn on the stirrer speed control and listen for the stirrer motor.
9.6.2.5. Turn off the stirrer speed control.

9.7. ***Install Pellicon XL Device***

9.7.1. Remove the plugs from FEED, RET, PERM 1, and PERM 2 ports on the Pellicon XL Device.
9.7.2. Align the Pellicon XL device ports with Labscale 500 ml Reservoir ports being sure the PERM and RET DEVICE ports of the Pellicon XL Device and reservoir match. Press the device firmly onto the reservoir ports. Turn the lock nuts until snug.

9.8. ***Flushing of TFF/Pellicon.***

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.

9.8.1. 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).
9.8.2. Remove the 4 plugs on the Pellicon cassette and attach the Pellicon cassette to the Labscale apparatus.

SOP: End-of-Run Anti-IL8 mAb Process: Harvest, Centrifugation, TFF Concentration

9.8.3. Add 500mL MilliQ water to the reservoir and flush the cassette as described in section 9.4.4.

9.8.4. ***Flushing the Pellicon cassette.***

9.8.4.1. Disconnect retentate silicone (translucent) tubing from RET IN port and place end of retentate tubing in waste collection vessel.

9.8.4.2. Place end of permeate silicone (translucent) tubing into waste collection vessel. Open retentate valve by turning the counterclockwise.

9.8.4.3. Remove the reservoir cover and fill reservoir with 500mL of MilliQ water. Remove the plug from VENT port and open tank outlet valve.

9.8.4.4. Turn the pump on and increase the speed until the feed pressure gauge reads 1.38Bar (20psi).

9.8.4.5. Continue pumping to the waste collection vessel until the level in the reservoir drops to 350mL and then turn the pump off.

9.8.4.6. 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 1.38Bar (20psi). Check the system for leaks and tighten connections if leaks are found.

9.8.4.7. Adjust retentate valve restriction by slowly turning retentate valve clockwise until the retentate pressure gauge reads 0.69Bar (10psi).

9.8.4.8. Adjust pump speed and retentate valve restriction to achieve 2.07Bar (30psi) feed pressure and 0.69Bare (10psi) retentate pressure.

9.8.4.9. Allow to run until 50mL remains in the chamber.

9.8.4.10. Disconnect the pump outlet (Sta-pure, white) tubing from the pump outlet port and place in waste collection vessel.

9.8.4.11. 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.

9.8.4.12. 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).

9.8.4.13. Disconnect FEED IN tubing and place in collection vessel. Open tank outlet valve, turn pump speed up to drain reservoir.

9.8.4.14. Reconnect the pump outlet tubing (Sta-Pure, white) to the Feed In port.

9.9. ***Pre-conditioning***

9.9.1. Place end of permeate tubing silicone (translucent) in the waste collection vessel.

9.9.2. Remove reservoir cover and fill the reservoir with 50mL of PBS containing 0.1% Tween 80 (or other appropriate buffer) and then remove the Vent port plug.

9.9.3. Open the tank outlet valve. Turn the pump on and increase the pump speed until the feed pressure gauge reads 1.38Bar (20psi at its maximum; the needle will pulse as the pump turns). Check all system connections for leaks and tighten any connections as necessary.

SOP: End-of-Run Anti-IL8 mAb Process: Harvest, Centrifugation, TFF Concentration

9.9.4. 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.

9.10. ***Transfer of culture from bioreactor to centrifuge bottles.***

9.10.1. 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.

9.10.2. Transfer the culture to three 250mL centrifuge bottles using a 50mL pipet and PipetAid. Residual culture can be transferred to an Ehrlenmeyer flask for temporary storage.

9.10.3. Centrifuge cells in pre-chilled Sorvall centrifuge, fitted with a SLA1500 rotor, at 500 x *g* for 5 min, 4 degrees C.

9.10.4. Transfer conditioned medium (CM) from centrifuge bottle to storage vessel/bottle by carefully decanting the supernatant to appropriately labeled 250mL Corning bottles.

9.10.5. Add protease inhibitors and Tween 80 as follows. To each 250mL bottle of CM supernatant, add 1mL 10mg/mL PMSF, 50 µl of 10mg/mL Aprotinin stock and 62.5µl 2mg/mL Leupeptin stock. Also add 2.5mL 10% Tween 80 (final concentration will be 0.1%).

9.10.6. 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.

9.11. ***Concentrate the Sample***

9.11.1. Remove the reservoir cover and fill the reservoir with the sterile filtered condition medium (up to 500mL) to be concentrated.

9.11.2. 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.

