Biochemistry Lab

Expression, Lysis and Purification Lab

This handout will serve to direct each student group on the overall workflow and expectations for the purification and characterization of their MDH and CS recombinant proteins. Details of buffers, volumes of wash and elution and other processes are found in the section on expression, purification and characterization. Students are expected to review the video presentation on expression and purification, read the handouts and use this information to plan their experiments.

Each group will express One of the wild-type MDH, wild-type CS and their selected mutant. This translates to each group of three or four students will be purifying three proteins at one time.

Use the information below plus the information in your handouts to prepare a lab notebook. Even though some of the bacterial culture, expression and lysis was performed for you, write up the laboratory protocol/procedure and results as if you conducted all of the experiment.

The overall goal is to have each group purify, dialyze, protein assay, and characterize using SDS PAGE and Westernblot. Each group will later be responsible to determine the specific activity of each wild-type and mutant MDH as well as the kinetic parameters (Km and Vmax). These samples will be further used to measure interaction using your pulldown assays.

Group 1 : MDH1, CS and mutant Group 2: MDH2 CS and mutant Group 3: wgMDH, CS and mutant Group 4: MDH1, MDH2 and mutant

Expression: Each group will be given 500 ml of cultured lysed cells. The culture started with plasmids for human MDH1v3, MDH2, wgMDH (watermelon MDH) or CS transformed into a BL21(DE) competent cell strain. Each strain was cultured on LB agar with Kan. A single colony was selected and used to start an overnight 5 ml culture. The culture was then expanded the next morning into 500 ml of LB with Kan. The 500 ml culture OD was monitored at 500 nm until reaching an OD of 0.6. At that time each culture was chilled to 20°C and induced with 1 mm IPTG for 24-28 hours. Cells were centrifuged at 3000 x g for 15 min and the pellet retained.

Lysis: Cell pellets from each 500 ml culture is resuspended in 25 ml of lysis His tag buffer (found on purification handout) with 0.0015 mg DNaseA, 0.25 mM $MgCl_2$, 0.01 mM $CaCl_2$ and 1 mM PMSF (from lysis handout). Lysozyme (0.5 mg/ml) was added and incubated for 30 min at 4°C. Final disruption of cell membranes was accomplished by three repeats of sonication for 30 seconds each. The resulting lysate was centrifuged at 8,000 x g for 20 min at 4°C. The resulting supernatant contained the soluble bacterial proteins to be used for purification of affinity his tagged protein.

Purification: We will be using 8 ml columns for your purifications. Each group will be responsible for planning and conducting the purification using the information on the handout/protocols/videos as well as the particular information provided here.

- Each student will perform one of the purifications.
- Be very careful in preparation AND labeling of your buffers. Don't mix up the elution buffer or you will be starting from scratch on your own – we only have one pellet prepared for each group!
- Each group should prepare 500 ml of each buffer from stock starting conditions. Record calculations and composition of each buffer in your eNotebook. Your instructor will help you use the pH Vernier modules to adjust the pH of the buffers.
- Follow the column purification vs batch binding for your column. No need to re-apply the flow through.
- *Each column has been washed with 20 ml of elution buffer. BEFORE USE, wash each column with 80 ml of His-Binding Buffer.*

- Save ~100 µl of lysate in a labeled microfuge tube and store in -20°C freezer for later analysis by SDS PAGE. Hint: an average drop of water is about 20 µl
- Collect the flow through as a single fraction in a labeled beaker. Save 100-200 µl of flow through.
- Adjust the volumes for His-Binding and then His-Wash Buffer from the protocol by dividing by a factor of three. The volumes in the protocol are for a 1000 ml culture / 25 ml column. Your column is 8 ml.
- Wash with enough His Wash Buffer until the non-specific binding protein is no longer eluting through the column. Use the Bradford quick method to know when it is time to switch buffers.
- Elution: Collect in 5 ml fractions. Fill a test tube with 5 ml of water and mark to have a level that indicates approx 5 ml. Use the Bradford microfuge tube method to know when the protein has started and finished eluting (video covered this!).
- Test the total protein in each elution tube: Using parafilm on the top of the tube, invert to mix, then mix 20 µl of fraction plus 500 µl 1X Bradford protein assay agent to measure the relative protein concentration.
- Capture an image of these fractions on a white background and save to your laboratory notebook. Indicate which tube represents which elution fraction.
- Determine which fractions to pool. Pool the fractions (combine those you wish to mix together) and conduct a quick Bradford protein assay. Use 20 µl of sample mixed with 500 µl 1X Bradford reagent. Do the same with 0, 0.5, and 1.0 mg/ml BSA standard your group made and froze when measuring protein concentration. If the protein is above 1 mg/ml protein, dilute using dialysis buffer until just below 1 mg/ml. This will avoid a crashing (aggregation) of your protein. Save a 100-200 µl fraction for SDS PAGE analysis.
- Work with your instructor to prepare your samples in dialysis tubing for overnight dialysis of MDH Storage Buffer (10 mM KPi, 0.1 mM EDTA, 0.1 mM beta mercaptoethanol) 500 ml x two changes. The following laboratory period, collect the sample measure the final volume, protein concentration and store in a well labeled 20 ml conical tube at 20oC (refrigerator NOT the freezer).