

SOP: Millipore Tangential Flow and Diafiltration Using Pellicon XL Device of tPA

Approvals:

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Date: 07JAN14
Date: 08JAN14
Date: 26MAR15

1. Purpose:

1.1. To concentrate and perform buffer exchange of protein products using tangential flow and diafiltration processes.

2. Scope and Applicability:

2.1. Applies to performing Tangential Flow Filtration with the Millipore Pellicon XL Device to concentrate and perform buffer exchange.

3. Summary of Method:

- 3.1. Preparation of solutions
- 3.2. Install resonant and permeate tubing and tank outlet valve on the Labscale 500ml Reservoir and add the stir bar
- 3.3. Connect the Labscale 500 ml Stir Base to power and check operation
- 3.4. Install the Pellicon XL Device
- 3.5. Flush and precondition the Labscale Tangential Flow Filtration System
- 3.6. Concentrate the sample, perform a buffer exchange on the sample, and then retrieve the sample.
- 3.7. Flush, clean, and drain the system.
- 3.8. Flush and prepare the Pellicon XL Device for storage.
- 3.9. Clean the Labscale Tangential Flow Filtration System.

4. References:

4.1. pH meter SOP

5. Definitions:

- 5.1. Permeate- the material that passes through the membrane.
- 5.2. Retentate- the material that does not pass through the membrane.

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.

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. 0.1N NaOH (sodium hydroxide)
- 8.2. 0.05N NaOH (sodium hydroxide)

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- 8.3. NaH_2PO_4 (sodium phosphate monobasic, anhydrous)
- 8.4. $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ (sodium phosphate dibasic, heptahydrate)
- 8.5. preconditioning buffer
- 8.6. pH Meter and pH paper
- 8.7. 2 L filter unit
- 8.8. 2 magnetic stir plate and stir bars
- 8.9. Millipore Tangential Flow Filtration System and Pellicon XL Device and Accessories
- 8.10. 3 containers, 500mL
- 8.11. 50 mL graduated cylinder
- 8.12. MilliQ Water
- 8.13. 2L graduated cylinder
- 8.14. 2L Flask
- 8.15. 10ml graduated cylinder
- 8.16. 25 ml beaker

9. Procedure:

9.1. Preparation and Set Up

- 9.1.1. Prepare 0.1N NaOH for cleaning.
 - 9.1.1.1. Using a 1L graduated cylinder, measure 1L of MilliQ water.
 - 9.1.1.2. Transfer water to a 1L flask.
 - 9.1.1.3. Weigh $4.0 \pm 0.05\text{g}$ of NaOH.
 - 9.1.1.4. Transfer NaOH to flask.
 - 9.1.1.5. Add magnetic stir bar and stir to dissolve.
 - 9.1.1.6. Sterile filter the solution and label container: 0.1N NaOH, [date], [initials], [group number], Storage: room temp, Disposal: adjust to pH 7 then drain.
- 9.1.2. Prepare 0.05N NaOH for Pellicon XL Device Storage
 - 9.1.2.1 Using a 10 ml graduated cylinder, measure 5 ml of MilliQ water
 - 9.1.2.2 Transfer MilliQ water to 25 ml beaker
 - 9.1.2.3 Using a 10 ml graduated cylinder, measure 5 ml of 0.1N NaOH
 - 9.1.2.4 Transfer 5 ml of 0.1N NaOH to 25 ml 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
- 9.1.3 Diafiltration Buffer Preparation (20mM Phosphate Buffer pH 7.1)
 - 9.1.3.1 Weigh out $0.80 \pm 0.02\text{g}$ NaH_2PO_4 and place into 1L flask
 - 9.1.3.2 Weigh out $3.60 \pm 0.2\text{g}$ $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ and place into the 1L flask containing NaH_2PO_4 .
 - 9.1.3.3 Using a 1L graduated cylinder, measure 1L of MilliQ water.
 - 9.1.3.4 Add the water to the 1L flask containing the phosphates.
 - 9.1.3.5 Add a magnetic stir bar and stir to dissolve.
 - 9.1.3.6 Adjust pH to 7.1 ± 0.1 .
 - 9.1.3.7 Sterile filter the solution and label container: 20mM Phosphate Buffer pH 7.1, [date], [initials], [group number], Storage: room temp, Disposal: drain

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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 retenate outlet (RET OUT) and retenate inlet (RET IN) ports.

