



Ultrafiltration Protocol



Theory and Introduction: Ultrafiltration - Ultrafiltration is a method to concentration protein or other macromolecules through a membrane with defined pores. The membranes will have a molecular weight cut-off (MWCO). This is the limit of size (or range) of a protein that can fit through the membrane. Each membrane also has a chemical characteristic for binding proteins and stability in various solvents. There are two main methods to force the protein containing fluid through the membrane, pressure from an inert gas or centrifugal force. Larger volumes are more suited to the pressure stirred cells. For smaller volumes the centrifugal devices are easier and more practical to use. In centrifugation, the hydrostatic pressure during centrifugation forces the fluid through the membrane. Proteins larger than the MWCO are retained and the smaller molecules, like the salts, buffers, water and small proteins will pass through. THINK about what is going where before dumping any of your samples. See the Millipore handout on the lab website or (www.millipore.com) for details on this type of method.



This method can also be used to exchange buffers. By repeated concentrations and dilutions into a new buffer, your protein can be exchanged into a new buffer system.

Location - The ultrafiltration tubes (centriprep filter devices) are to be stored in PBS at 4°C (in the refrigerator).

Protocol - (see pages 9 - 12 in the Millipore Centrifuge Handout).

1. Twist off the lock cap and remove the inner tube (filtrate collector). BE CAREFULL not to touch the white membrane or it will not work. Check the membrane for obvious defects or damage.
2. Add solution into the larger tube (sample container). The max volume is 15 ml. If the protein concentration is high, use 5 ml.
3. Twist the inner filtrate collector tube back onto the sample container.
4. Place the device (with a counterbalance) in the swinging bucket rotor. Inspect to see if the tube will rotate without damaging the top of the device.
5. Centrifuge the device for 10 min in at 2000 x g. This is about maximum RPM for the centrifuge in the biochem laboratory. When the volume inside the tube and the volume outside of the inner tube are at equilibrium, the sample must be decanted.
6. Decant the sample in the INNER tube (filtrate collector) through the air seal cap. Your protein is in the larger, sample container tube.
 - If the volume is not low enough, repeat step 1 through 5.
 - If you have more sample to concentrate, add it to the large tube and repeat step 1 through 5.
 - It is possible with three spins to concentrate the sample to 0.6 ml.
7. Rinse the tube several times with milliQ water and store the tube in PBS in the refrigerator.

