

Sample Preparation for Analytical Ultracentrifugation:

Protein should be as pure as possible. It is a good idea to run gel filtration to remove aggregates. Samples also should be at "osmotic" equilibrium with their respective buffers. This can be achieved by extensive dialysis, at **least 24 hours**. After extensive dialysis, filter sample and 50 ml dialysis buffer with a 0.22 μ M filter.

For initial runs, sample concentrations should be from 0.3 to 1.0 mg/ml for use with the Rayleigh optical system (refractive index). In our AUC setup, four concentrations can be run at once. A good starting point for protein concentrations is 1.0, 0.75, 0.5, and 0.25 mg/ml. To ease with loading samples into cells, it is best to have at least 500 μ l of sample at each concentration. Sample dilutions should be made by diluting concentrated the sample stock, which has been dialyzed extensively, with dialysis buffer.

Note: We are subtracting two very large numbers (the refractive index of the dialysate from the refractive index of the buffer that the sample is in) to get the refractive index contribution of the protein. So, an exhaustive dialysis is required against the last buffer change.

Components Diagram

Hover the diagram for more information.



1. Place window gasket at bottom of window holder
2. Position window liner inside the window holder so that the **opening of the window liner is opposite the keyway**
3. Place window inside the window holder, **aligning the arrow on window with the keyway of window holder. Arrow should point towards holder.**
4. Repeat steps 1-3 for second window
5. Slide window assembly (**with interference slits**) into the cell housing with window facing up
6. Insert centerpiece into cell housing with meniscus capillaries facing up. **Important: be careful not to use tool to push the centerpiece into housing with out first covering with lens paper.**
7. Insert second window housing (**with wider openings**) into cell window facing centerpiece. Hold the cell housing on its side and position the second window assembly so that keyway is aligned.
8. Place screw ring gasket on top of second window assembly.
9. Screw in screw ring, with up facing up. For storage wrap cell in lens paper. **Directly before use put small amount of Spinkote on threads and work back and forth into cell housing to spread evening**