9.2.1.3. 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.

9.2.2. 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.

9.3. Install Tank Outlet Valve

9.3.1. 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.

9.3.2. Install Vent Filter (If required)

9.3.3. 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.

9.4. Install Stir Bar

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

9.5. Labscale Stir Base Set Up

9.5.1. Power Connection

9.5.1.1. Turn Stirrer and pump speed controls to the off position.

9.5.1.2. Connect power cord to the power cord receptacle located at the rear of the system base.

9.5.1.3. Align detent on connector with receptacle.

9.5.1.4. Press connector into receptacle and turn lock ring to secure.

9.5.2. Check Operation

9.5.2.1. Remove the plugs from the pump inlet and pump outlet ports.

9.5.2.2. Turn on the pump speed control, set to 2, and listen for pump motor.

9.5.2.3. Turn off the pump speed control.

9.5.2.4. Turn on the stirrer speed control and listen for the stirrer motor.

9.5.2.5. Turn off the stirrer speed control.

9.6. Install Pellicon XL Device

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- 9.6.1. Remove the plugs from FEED, RET, PERM 1, and PERM 2 ports on the Pellicon XL Device.
- 9.6.2. 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.

9.7. Flushing

- 9.7.1. Disconnect retentate silicone (translucent) tubing from RET IN port and place end of retentate tubing in waste collection vessel.
- 9.7.2. Place end of permeate silicone (translucent) tubing into waste collection vessel. Open retentate valve by turning the counterclockwise.
- 9.7.3. Remove the reservoir cover and fill reservoir with 500 ml of MilliQ water. Remove the plug from VENT port and open tank outlet valve.
- 9.7.4. Turn the pump on and increase the speed until the feed pressure gauge reads 1.38 Bar (20 psi).
- 9.7.5. Continue pumping to the waste collection vessel until the level in the reservoir drops to 350 ml and then turn the pump off.
- 9.7.6. 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.
- 9.7.7. Adjust retentate valve restriction by slowly turning retentate valve clockwise until the retentate pressure gauge reads 0.69 Bar (10 psi).
- 9.7.8. Adjust pump speed and retentate valve restriction to achieve 2.07 Bar (30 psi) feed pressure and 0.69 Bar (10 psi) retentate pressure.

9.8. Pre-conditioning

- 9.8.1. Place end of permeate tubing silicone (translucent) in the waste collection vessel.
- 9.8.2. Remove reservoir cover and fill the reservoir with 50 ml of an appropriate buffer and then remove the Vent port plug.
- 9.8.3. 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). Check all system connections for leaks and tighten any connections as necessary.
- 9.8.4. Continue pumping to the waste connection 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.

9.9. Drain Permeate Tubing

- 9.9.1. Disconnect the permeate tubing (silicone, translucent) from the permeate outlet (Perm 2) port. Drain the permeate tubing into the waste collection vessel.
- 9.9.2. Reconnect the permeate tubing to the PERM 2 port.

9.10. Concentrate the Sample

- 9.10.1. Remove the reservoir cover and fill the reservoir with tPA sample to be concentrated.

