5.36 Biochemistry Laboratory
Spring 2009

SESSION 4

Laboratory Procedures (lab open 1-5 pm)

1.) Preparation of Ni-affinity column buffers, dialysis stock buffer solution, and SDS-PAGE buffers: solutions for isolation and analysis of the H396P Abl kinase domain.

The Ni-NTA buffers and SDS-PAGE buffers will be shared by the class. Your TA will therefore assign each group a total of three class buffers to prepare. Some of the buffer preparation protocols contain blanks, and for these you are must to calculate the required amount of each reagent (in grams) to achieve the specified concentration. CONFIRM THAT YOUR CALCULATIONS ARE CORRECT WITH YOUR TA BEFORE YOU START TO PREPARE YOUR BUFFERS.

Your TA will assign you to prepare one of the three Ni-NTA purification buffers described below:

a) Ni-NTA binding buffer (50 mM Tris, 300 mM NaCl, pH 7.8). In a 1 L bottle, combine _____ g of Tris base (FW 121.1), _____ g NaCl (FW 58.44), and 800 mL of cold water. Adjust the pH to 7.8 with HCl, and then adjust the final volume to 1L.
b) Ni-NTA washing buffer (50 mM Tris, 300 mM NaCl, 30 mM imidazole, pH 7.8). In a 500 mL or 1L bottle, combine _____ g Tris base (FW 121.1), _____ g NaCl (FW 58.44), _____ g imidazole (FW 68.08), and 400 mL of water. Adjust the pH to 7.8 with HCl, and then adjust the final volume to 500 mL.
c) Ni-NTA elution buffer (50 mM Tris, 300 mM NaCl, 200 mM imidazole, pH 7.8). In a 500 mL or 1L bottle, combine _____ g Tris base (FW 121.1), _____ g NaCl (FW 58.44), _____ g imidazole (FW 68.08), and 400 mL of water. Adjust the pH to 7.8 with HCl, and then adjust the final volume to 500 mL.

EACH group must prepare a 10X stock of TBS for dialysis as indicated:

d) 10X TBS (dialysis buffer) (200 mM Tris, 1.37 M NaCl, pH 7.5). In a 1 L bottle, combine _____ g Tris base, _____ g NaCl, and 800 mL of water. Adjust the pH to 7.5 with HCl, and then adjust the final volume to 1 L.

You will be assigned two of the SDS-PAGE gel buffers below to prepare for the class to share. Several of the buffers will be prepared by the TAs, as indicated:

e) 10X electrophoresis buffer (Tank buffer). In a 2 L bottle, combine 60.4 g Tris base, 288 g glycine, and 10 g SDS. Add water to a final volume of 2 L.
f) Coomasie staining solution **(provided by TA)**. 0.25 % Coomassie Brilliant Blue, 50 % methanol, and 10 % glacial acetic acid.

g) 6X reducing protein loading buffer. For 15 mL of solution, combine 3.5 mL water, 3.5 mL of 1 M Tris-HCl, pH 6.8, 3.6 mL glycerol (use a positive displacement pipette for this viscous liquid), 1.0 g SDS, 930 mg DTT, and 1.2 mg bromophenol blue. Store this solution at – 20 ºC.
h) Fast destain solution **(provided by TA)**. 40% methanol and 10% glacial acetic acid. This solution can destain gels in 1-3 hours.
i) Slow destain solution **(provided by TA)**. 5% methanol and 10% glacial acetic acid. This solution is for destaining overnight.
j) 1.0 M Tris-HCl, pH 8.8. In a 1 L bottle, add _____ g of Tris base and 800 mL of water. Adjust the pH of the solution to 8.8. Add water to a final volume of 1 L.
k) 0.38 M Tris-HCl, pH 6.8. In a 1 L bottle, add _____ g of Tris base and 800 mL of water. Adjust the pH with HCl to 6.8. Add water to a final volume of 1 L.
l) 50 % sucrose, 100 mL
m) 10 % ammonium persulfate (APS), 10 mL. store at 4 °C.
n) 40% acrylamide/ bisacrylamide solution **(provided by TA)**

2.) Preparation of BSA solutions and creation of a standard curve for the Bio-Rad protein quantification assay. (*This can be completed anytime before session 6. At a minimum, you should prepare the 1 mg/mL BSA stock solution today.*)

Prepare 5 mL of a 1 mg/mL aqueous solution of bovine serum albumin (BSA). To do this, weigh out 5 mg of BSA into a 15-mL conical tube on the balance. Add 5 mL of water and invert or vortex the tube to completely dissolve the BSA. Divide the stock solution into 1-mL aliquots in 1.5-ml eppedorf tubes and store at -20 °C for use in protein quantification assays throughout Modules 4 and 5. Prior to each assay, you must prepare five dilutions of your BSA stock solution to create a standard curve. The linear range of the Bio-Rad protein assay is 0.2 to 0.9 mg/mL. Therefore in six separate 0.65-mL eppendorf tubes, label and add the following: 270 µL of the BSA stock and 30 µL of deionized water (to give 0.9 mg/mL), 210 µL of BSA and 90 µL of water (to give 0.7 mg/mL), 165 µL of BSA and 135 µL of water (to give 0.55 mg/mL), 120 µL of BSA and 180 µL of water (to give 0.4 mg/mL), 60 µL of BSA and 240 µL of water (to give 0.2 mg/mL), and no BSA and 300 uL of water (to use as your "blank").

Prepare 20 mL of the assay dye reagent by diluting 1 part Bio-Rad dye concentrate with 4 parts DI water. Filter the resulting solution and store any unused reagent for up to two weeks at room temperature. To create a standard curve using your BSA standards, pipette 20 µL of each standard into an eppendorf tube. Add 980 µL of the prepared dye reagent to each tube and vortex briefly. Incubate the tubes at room temperature for 5 to 50 minutes. Measure the absorbance of each solution at 595 nm by UV/Vis spectrometry using disposable polystyrene cuvettes. See Appendix A2 for instructions on using the Varian Cary 100 spectrophotometer. The sample with 20 µL of water in 980 µL of assay reagent should be used as your "blank" in the spectrophotometer. Plot the absorbance vs. concentration for the BSA standards using

Microsoft Excel or another graphing program and determine the R-squared value and slope of the best-fit line to establish that you can obtain a strait line with your standards. Print a copy for your lab report. If your R-squared value is less than 0.9, repeat the exercise until you achieve an acceptable line. Good laboratory practice calls for the creation of a new standard curve each time you run a protein assay.

3.) Hand in the order form for your primers. See your TA about entering your primer information onto an Invitrogen order form.