

Title: Millipore Pellicon XL Tangential Flow Filter SOP

Approvals:

Preparer: _____ Deb Audino _____ Date _____ 03Apr08 _____
Reviewer: _____ Bob O'Brien _____ Date _____ 03Apr08 _____

1. Purpose:

1.1. To perform tangential flow filtration.

2. Scope:

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

3. Responsibilities:

3.1. 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.

3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

4. References:

4.1. Millipore Pellicon XL Operations Manual

4.2. 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. 0.1M 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 Tangential Apparatus is stored and flushed with 0.1M 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. Materials:

7.1. 0.1M NaOH (sodium hydroxide)

7.2. preconditioning buffer

7.3. pH Meter and pH paper

7.4. 1L filter unit

7.5. magnetic stir plate and stir bars

7.6. Millipore Pellicon XL Tangential Apparatus

7.7. peristaltic pump

7.8. two pieces of ~40 cm long tubing

7.9. 1 Masterflex ~60 cm long thick wall tubing. (Masterflex 96400).

7.10. 3 fittings and 1 clamp

7.11. 3 containers, 500mL

7.12. 50 mL graduated cylinder

7.13. 3 cable ties

7.14. biopure water

8. Procedure:

8.1. **Preparation and Set Up**

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- 8.1.1. Prepare 0.1M NaOH for cleaning (if needed).
 - 8.1.1.1. Using a 1L graduated cylinder, measure 1L of deionized water.
 - 8.1.1.2. Transfer water to a 1L flask.
 - 8.1.1.3. Weigh 4.0 ± 0.05 g of NaOH.
 - 8.1.1.4. Transfer NaOH to flask.
 - 8.1.1.5. Add magnetic stir bar and stir to dissolve.
 - 8.1.1.6. Sterile filter the solution and label container: 0.1M NaOH, [date], [initials], [group number], storage: room temp, disposal: adjust to pH 7 then drain.
 - 8.1.2. Collect two pieces of ~ 40cm long tubing, and one piece of ~60cm long thick wall tubing (Masterflex 96400-16).
 - 8.1.3. If necessary, attach fittings to one end of each tubing and cable tie to secure.
 - 8.1.4. Obtain the Millipore Pellicon XL Tangential Filter from the 2-8°C refrigerator.
Note: the best filter composition for protein purification is regenerated cellulose, check for the filter composition on the label.
 - 8.1.5. Take off the caps to the Feed, Retentate and the Permeate 2 port.
 - 8.1.6. Attach the tubing to the Pellicon filter, making sure that the long tubing is placed on the Feed port.
 - 8.1.7. Loosely attach a thumbscrew clamp to the retentate tubing.
 - 8.1.8. Open the rotary assembly latch in the front of the peristaltic pump, place the feed tubing around the top of the rotor assembly and close latch. Secure the tubing in place with the two black clips.
 - 8.1.9. Place the end of the Feed and Permeate tubing into separate 500mL containers and the Retentate tubing into a 50mL graduated cylinder.
- 8.2. Flushing**
- 8.2.1. Fill Feed container with approximately 500mL of biopure water.
 - 8.2.2. Make sure that the speed dial on the peristaltic pump is set at zero and that the CW/CCW switch is in the central "OFF" position.
 - 8.2.3. Place CW/CCW switch to appropriate setting.
 - 8.2.4. Adjust speed dial until the rotor assembly begins to move starting the pump.
 - 8.2.5. Set the retentate flow rate to 30-50 mL/min.
 - 8.2.5.1. To measure the flow rate, collect water for 1 minute. Measure the volume collected in the graduated cylinder to obtain flow rate. Adjust pump speed if necessary and measure again. Once the correct flow rate is obtained, note the speed setting for future reference.
Note: DO NOT CHANGE the speed setting for the remainder of this SOP.
 - 8.2.6. Flush about 150mL of the biopure water through the retentate tube until the pH of the fluid flowing directly from the retentate tubing is near neutral as determined with pH paper.
 - 8.2.7. Tighten the thumb screw clamp on the retentate tube (not completely).
 - 8.2.8. Continue flushing until about 300mL of biopure water are collected in the permeate container and the fluid flowing directly from the permeate tubing is near neutral as determined with pH paper.
 - 8.2.9. Turn off pump and empty feed, permeate and retentate containers into the sink.

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8.3. Preconditioning

- 8.3.1. Place the end of the retentate tube into the feed container with the feed tube to recirculate the material.
- 8.3.2. Place the permeate tube into the 50mL graduated cylinder.
- 8.3.3. Fill feed container with 100-200mL of the appropriate buffer (see process SOP).
- 8.3.4. Turn on the pump and pump until approximately 30-50mL of buffer are collected through the permeate tube.
- 8.3.5. Turn off pump and empty feed and permeate containers into the sink.