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- 9.10.2. 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.
- 9.10.3. 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.
- 9.10.4. Adjust the retenate valve restriction by slowly turning the retenate valve clockwise until the retenate pressure gauge reads 0.69 Bar (10 psi).
- 9.10.5. Adjust the pump speed and retenate valve restriction to achieve desired feed retenate pressures (2.07 Bar (30 psi feed / 0.69 Bar (10 psi) retenate). Do not exceed 4.14 Bar (60 psi) feed pressure.
- 9.10.6. Filter the solution until the desired volume is reduced 10 fold.
- 9.10.7. Turn off the pump and empty the permeate container into a large bottle with a cap and label ad: tPA Permeate Waste, disposal; bleach then drain, [initials], [date].
- 9.11. ***Perform a Buffer Exchange on the Sample***
 - 9.11.1. Add the 20mM Phosphate Buffer to the sample to bring the volume back to the pre-concentrated volume.
 - 9.11.2. Repeat Concentrate the Sample (Number will go here) until the pH of the concentrated retenate is 7.1 ± 0.1 as measured with a pH meter.
- 9.12. ***Retrieve the Sample***
 - 9.12.1. Disconnect the pump outlet tubing (Sta-Pure, white) from pump outlet port and place in product recovery collection vessel.
 - 9.12.2. Disconnect the retenate tubing (silicone, translucent) from the retenate in port. Fluid should now drain by gravity. If additional drainage is required, a syringe can be placed on the end of the retenate tube and fluid can be blown down.
 - 9.12.3. Replace retenate tubing (silicone, translucent) in retenate port. Reconnect pump outlet tubing (Sta-Pure, white).
 - 9.12.4. Disconnect FEED IN tubing and place in collection vessel. Open tank outlet valve, turn pump speed up to drain reservoir.
 - 9.12.5. Reconnect the pump outlet tubing (Sta-Pure, white) to the Feed In port.
 - 9.12.6. Label the recovery collection vessel Concentrated tPA, [date], [initials].
 - 9.12.7. Store in 2°C-8°C refrigerator for use in further purification steps.
- 9.13. ***Flushing***
 - 9.13.1. Repeat Flushing as described in 9.7
- 9.14. ***Cleaning***
 - 9.14.1. Disconnect the retenate 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.
 - 9.14.2. Open the retenate valve by turning it counterclockwise.
 - 9.14.3. 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.
 - 9.14.4. Open the tank outlet valve.

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- 9.14.5. 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.
- 9.14.6. 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 retenate (silicone, translucent) tubing to the RET IN port.
- 9.14.7. 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 pressure gauge reads 1.38 Bar (20 psi). Check all system connections for leaks and tighten any connections as necessary.
- 9.14.8. Adjust the retenate valve restriction by slowly turning the retenate valve clockwise until the retenate pressure gauge reads 0.69 Bar (10 psi). Adjust the pump speed and retenate valve restriction to achieve 2.07 Bar (30 psi) feed pressure and 0.69 Bar (10 psi) retenate pressure.
- 9.14.9. Recirculate the cleaning solution for 30-60 minutes and then turn the pump off.
- 9.15. ***Drain the System***
 - 9.15.1. Disconnect the pump outlet (Sta-pure, white) tubing from the pump outlet port and place in waste collection vessel.
 - 9.15.2. Disconnect the retenate 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 retenate tube and fluid can be blown down.
- 9.16. ***Flushing***
 - 9.16.1. Repeat Flushing as described in 9.7
- 9.17. ***Pellicon XL Device Storage***
 - 9.17.1. Turn all of the lock nuts until you are able to remove the Pellicon XL Device.
 - 9.17.2. Fill a syringe with 0.05N NaOH Storage solution.
 - 9.17.3. Attach the syringe to the retenate 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.
- 9.18. ***Clean Base***
 - 9.18.1. Disconnect the power cord.
 - 9.18.2. Clean exterior surfaces, reservoir, and Labscale System Base with a mild soap and water solution.

