Instructions for the New Concentrator

This is a new system from Pall Life Sciences. This is the Centramate PE (polyethylene) lab scale tangential flow system. The filter holder is:

Prodcut number 29301-354, 3-gauge system, $2,775
The filters are the centramate cassettes with Omega Membrane, suspended screen option. The 10K cutoff order number is 29301-440, $414.00. We are currently working with three membranes.

Unlike the previous systems this one apparently doesn't need the same filtration stringency. Therefore it is no longer necessary to use an 0.2 um filter before loading into the system. This may even increase your yields and save on the headaches of filtering. The representative said a 1-10 um filtration is all that is necessary. We don't have these kinds of filters so I'm still using 0.45 but that seems fine thus far. For the most rapid filtration use an 0.8 um filter with a couple of prefilters and then an 0.45 um without a prefilter. You need to be more stringent when your baculovirus supes are cloudy and contain precipitate. If they're VERY clear an 0.8 um filter is fine.

I do not recommend concentrating more than 4 liters of baculovirus supernatant at one time, or more than 10 liters of CHO cell or similar type growth media. The solutions become too soupy towards the end and loss is inevitable.

IMPORTANT: Do not let the drainage tubing (left side of the 3-way valve) hang below the system. There is a slow leak in the valve and the chamber contents will drain all over the place. Make sure the tube is wrapped to the side of the chamber and that the clamp is attached tightly. You'll want to do this during the concentration and exchange procedures too.

To use this system follow this procedure:

NOTE: PLEASE DO NOT USE PHOSPHATE BUFFERS IN THIS SYSTEM. WE'VE TRIED THIS AND IT'S A LITTLE SCARY HOW CLOUDY THE PERMEATE, WHICH MADE IT THROUGH A 10 KD FILTER, BECAME. SUPERNATANTS CONTAIN ALL KINDS OF MATERIALS, MANY OF WHICH CAN PROBABLY FORM INSOLUBLE PHOSPHATES. PLEASE EXCHANGE INTO TRIS, HEPES, OR SOMETHING THAT CANNOT FORM AN INSOLUBLE SALT COMPLEX. THE ADDED COST OF AN ORGANIC BUFFER PALES IN COMPARISON TO REPLACING ALL THREE CARTRIDGES AT A COST OF NEARLY $1,300.

Also, in the cleaning procedures you can use KOH instead of NaOH if you want. KOH dissolves a lot faster.

1) Let your samples warm to roughly room temperature. Running cold supernatants will change the shape of the cartridges and may introduce leaks. It can be cool to the touch but certainly not 4 degrees. During this phase it may be a good idea to clean the system with NaOH (see the end of these instructions) to get a better flow rate. You can wash once with 400 mM NaOH, and a couple liters of water. To get the pH to an acceptable level it is ok to pour in a little stock buffer.

2) The filtration cassettes are held in place and under pressure by 4 large bolts on the top of the cell holder. You MUST check the torque on these bolts before each use of the system as they slowly loosen between uses. Lately I have often found the bolts overtightened. If you find the bolts are already at the pressure setting of the torque wrench, loosen them slightly and then retighten. Overtightening will shorten membrane life and can introduce leaks.. The torque wrench needs to remain nearby and anyone who removes or loses it will be subjected to whatever form of medieval torture Pamela deems appropriate. The wrench is set at 55 in/lb. Make sure that setting is still there and DON'T change it. Torque down each bolt in numerical succession (1,2,3,4). The bolts have been labeled for ease. After you do all four, go back and do them all again. Repeat until none of them move. If you don't know how to use a torque wrench ask someone who does. It's easy but you could wreck the system if you do it wrong.

3) Make sure the machine has been cleaned well before you begin. One of the better tests is to take the lid off the top of the reservoir and sniff the chamber. There will most likely be a slight odor, but it should not be strong or overpowering. If it is you will need to do a more thorough cleaning before beginning. You may also punish the person who used it before you.

4) Before running your sample you must test the permeate flow rate. With the machine in water start the pump running at speed setting=5. Manipulate the back-pressure valve until the pressure on gauge number 2 (again labeled for you) reads 10 PSI. Transfer the permeate line (the one with green tape on it that says 'permeate') into a 500 ml grad cylinder and wait one minute. Record the rate you find in the notebook that should be near the machine. Also record your name, date, volume of what kind of supernatant you're concentrating, your observations about the odor, and anything else you deem appropriate.

