

Names: _____

Datum Sheet for Laboratory 4 Determining Protein Concentrations by Spectrophotometry

A. Preparation of a Food Source Extract (1 point each, 4 points)

1. What was the food source that you used?

2. What was the designated serving size?

3. What was the labeled protein content in grams/serving?

4. How many ml of extract did you obtain after homogenizing one or part of one serving? If you used less than an entire serving note that also.

B. Setting up a Protein Standard Curve

1. What is the purpose of making the BSA standard curve? (1 point)

2. Enter your raw data for the absorbance measurements of the samples from the bovine serum albumin (BSA) standard curve (*6 points*)

Tube	Volume BSA (μ l)	A(595 nm)	Average A(595 nm)	μ g of protein
1	0			0
2	5			
3	5			
4	10			
5	10			
6	15			
7	15			
8	20			
9	20			
10	30			
11	30			
12	40			
13	40			
14	50			
15	50			
16	60			
17	60			

3. **Attach to this datum sheet a graph of the BSA standard curve.** Draw the best fit straight line that is possible through most of the datum points. The line should go through the origin (0,0) since all of the samples were read against a “blank” containing 0 protein that was set to 0.000 A. You can make the graph either on a piece of graph paper or with a software package such as Excel (*2 points*)
4. Define a conversion factor relating absorbance to the amount of protein. This is the same as the slope of the line from your graph. (*1 point*)

$$1 \mu\text{g} = \text{_____} A_{595}$$

C. Protein Concentration of the Food Source Extract

1. Enter the raw data for the unknown protein sample that you analyzed (*3 points*)

Tube	Water (μl)	Sample	Sample Volume (μl)	A (595 nm)
1	100	None	0	
2	95	Undiluted	10	
3	95	Undiluted	30	
4	90	Undiluted	70	
5	90	1/10 Dilution	10	
6	85	1/10 Dilution	30	
7	85	1/10 Dilution	70	
8	80	1/100 Dilution	10	
9	80	1/100 Dilution	30	
10	70	1/100 Dilution	70	
11	70	1/1000 Dilution	10	
12	60	1/1000 Dilution	30	
13	60	1/1000 Dilution	70	

2. Show your complete calculations for the protein concentration (mg/ml) of this unknown sample (*4 points*)

D. Final Calculations

1. Show your calculation of the protein content of your sample per serving (*2 points*)
2. Compare your value for the protein content with that shown in the nutritional label? How can you explain any differences between these values? (*2 points*)

E. Additional Questions

1. A scientist wanted to determine the protein concentration of an unknown sample. He added varying amounts of a 1 mg/ ml solution of bovine serum albumin and water to a series of tubes as shown in the following table. He also added 100 μ l portions of several dilutions of an unknown sample to other tubes. 2.0 ml of the Bradford Reagent were added to each tube and the color was allowed to develop. The absorbance at 595 nm was then determined. The results are shown in the following table.

Tube	BSA (μ l)	Sample	Water (μ l)	Bradford Reagent (ml)	A (595 nm)
1	0	-	100	2.0	0.000
2	1	-	99	2.0	0.076
3	5	-	95	2.0	0.378
4	10	-	90	2.0	1.810
5	20	-	80	2.0	1.610
6	25	-	75	2.0	1.920
7	-	100 μ l (undiluted)	0	2.0	3.600
8	-	100 μ l (1/5 dilution)	0	2.0	1.600
9	-	100 μ l (1/10 dilution)	0	2.0	0.750

- a. Calculate the amount of BSA in each of the first six tubes. *(1 point)*

Tube	μg protein (BSA)
1	
2	
3	
4	
5	
6	

- b. Make a BSA standard curve and determine the slope or conversion factor. **Attach your graph to this datum sheet.** *(1 point)*
- c. Determine the amount of protein in the unknown sample. Show your complete calculations. *(1 point)*

2. A scientist wished to measure the amount of the metabolite creatinine in a sample of human serum. She combined varying amounts of a stock solution of creatinine at a concentration of 1 mg/ml with water or serum as shown in the following table. She then added 3.0 ml of picrate to each tube and measured the absorbance of the solution at 500 nm. The results are also shown in the table.

	<i>Reagent/Tube</i>					
	1	2	3	4	5	6
Creatinine (1 mg/mL)	0	0.5	1.0	2.0	3.0	—
Water	3	2.5	2.0	1.0	0	2.0
Serum	—	—	—	—	—	1.0
Picrate	3	3	3	3	3	3
A_{500}	0	0.1	0.2	0.4	0.6	0.5

- a. Using the data in tubes #1 to #5, construct a creatinine standard curve. Calculate the amount of creatinine in mg in each tube and plot A_{500} as a function of the amount of creatinine. Determine the slope of the best fit straight line. Attach your graph to this datum sheet. **(1 point)**
- b. Using this standard curve, determine the creatinine concentration in mg/ml of the serum sample. Show your complete calculations. **(1 point)**