Biochemistry Lab
Protein Assay Lab

Introduction: *This lab will introduce you to the basics of protein assay using a micro volume (plate reader) format. Students will work* ***independently*** *for the experimental and write-up portion of the lab. Each student will be given three samples and will determine the protein concentration of the proteins using the Bradford protein assay method.*

Lab Notebook: Be certain to enter into your notebook the purpose, procedure, results/calculations and conclusions sections. More details are provided in task 3.

Safety and Waste: The Bradford assay is slightly acidic and contains methanol and low concentration of phosphoric acid. Waste and unused Bradford reagent will be placed in the Bradford waste container in the hood. Label your unused BSA protein standard with concentration and your initials. Store the standards in the lab freezer for later use in the semester.

Protein Assay Assignment: You will not be required to submit a formal, complete laboratory write-up for this experiment. Instead each student will complete the protein assay assignment, linked at the bottom of the class web page. Use the data and information from your laboratory notebook to answer the questions.

Materials: 10 ml, 1X BioRad Bradford Assay Solution in falcon tube, 1 ml BSA (1.00 mg/ml), 200 µl unknowns (see below), 96 well plates (rinse with methanol and water for re-use) and Bradford Waste Container.

Task 1) Review the links on protein assays. Links will be found on the web page. This will introduce you to the basics of protein assays. *Should be completed prior to class*.

Task 2) Carefully read the Protein Assay Protocol. The protocol includes detailed background for the Bradford assay and specific instructions for conducting a protein assay using a 96 well plate reader format. *Should be completed prior to class*

Task 3) Conduct the protein assay. You will be given three unknown protein samples. Using the protein assay protocol, you will design a standard curve using BSA and determine the protein concentration of each sample. Record the unknowns in your lab book. All dilutions and steps should be included in the laboratory notebook.

- Unknowns: Unknown (A-E) is a pure protein sample of BSA. There are two other unknowns. Each is the soluble portion (lysate) of bacterial cells cultured and induced to express MDH and a non-induced control bacterial lysate. You will determine the protein concentration of all three unknowns. You will likely have to dilute one or more of the unknowns to keep the absorbance of the sample within the standard curve.
- Notebook: Record all calculations for dilutions of standards and unknown samples, generate a standard curve using a program of your choice and calculate and clearly identify the final concentration of each unknown in your laboratory notebook.