BECKMAN XL-I AUC FAQ

How do I get started?

Before you make an appointment for training, do some background reading to familiarize yourself with the principles of the technique and determine whether it’s likely that it will work for your system. Finding a relevant paper in the literature or checking out one of the references on this website would be a good start.

How do I arrange for a training session for the instrument?

Contact Bradley Turner (bif@mit.edu) or 617-452-2051 to find out how long training will take and to make an appointment when you and he and the instrument are all free at the same time.

How do I make reservations for the instrument?

The reservation for the initial training session will be made by Brad. After you’re trained, you’ll make reservations online through the Exchange calendar if you’re an MIT person. People from outside MIT will need to ask Brad to make reservations for them.

Are there any restrictions on what materials I can put in the AUC?

The BIF is a BL1 facility so no prions or other BL2 materials are allowed. Some organic solvents are compatible with our centerpieces so talk to Brad about which ones.

How do I decide which kind of AUC run to do?

That depends on the question you want to answer. See the first page of the SOP to see which runs provide which information.

If I just want to know if I have monomer or dimer, which type of run should I do?

Try starting with a SV run using three different concentrations of your protein. We recommend $A_{280}=0.33$, 0.66 and 1.0, for instance. You want to stay within the linear range of the instrument (0.2 – 1.2 AU). This may give you your information with a single run. Or you may need to continue with a SE run.

What is special about SE runs?
You run your sample at three different speeds, waiting for it to come to equilibrium each time. This usually takes three days. You run three different concentrations of each sample, as above. To calculate which speeds are appropriate for your sample, use the monomer weight and read the speeds off a special chart Beckman provides.

**How clean does my sample have to be?**

If your protein is coming off a SEC, you can use it straight for an absorbance run and use the equilibration buffer as your blank. If you’re going to do an interference run, you still need to dialyze overnight to 24 hours, changing the buffer three times, and using the dialysis buffer as your blank. Dialysis is also required for samples not off SECs.

**How much sample do I need?**

To fill the SV sectors, you need 430 ul of sample and 440 ul of buffer as a blank. For the SE sectors, you need 110 ul of sample at each concentration and 120 ul of buffer as a blank for each one. Runs can be performed with lower volumes than these, but the larger the volume, the better the resolution of the data.

**How do I decide which optics to use, absorbance or interference?**

If you have proteins larger than 10 kD with normal extinction coefficients and you have concentrations of 0.2 – 1.2 AU at 280 nm, then absorbance will work fine. If you have small proteins with low extinction coefficients (3840-5120 M⁻¹·cm⁻¹) and low concentrations (0.1 – 0.3 mg/ml), then interference optics will give you cleaner results.

With small amounts of sample, you could work at 232 nm where peptides absorb but your buffer might absorb at that wavelength. You’d need to run a spectrum of your sample and see if the buffer signal swamps out the protein signal. The absorbance data may be noisy down at 232 nm. You want to keep the A₂₃₂ < 1.0.

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