

The Crucial Concentration

Investigating Unknown Quantities of Protein Using the Lowry Assay

www.mdbiolab.org • info@mdbiolab.org

Adapted from *The Crucial Concentration*, written by Donald A. DeRosa and B. Leslie Wolfe

It is often very useful for scientists to measure the concentration of something in liquids. For example, they may measure the amount of lead levels in drinking water, a patient's cholesterol, or the amount of dissolved oxygen in the Chesapeake Bay.

The concentration of a component in a solution is expressed as mass over volume. The nutrition facts label on food and drinks contains information about nutrients in the product.

Several companies are competing to produce a new product, *The Power Drink*. It is a high protein drink for athletes for improved physical performance. Three companies advertise that they produce a drink with the highest concentration of protein.

Sharon works for an independent testing agency and has been hired to settle the dispute among the companies. She will test the concentration of protein in each drink.

You and your partner can help Sharon find out which of the three companies can actually say they make the drink with the highest amount of protein. Your job is to determine the concentration of protein in the three drinks. The results from each team will be compared and you will present your conclusion to the companies. You will have to defend your conclusion with data, especially to the companies that lost.

Protein is colorless – you cannot look at each sample to see the amount of protein. Sharon has decided to use the Lowry Assay. The Lowry Assay uses a chemical reaction to add color to protein in solutions - the darker the color, the greater the amount of protein in the sample

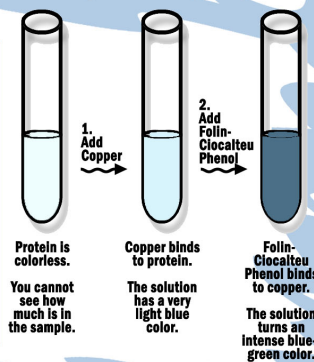
The Lowry Assay

Protein is colorless in solution. But scientists found that they could get *colored* chemicals to bind to protein. They could then find out how much protein was in a solution.

That method is called the **Lowry Assay**. It is a *colorimetric assay* which means that it uses color to measure something.

The Lowry Assay uses copper (Cu^{2+}) and Folin-Ciocalteu Phenol. The copper binds to protein. The Folin-Ciocalteu Phenol binds to copper. When the phenol binds with copper, they turn blue-green.

If a sample is compared to a set of standards, the protein concentration can be determined.



Laboratory Protocol

Think of the activity you did with your class using food coloring and bottles. Your teacher asked you to develop a method to determine how many drops of food coloring are dissolved in a mystery solution. Sharon will use this same method to determine how much protein is dissolved in each of the three sports drinks. You and your partner will use standards, each with a known concentration of protein, to compare the three sports drinks to.

To do *The Power Drink Challenge*, you will need several materials. The following is a list of the materials you will be using for the experiment:

Unknown A
Unknown B
Unknown C
Timer

Stock Protein
Folin-phenol reagent
Distilled Water
Micropipettes and tips

Spectrophotometer
Copper reagent
Test tubes

Prepare Unknown Samples

- 1. Label three of the empty test tubes A, B and C at the top of the tube, and label five of the empty test tubes 1, 2, 3, 4 and 5 at the top of the tube.
- 2. Add 1,000 μL of unknown A to the empty test tube labeled A.
- 3. Add 1,000 μL of unknown B to the empty test tube labeled B.
- 4. Add 1,000 μL of unknown C to the empty test tube labeled C.

Prepare Protein Standards

- 5. To create each standard, add distilled water (dH_2O) to the tubes marked 1,2,3,4, and 5:

Tube Number	Amount of dH_2O (μL)
1	1000
2	975
3	950
4	925
5	900

- 6. Using the table below, add the appropriate amount of stock protein to each test tube (The concentration of the stock protein is 1 $\mu\text{g}/\mu\text{l}$) to create your standards.

Tube Number	unt of Protein Solution
1	--
2	25
3	50
4	75
5	100

Why are the five tubes called “standards”? How will you use them for this experiment?

Lowry Assay Reaction – Step 1

- 7. Add 1,000 μL of the Cu^{2+} reagent to each test tube. Vortex the tubes for three (3) seconds. Hold the tubes by their top between your thumb and forefinger to mix the samples.
- 8. Incubate the sample tubes at room temperature for five minutes.

Compare the eight tubes to each other. Did anything change visually after adding the copper reagent? What sort of reaction is happening in the tube?

Lowry Assay Reaction – Step 2

- 9. Add 200 μL of the Folin-Phenol reagent to each test tube. Vortex the tubes for three seconds to mix the samples.
- 10. Incubate sample tubes at room temperature for five minutes.

Compare the eight tubes to each other. What happened to the samples when you added the Folin-Phenol reagent? Which of the unknowns A, B or C appears to have the largest amount of protein? How do you know?

Analysis of the Protein Standards and Unknown Samples

- 11. Take your samples to the spectrophotometer. An instructor will demonstrate how to use the spectrophotometer. The spectrophotometer measures absorbance, which is the amount of light absorbed by the color in the tubes.
- 12. Make sure the wavelength of the spectrophotometer is set to 750nm.
- 13. Insert sample tube 1 into the cuvette holder and press the 0 A/100 %T button to blank the spectrophotometer (Note A=Absorbance; T=Transmittance). Wait until the display reads 0.000. Each group must use their own tube 1 to blank the machine before reading their other tubes.

Why is sample tube one (1) used to “blank” the spectrophotometer?

- 14. Take sample tube one (1) out of the cuvette holder and insert sample tube two (2) (DO NOT PRESS ANY BUTTONS). Record the absorbance displayed on your data sheet.
- 15. Remove sample tube two (2) and read tubes the rest of the tubes, including A, B and C. Record the absorbance for each sample on your data table.

Data Analysis

Use this chart to record the absorbencies indicated by the spectrophotometer.

Test Tube	Protein ($\mu\text{g}/1000 \mu\text{L}$)	Absorbance
1	0	
2	25	
3	50	
4	75	
5	100	
A		
B		
C		

Using the absorbance values for the standards with known protein concentrations (0, 25, 50, 75, and 100 μL of protein in 1000 μL of solution), **create a graph with a line of best fit**. Make sure to fill in labels for each axis (what you are measuring *and* the units you are using). Use your graph to determine the concentration of protein in your unknown samples by marking their absorbance on the graph, then estimating how many microliters of protein are in each sample. Record your answers in the table above.



Which of the samples, A, B or C had the highest concentration of protein? How do you know?

Review Questions

What is a standard?

How were standards used for this experiment?

Why is the copper reagent (Cu^{2+} Reagent) added to the test tubes?

What happened when the Folin-Phenol was added to the test tubes? Explain why.

How does the amount of color in the tube relate to the amount of protein?

What does the spectrophotometer do?