9.11.3. Turn the pump on and increase the pump speed until the feed pressure gauge reads 1.38Bar (20psi). Check all system connections for leaks and tighten any connections as necessary.

9.11.4. Adjust the retentate valve restriction by slowly turning the retentate valve clockwise until the retentate pressure gauge reads 0.69Bar (10psi).

9.11.5. Adjust the pump speed and retentate valve restriction to achieve desired feed retentate pressures [2.07Bar (30psi feed / 0.69Bar (10psi) retentate]. Do not exceed 4.14Bar (60psi) feed pressure.

9.11.6. Filter the solution until the desired volume is reduced 10 fold or greater, but ideally down to about 20mL.

9.11.7. Turn off the pump and empty the permeate container into a large bottle with a cap and label as:Permeate Waste; bleach then dispose of.

9.12. ***Retrieve the Sample***

SOP: End-of-Run Anti-IL8 mAb Process: Harvest, Centrifugation, TFF Concentration

9.12.1. Disconnect the pump outlet tubing (Sta-Pure, white) from pump outlet port and place in product recovery collection vessel (beaker with small stir bar is preferred; or 50mL tube).
9.12.2. 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.
9.12.3. When drainage ceases, rinse the Pellicon innards by injection of 5mL PBS/0.1% Tween 80 from the retentate tube using a 10mL syringe. Additional drainage is required; a syringe can be placed on the end of the retentate tube and fluid can be blown down.
9.12.4. Replace retentate tubing (silicone, translucent) in retentate port. Reconnect pump outlet tubing (Sta-Pure, white).
9.12.5. Disconnect FEED IN tubing and place in collection vessel. Open tank outlet valve, turn pump speed up to drain reservoir.
9.12.6. Stop the pump, close the outlet valve, and add 5mL PBS/Tween80 to the chamber to rinse sides and effect collection of residual tPA. Pipet the solution along the walls repeatedly to rinse, then collect and transfer to the collection vessel.
9.12.7. Reconnect the pump outlet tubing (Sta-Pure, white) to the Feed In port.
9.12.8. Label the recovery collection vessel Concentrated tPA, [date], [initials].

9.13. ***Perform a Buffer Exchange on the Sample***

9.13.1. Add the 20mM Phosphate Buffer to the sample to bring the volume back to the pre-concentrated volume.(i.e. BTV 500ml)
9.13.2. Repeat step 9.7 and 9.8 for sample concentrate and sample retrieving.

9.14. ***Flushing***

9.14.1. To begin cleaning the Millipore TFF apparatus and Pellicon filter, repeat Flushing as described in 9.8.4

9.15. ***Cleaning the Labscale TFF/Pellicon cassette.***

9.15.1. 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.
9.15.2. Open the retentate valve by turning it counterclockwise.
9.15.3. Remove the reservoir cover and fill with 500mL 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.
9.15.4. Open the tank outlet valve.
9.15.5. Turn the pump on and increase the pump speed until the feed pressure gauge reads 1.38Bar (20psi). Check all system connections for leaks and tighten any connections as necessary.
9.15.6. Continue pumping to the waste collection vessel until the level in the reservoir drops to 250mL and then turn the pump off. Reconnect the retentate (silicone, translucent) tubing to the RET IN port.
9.15.7. 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

SOP: End-of-Run Anti-IL8 mAb Process: Harvest, Centrifugation, TFF Concentration

pressure gauge reads 1.38Bar (20psi). Check all system connections for leaks and tighten any connections as necessary.

9.15.8. Adjust the retentate valve restriction by slowly turning the retentate valve clockwise until the retentate pressure gauge reads 0.69Bar (10psi). Adjust the pump speed and retentate valve restriction to achieve 2.07Bar (30psi) feed pressure and 0.69Bar (10psi) retentate pressure.

9.15.9. Recirculate the cleaning solution for 30-60 minutes and then turn the pump off.

9.16. ***Drain the System***

9.16.1. Disconnect the pump outlet (Sta-pure, white) tubing from the pump outlet port and place in waste collection vessel.

9.16.2. 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.

9.17. ***Flushing***

9.17.1. Repeat Flushing as described in 9.4.4.

9.18. ***Pellicon XL Device Storage***

9.18.1. Turn all of the lock nuts until you are able to remove the Pellicon XL Device.

9.18.2. Fill a 10mL syringe with 0.05N NaOH Storage solution.

9.18.3. 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.

