Biochemistry Lab

Protein Characterization
Experimental Plans

Goal: You have a performed an expression and purification of recombinant MDH and CS. Now you need to characterize the enzyme in each fraction and the overall impact of each expression condition. To do this, you will assay the protein concentration for each fraction, observe the quality of the purification procedure, and measure the enzyme activity in your fractions.

General Procedure:

- *Protein Assay*: Determine the protein concentration of each fraction. Use the BSA standards you prepared earlier this semester to create a new standard curve. Refer to your notebook and handouts to create the procedure/method for your laboratory notebook. Record both the total protein and the protein concentration (mg/ml).

- *SDS PAGE Analysis*: Refer to the posted SDS PAGE general protocol for how to perform the SDS PAGE analysis of your purification. Each student group will run one gel with 15 available lanes. Run two identical gels. One for staining for protein, the second to conduct a western blot. Plan which samples to load using the information below. Load your gel with the following order for each expression condition. Stain ONE gel with Coomasie Stain and de-stain follow the next instructions for westernblot.

  - Lane 1: 10 µl Molecular Weight Standards (record manufacturer and specific information for each)
  - Lane w: 10 µl Lysate
  - Lane x: 10 µl Flow Thru
  - Lane y: 15 µl Peak Fraction
  - Lane z: 15 µl Pooled Protein

  - Remaining lanes – 15 µl of lysate, flow thru, peak fraction and pool fractions for the other purified proteins.

- *Westernblot (immunoanalysis)*: Using the second identical gel, perform a westernblot analysis using the information on the posted protocol page.

- *Enzyme Activity*: Measure the enzyme activity in the pooled fraction of wild type wgMDH, MDH1, MDH2 and your mutant. You will measure the specific activity as well as the kinetic parameters, Km and Vmax for OAA + NADH. This will be conducted in the following weeks of lab.