5) Prepare 3 liters of your buffer ahead of time. The machine goes fast and filters out permeate faster than a vacuum filter can process your buffer. Add your supernatants and begin concentrating. The pressure on valve #1 should NOT exceed 20 PSI. The other pressure gauges are less interesting and you can read about them in the manual. I highly recommend using higher flow rates and a lower setting on the backpressure valve to get the most efficiency. A viscous film forms on the surface of the membranes during concentration and higher flow rates keeps it thinner and speeds filtration efficiency. However, check back often if you're running near 20 PSI as the pressure can change a lot during the concentration procedure and you don't want to blow the tubing.

6) When your supernatants have reached a sufficiently small volume (I recommend about 400mL transfer the diafiltration line into your buffer and seal the reservoir lid to the chamber by turning the white knob clockwise until the black gasket seals. You can use a syringe or a vacuum line to pull a slight vacuum on the chamber using the 3-way stopcock. The stopcock has been labeled green on one side and red on the other. Never turn it to red or you'll suck buffer out of your buffer tank and into the vacuum lines. One a partial vacuum has been established, restart the pump and open the stopcock to the green position. Buffer should now flow from your tank into the reservoir diluting your protein at the same rate fluid is filtered out. To effect a 99% dilution of the sample you need to run 4.6x the volume present in the chamber (roughly 2.5 liters) through the machine. This will not take long. Be sure to monitor the vessel containing the permeate as it will overflow quite quickly and create a huge mess. Also, the seals may not be perfect so you should monitor the volume in the chamber. It could be decreasing.

6) A note about chamber volumes: Unlike the old system, air will hurt your sample if it gets into the filter. Because of the speed of this system a vortex will form when there is about 200-300 ml of volume in the reservoir chamber. This will allow air to enter the system and the air can start creating shear flow in the filters damaging proteins. Try to avoid this by keeping the volumes higher. If 400 ml is too much sample you can let it concentrate again after the dilution phase at a slower pump rate to get a smaller volume.

7) After you have diluted your sample to your satisfaction, concentrate it to whatever volume you want (it's hard to get below 200ml without adding a lot of air to the system. This phase may require you to work slowly, but it will still be fairly fast compared to the old system.

8) Want your sample back? I recommend first transferring the recirculation tube and the drain tube into a new container. Then drain the chamber using the 3-way valve at the base of the chamber. Then add 500 ml of buffer to the chamber, allow a little to drain out the drain valve to wash that (you don't need much). Then go ahead and set the valve towards the pump and run the remaining volume through the concentrator collecting the recirculated material this time. You can alternate between slow and fast settings on the pump during this phase to help strip off any films on the membranes that might contain your protein.

9) Once you have your material out, refill the chamber with 1 liter of your permeate (if it's still clear and not precipitating). Turn the backpressure valve off so there is no back pressure and run the pump at a high flow rate for a few minutes. Drain the sample and repeat with fresh permeate. This step strips the viscous coating that builds up on the membrane surface.

10) After you drain the chamber again, refill it with 1 liter of 250 mM NaOH and recirculate for a while. You'll need to add some backpressure so the cleaning solution gets all the way through the membrane. Going to 10 PSI should be enough. Try to use a higher flow rate. Drain the chamber and add 1 liter of 400 mM NaOH and recirculate. I recommend letting this wash go for a long time if possible (30 minutes to an hour). This seems to clean the membrane more effectively. Also, you are welcome to change the NaOH solution again.

11) After the NaOH washes, do two washes with 1 liter of water . The idea here is to get out all the protein and the amount of foam forming is a good indicator of this. You'll also need some water in the system to repeat the permeate flow test (see step#4). Note this rate in the log book. It should be somewhat close to what you started with. If it's a little slower that's fine, we've noticed some fluctuations but another round of cleaning will usually increase the flow rate a little more. **Write your observations in the notebook.**

12) When you are done fill the chamber with 500 mL of 250 mM NaOH and 0.5% NaN_3 for storage.

13) Dry off anything that may have gotten wet, put everything back so the next person can find the torque wrench, etc.