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5.36 Biochemistry Laboratory  
Spring 2009

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## SESSIONS 7 and 8 (lab open 1-5 pm)

*Note: Sessions 7 and 8 can be combined into one laboratory day if you would prefer one long session instead of two short sessions.*

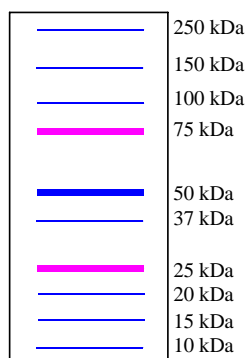
During Sessions 7 and 8 you will analyze the purified H396P Abl(229-511) by SDS-PAGE gel electrophoresis. You will also determine the concentration of your protein domain after purification and dialysis.

### 1.) Running an SDS-PAGE gel

Remove your cast gel from the storage container, remove the white strip of tape from the bottom of your gel, and place the cassette in the gel running apparatus. Two gels fit in a gel box, so two groups of students should use each box. Fill the apparatus with 1X electrophoresis (TANK) buffer such that the section between the two gels is filled to the top and the outer sections are filled halfway with buffer. Carefully remove the gel comb. Using gel-loading pipette tips on a 20p pipette, load 5 uL of protein ladder (provided by your TA) into a corner well of your gel. The protein ladder consists of proteins with known molecular weights conjugated to visible dyes and does not need to be mixed with the sample loading buffer. See the posting on the -20 freezer or the figure below to identify the molecular weight markers in the protein ladder. In the next two wells, load 10 uL of the pre and post-induction samples. In the final 7 wells, load your Ni-NTA elution samples. Run the gel at 200 V until the blue dye reaches the bottom of the gel (approximately 45 to 60 min).

Once the gel running is complete, disassemble the gel apparatus, and carefully pry the cassette apart with a spatula. Transfer the gel into a container for staining. Running a light stream of water over the gel can help prevent gel tearing during cassette separation and gel transfer. Add enough Coomassie blue staining solution to cover the gel (10 to 25 mL), and place the container on the gel rocker. Rock the gel in the stain for 30 minutes. Pour the used staining solution into a designated bottle, and rinse the gel with water. To destain, cover the gel with fast destaining solution and add a balled-up Kimwipe to the corner of the gel container. Rock the gel at room temperature for 30 to 50 min, then replace the fast destain with the slow destain solution and rock the gel overnight. For the overnight destain, add a fresh Kimwipe and cover the container with saranwrap to avoid excessive evaporation (especially during the winter months).

Bio-rad precision plus  
Molecular weight markers



The H396P Abl kinase domain should appear as a band at approximately 33 kDa. Depending on the protein concentration, some impurities may be visible in the more concentrated elution fractions. In the literature, these impurities have been reduced or eliminated by running a second column. However, we will use the singly purified protein for our assays, since we are interested in comparing the activity with vs. without inhibitor rather than determining absolute activity.

## **SESSION 7B** (lab open 1-2 pm)

After destaining, pour the destain solution in a designated waste bottle and check your gel for protein bands. You should rinse the gel with water and take a digital picture for your laboratory report. Once you have a decent picture of your gel, you may discard it.

## **SESSION 8**

### **1.) H396P Abl(229-511) Protein Concentration**

Using a syringe, transfer the dialyzed protein solution from the dialysis cassette into a 15-mL conical tube. The cassette cannot be reused and should be discarded after use.

Concentrate the protein solution to a final volume of approximately 500  $\mu$ L using a Millipore 10-KDa MWCO centrifugal concentrator. For concentration, add up to 12 mL of your dialyzed protein solution to the concentrator tube and replace the cap. Place the concentrator tube with the volume gradations facing up in the centrifuge. Add a counterbalance, either another group's sample or a 50-mL conical tube with the appropriate volume of water to balance your concentrator tube. Spin at 500 x g until you reach the desired volume, approximately 15 to 45 minutes. Use a pipette to recover your concentrated protein from the filter unit in the concentrator tube. For maximum protein recovery, withdraw the protein from the bottom of the filter unit and use a "side-to-side sweeping motion". You should remove the concentrated protein solution as soon as possible following centrifugation.

Store your protein in a clearly labeled eppendorf at 4 °C for use in Sessions 13 and 14.

### **2.) Protein Quantification**

Use the Bio-Rad quantification assay (Session 4) to determine the protein concentration of the H396P Abl kinase domain after purification, dialysis, and concentration. See Appendix A1 for a review on preparing your BSA samples from the 1 mM BSA stock you made in Session 4.

**Clearly label the dialyzed protein with your names, your TA's name, and the date. Store the solution at 4 °C.**