9.19. ***Clean Base***

9.19.1. Disconnect the power cord.

9.19.2. Clean exterior surfaces, reservoir, and Labscale System Base with a mild soap and water solution.

10. Attachments:

SOP: End-of-Run Anti-IL8 mAb Process: Harvest, Centrifugation, TFF Concentration

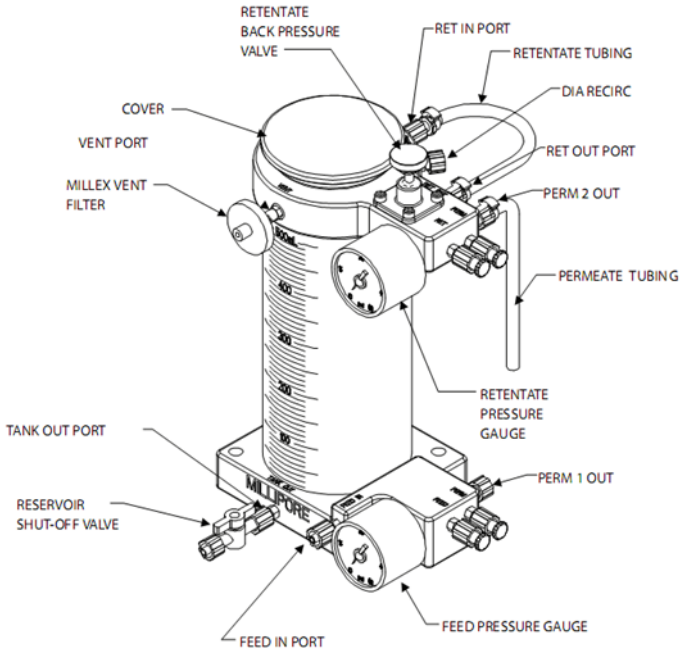
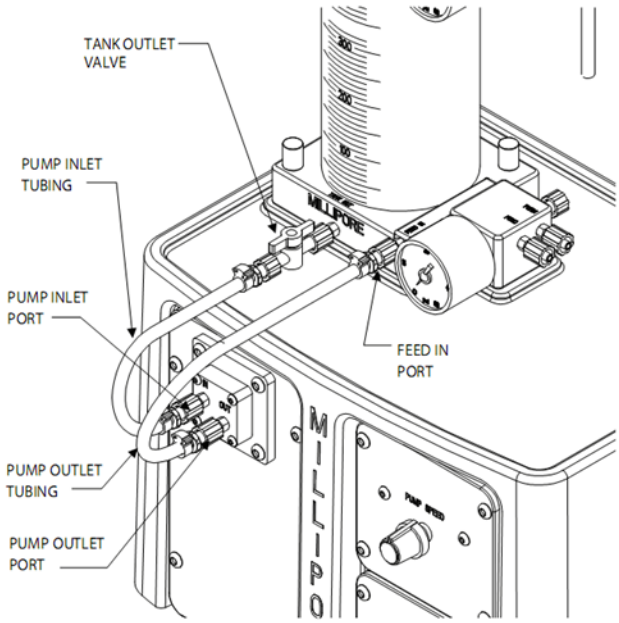


Figure 1: Reservoir Set Up
(http://www.millipore.com/userguides.nsf/docs/p60085)



SOP: End-of-Run Anti-IL8 mAb Process: Harvest, Centrifugation, TFF Concentration

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

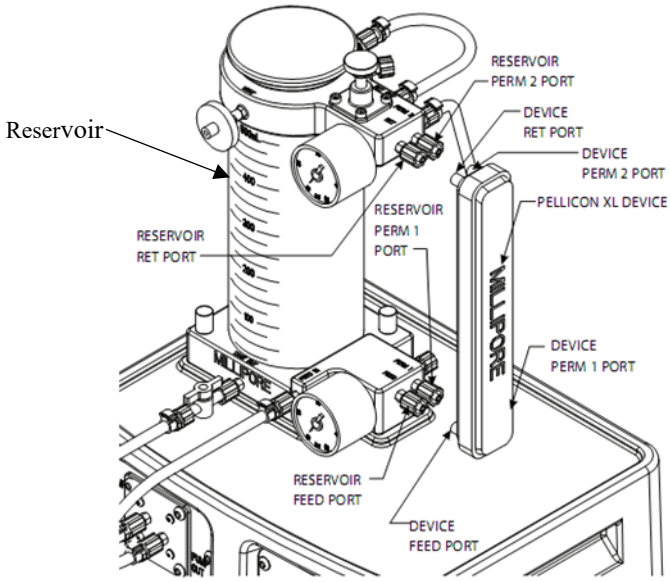


Figure 3: Installation of Pellicon XL Device
(http://www.millipore.com/userguides.nsf/docs/p60085)

History

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	16JUN17	Hetal Doshi	Initial release
1	20DEC18	Hetal Doshi	Added Buffer exchange step.