Module 7: Protein Detection by Western Blot
Prelab handout

This handout must be completed BEFORE coming to lab. It will be collected at the start of lab. Be sure to have the necessary calculations (from 1 and 2) available in your ELN for reference.

1. Using the concentrations of your cell lysates calculated in the Module 6 assignment, calculate the volume of lysate needed for 20 µg of protein (calculate this for both lysates). The total volume of sample to be loaded on the gel should be 30 µL. You will prepare the samples with 4X sample buffer, lysate, and bring the final volume to 30 µL with water. Record these calculations in your ELN.

2. Using the amounts of lysate, 4X sample buffer, and water calculated in part 1, calculate the amounts needed for a “master mix” with enough sample to load the gel 3 times (see Figure 1 in Module 7 handout). A master mix is often used to reduce variability and save time on sample or reagent prep. A good rule of thumb is to make 10% or more volume than needed to account for sample lost on the side of the tube etc. Record these calculations in your ELN.

3. Name all of the wastes that need to be collected for DRS.
4. During what parts of the lab must you wear safety goggles? Why?

5. Given what you know about the two primary antibodies we are using (see product sheets in the ELN), draw what banding pattern you EXPECT to see on your two Western blots (include both sample lanes, see sample layout in the lab handout), including the protein standard and label all sizes of bands.