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Biology 105 Laboratory Fall 2013 *15samples* Instructor: Ayça Akal-Strader

Revised Bradford Assay Procedure for Lab 2 (For 2 mL Cuvettes):

1. Obtain 5 microfuge tubes. These will be used to prepare the dilutions of BSA from 1 mg/ml BSA.

2. Obtain a stock solution of BSA (1 mg/ml): = 1 mg/mL BSA = 1000 mg/mL BSA

3. Using the Table 1 below, make dilutions of varying concentrations from the original BSA stock solution. Label each tube with the concentration it contains.

Table 1: Preparing BSA Concentrations from Stock

	Amount of BSA Stock (1 mg/ml)	Amount of Water	Final [BSA] µg/ml
1	50 µl	950 µl	50
2	100 µl	900 µl	100
3	200 µl	800 µl	200
4	300 µl	700 µl	300
5	400 µl	600 µl	400

4. Obtain 15 glass tubes.

5. You will now prepare each BSA concentration in **duplicate** to analyze in the spectrophotometer and then average the results. You will need <u>two glass tubes for each sample and each unknown sample, and one glass tube for a blank.</u> Label each tube with a marker. You should have a total of 15 labeled glass tubes. (10 samples) (1 sample) (15 samples total!)

6. Add 200 µl of the appropriate BSA concentration from the table above to the bottom of each tube.

7. Add 200 µl of the unknown BSA to the appropriate tubes.

8. Prepare a blank by adding 200 µl of water to the tube labeled "Blank".

At this point you should have 15 samples in microfuge tubes that you will analyze using the spectrophotometer after adding the Bradford Reagent as described below.

9. Gently mix the Bradford Reagent. Add 1800 µl of the reagent to each of the 15 glass tubes prepared in steps 4 – 8. One partner should add the reagent while a second partner seals the tube with parafilm and vortexes (or mixes by gently inverting up and down five times) the protein/reagent mixture.

10. Wait 5 minutes for full color development. The protein/dye complex will be stable for about an hour.

11. Transfer 2 ml of your water sample into the plastic cuvette and avoid introducing bubbles into the sample. Use a Kimwipe to remove any fingerprints or liquid from the outside of the cuvette before placing the tube in the instrument to avoid distorted readings. Have a TA show you how to blank the spectrophotometer using the water sample.

12. Wash out the cuvette using a P1000 to rinse it with water into your waste beaker & tap dry on a kimwipe (Alternatively, you can pipette out all the liquid and avoid draining). Add your first protein

sample to the cuvette and measure the absorbance at 595 nm. Use a Kimwipe to remove any fingerprints or liquid from the outside of the cuvette before placing the tube in the instrument to avoid distorted readings. Record your findings in the table provided below (Table 2). Wash & dry the cuvette after each reading (or pipette out all the liquid to avoid draining) and repeat for all of your protein samples, recording the absorbance values in the table below.

13. After your analyses are completed on the spectrophotometer **please rinse & save the cuvettes**. The protein + Bradford reagent can get washed down the sink, while pipette tips can go in the regular garbage. Glass tubes need to be discarded into the "glass only" disposal receptacle near the entrance.

14. Average the absorbance readings for each of the duplicate protein sample tubes. Fill in the appropriate column in Table 2.

Table 2: Results

	Concentration – (µg/ml)	Absorbance at 595 nm #1	# 2	Average A_{595}
1	50 µg/ml	.092	.096	
2	100 µg/ml	.172	.169	
3	200 µg/ml	.314	.297	
4	300 µg/ml	.455	.451	
5	400 µg/ml	.531	.503	
	Unknown BSA 1	.704	.678	
	Unknown BSA 2	.397	.425	

15. Using graph paper (on p.32 of your lab manual), plot the average A_{595} *versus* the concentration of the BSA in the standard sample tubes. The A_{595} should be on the Y-axis, and the BSA concentration on the X-axis. Plot the data for each of your samples on the graph. Draw a straight line that passes through (0,0) and approximates a best fit curve for your data points. This is the standard curve that can be used to determine the protein concentrations of your unknowns.

16. On the Y-axis of your graph, find the position for the average A_{595} for each unknown sample. Draw a line from the Y-axis at that position to meet the line of your graph. Now draw a line from that point down to the X-axis to determine the concentration of protein in your unknown samples. Record your results in Table 3.

Table 3: Protein Concentrations of Unknowns

Sample	Average A_{595}	Protein Concentration
BSA Unknown 1		
BSA Unknown 2		

µg/mL protein