8.4. Cleaning and Storing

- 8.4.1. Loosen the clamp on the retentate tube.
- 8.4.2. Perform the flushing procedure (step 8.2.6-8.2.9) with biopure water.
- 8.4.3. Clean with 0.1M NaOH.
 - 8.4.3.1. Place the retentate tube into the same container as the permeate tube. This will be the waste container.
 - 8.4.3.2. Loosen the thumbscrew clamp on the retentate tube.
 - 8.4.3.3. Fill the feed container with 500mL of 0.1M NaOH.
 - 8.4.3.4. Turn on the pump.
 - 8.4.3.5. Collect 250mL of solution in the waste container (containing the retentate and permeate tubes).
 - 8.4.3.6. Turn off the pump.
 - 8.4.3.7. Place retentate tube in the feed container.
 - 8.4.3.8. Turn on the pump.
 - 8.4.3.9. Tighten the thumbscrew on the retentate tube to increase the permeate flow rate.
 - 8.4.3.10. Recirculate the cleaning solution for 30-60 minutes.
 - 8.4.3.11. Turn off the pump off.
 - 8.4.3.12. Neutralize the NaOH in the waste container with HCl and flush down the drain.
 - 8.4.3.13. Carefully remove the tubes, place caps on the attachment fittings and store the device flat at 2-8°C.
 - 8.4.3.14. Rinse the tubing with deionized water before storing.

9. Attachments:

- 9.1. Figure 1: Pellicon XL with stand
- 9.2. Figure 2: Set Up

10. History:

Name	Date	Amendment
Sonia Wallman	1997	Initial release
SCP	2003	
Deb Audino	070505	Put into 2005 SOP format.
Deb Audino	12May06	Added attachments.
Deb Audio	04Apr08	College name change

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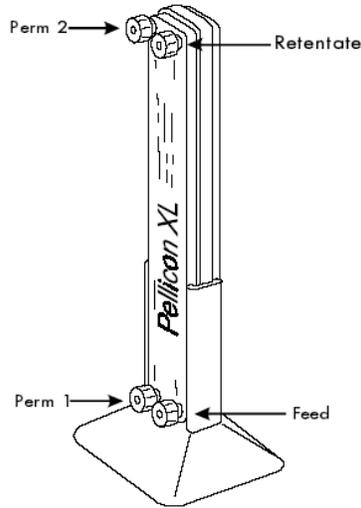


Figure 1: Millipore Pellicon XL Device
(<http://www.millipore.com/userguides.nsf/docs/p60085>)

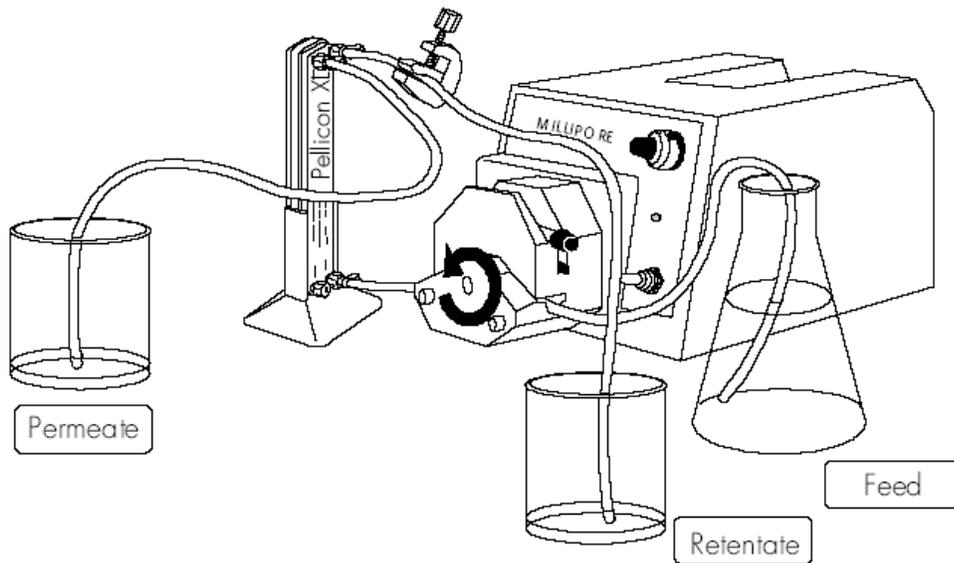


Figure 2: Set Up
(<http://www.millipore.com/userguides.nsf/docs/p60085>)