



## Dialysis Protocol



**Theory and Introduction: Dialysis** - If your sample has a small molecule (like a salt) that you wish to remove, or change the buffer, dialysis is a timely but easy and inexpensive method. Dialysis tubing is a semi-permeable membrane that can be purchased with a specified range of pore sizes. Keeping in mind that most proteins are larger than 10,000 Daltons and simple salts like NaCl (58.44 amu) or Tris-Cl (121.4 amu) are small, dialysis provides a mechanism to remove these impurities from your purification sample. Most dialysis tubing has a mwco (molecular weight cut-off) of 5,000 to 10,000 Daltons. Review dialysis in your textbook for a detailed background on this method.

**Location** - The tubing should be placed in the cabinet / shelves in the front (south) wall next to the chalkboard.

**Preparation** - Dialysis tubing comes dry in most cases. Unless otherwise stated, the tubing should be cleaned and prepared before use. See page 300 of *At the Bench* for instructions on preparation of tubing. The tubing we are using can simply be wetted by soaking in MilliQ water for 10 to 15 min.

### Protocol -

1. Cut enough tubing for the volume of solution you will use and then add 2 inches at each end for handling.
2. Use the orange dialysis clamps by folding one end of the tubing and pinching the tubing in the clamp.
3. Open the other end of the tubing by gently rubbing with your fingers. Pipet a small amount of buffer or water into the tubing and check for tears or leaks.
4. Rinse out the tubing with MilliQ water and a final rinse with the buffer of choice.
5. Fill the tubing with your sample using a plastic funnel or plastic transfer pipette.
6. Remove all but a small air bubble and clamp or tie off the open end. Leave a little slack in the tube if your sample has a high salt concentration.
7. Place the tubing in a large beaker or Erlenmeyer flask (at least 10 times the size of the solution you are dialyzing).
8. Fill the beaker with dialysis buffer, add a stir bar and place on a stir plate in the cold room.
  - The total volume of dialysis buffer should be about 10 -20 times the volume of sample is a good number, but you can use 5 times depending on the total volume.
  - If the tube bumps against the bottom of the beaker, you can tie the clip to a string and keep the string taped to the side of the beaker, OR add more solution. This is why I like to keep a small bubble in the tubing.
9. Change buffers at least once for small changes between the concentration of your sample and the new buffer. For a stringent dialysis, perform 2 to 4 buffer changes.
  - The first buffer change can take place 4 or so hours after starting.
  - Let the second buffer change dialyze overnight.