10. Attachments:

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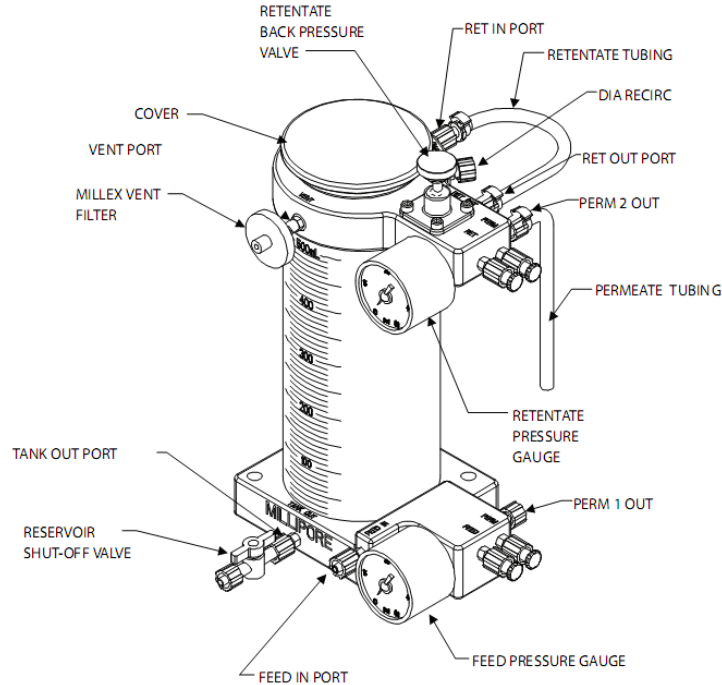
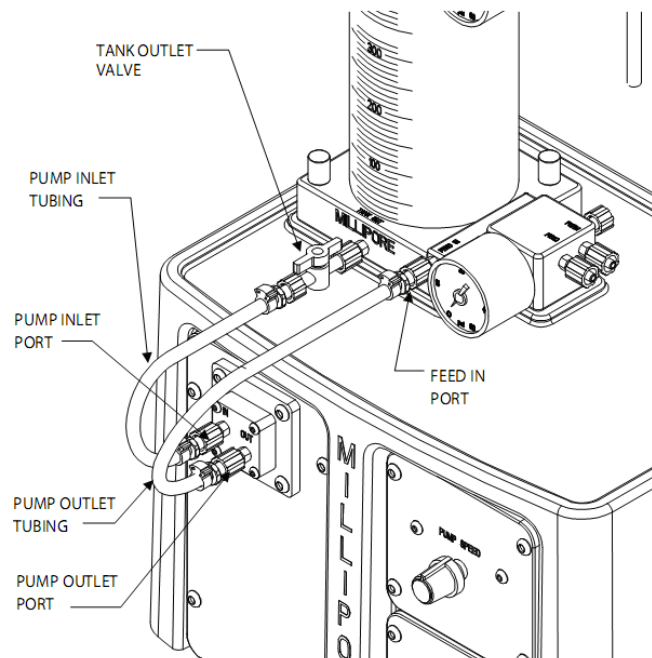


Figure 1: Reservoir Set Up

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



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Figure 2: Pump Base Set Up

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

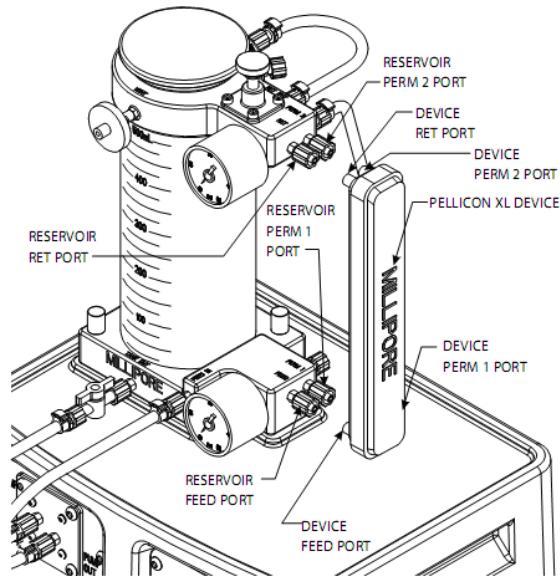


Figure 3: Installation of Pellicon XL Device

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

11. History

<i>Revision Number</i>	<i>Effective Date</i>	<i>Preparer</i>	<i>Description of Change</i>
0	07JAN14	Jason McMillan	Initial release