Lab # 12 The Microplate Bradford Assay

This experiment is designed to introduce a microplate format to the student and to build on previous experience making a serial dilution and determining protein concentration using the Bradford Assay. Read "Determining Protein Concentration" on pages 304-306 out of At The Bench.

Purpose:
To improve the students' ability to measure protein concentration accurately.

Below is a map of a 96-well plate. Prepare the Bradford Assay as outlined below. All values have the unit of mg/mL. Copy both tables in your lab book.

	0	0.25	0.5	0.75	1						
	0	0.25	0.5	0.75	1						
	1:1	1:10	1:100								
	1:1	1:10	1:100								
	1:1	1:10	1:100								

Absorbances

C. 96 Well Plate Assay Protocol
(5 µL of a 0.1-1.4 mg/ml protein sample is used) This assay is performed in a 96 well plate. With this assay it is possible to quickly assay multiple protein samples, while using a small sample volume (5 µL). It is also possible to automate your protein determination with this multiwell plate assay.

1. Prepare protein standards in water of 0.25 mg/mL, 0.5 mg/mL, 0.75 mg/mL, and 1 mg/mL using a supplied 1.4 mg/mL BSA standard.
2. Add 5 µL of the protein standards (0.25 mg/mL, 0.5 mg/mL, 0.75 mg/mL, 1 mg/mL) to indicated wells in the 96 well plate. To the blank wells, add 5 µL of water.
3. Prepare three dilutions of the unknown sample and add 5 µL of each dilution to the corresponding well. Dilutions should be at least 20 µL in volume and be undiluted, 1:10, and 1:100 dilutions in water.
4. To each well being used, add 250 µL of the Bradford Reagent to each well.
5. Let the samples incubate at room temperature in the dark for at least 15 minutes. Then measure the absorbance at 620 nm. The protein-dye complex is stable up to 60 minutes. The absorbance of the samples must be recorded before the 60 minute time limit and within 10 minutes of each other.
6. After you samples have incubated, bring your 96-well plate to the instructor to be read by the platereader.
7. Record absorbances in your lab book.
8. Calculate the average absorbance for each protein concentration of the standard (first two rows)
9. By hand, plot the average absorbance for each standard vs. the protein concentration of that standard.

10. Compare the average protein absorbance for each protein dilution and determine which dilution produced an absorbance reading closest to the middle of the absorbances of the standard curve.
11. Determine the average protein concentration of the chosen dilution of the unknown sample by comparing the average A_{620} values against the standard curve. Don't forget to correct for dilution.

Data:
Tape a copy of the gel picture in your lab book and label all lanes and the values of the protein ladder.

Conclusion:
1. What unknown sample were you given and what is the protein concentration for that sample?
2. Which dilution of the protein is the most accurate? Why?
3. What does BSA stand for and what animal does it come from?
4. Name five ways to determine protein concentration and indicate which one you think is the best.