



Introduction: This lab will introduce you to the basics of performing a protein assay using a micro volume (plate reader) format. Students will work **independently** for both the experimental and homework portion of the lab. Each student will be given two unknown samples and will determine the protein concentrations 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 2.

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. Clearly label your unused BSA protein standards with concentration and your initials. Store the standards in your lab team freezer box for later use in the semester.

Protein Assay Assignment: You will not be required to submit a formal laboratory write-up for this experiment. Instead each student will complete the protein assay assignment posted on Blackboard. Use the data and information from your laboratory notebook to answer the questions.

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

Task 1: Carefully read the Protein Assay Protocol provided. 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 2: Conduct the protein assay. You will be given unknown protein samples. Using the protein assay protocol provided, you will design a standard curve using BSA and determine the protein concentration of each unknown sample. Record the unknowns in your lab book! All dilutions and steps should be included in the laboratory notebook.

- **Unknowns:** Unknowns A - F are pure protein samples of BSA, you will receive two of these. You will determine the protein concentration of all your unknowns. You will likely have to adjust 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.