Determining protein content using a microplate reader

Abstract

Students will learn to quantify the amount of protein in a solution by the Coomassie Blue staining method and generating a standard curve based on absorbances read by a microplate reader. High school students with a general understanding of chemistry and molecular biology will be introduced to basic analytical chemistry techniques and biochemistry concepts. While building standard curve development techniques, student will learn to work with microscale equipment.

Instruments being donated:

- Statfax-2100 microplate reader (with computer and printer)

Materials: microplates, pipettors and tips, microcentrifuge tubes and rack

Introduction

Proteins are made up of many different amino acids linked up in a chain. This is called a protein's primary structure. Protein secondary structure forms alpha helices and beta sheets. Tertiary structure involves various chemical interactions between the amino acids which allow the chain form to take on a globular, 3D structure. Quaternary structure is the interaction of multiple tertiary structures. However, this 3D structure is often not permanent. Temperature and pH level can affect protein structure. You will learn to quantify the amount of protein in solution using the Coomassie blue by the Bradford assay staining method. The chemical in the staining solution interacts with pockets of hydrophobic amino acids formed by the 3D structure. Upon binding, the dye-protein complex gives off a blue color that can be measured using the microplate reader.

A microplate reader is often used in biochemistry labs to quickly read the absorbances of many samples. Not only is it time effective, microplate readers reduce the amount of sample and reagents needed to perform the experiment. In this experiment, you will learn to work precisely on a microscale level.

Materials

- Shallow well microplate
- Single Channel pipettors (100, 200 ul fixed)
- Pipet tips, 200 ul
- Distilled water
- Spatula
- Whey protein isolate

- Microcentrifuge tubes 1.7 ml
- Coomassie Blue
- Fine-tip sharpies to label microcentrifuge tubes
- Microtube rack
- Gloves
- Scale

Experimental procedure:

1. Obtain unknown protein solution from teacher.
2. Label two sets of microtubes with 0, 100, 500, 1000, 1500 and “unknown” ug/ul. Label one set as “initial” (I) and the other set as “diluted” (D). We need to dilute the original protein samples because the color intensity is too high for the microplate reader to be accurate.
3. Pipet 200 ul of each standard solution and the unknown protein solution into their respective tubes in the “initial” set of tubes. A standard of 0 ug/ml is the control well and will just contain distilled water. Change pipet tips between each solution.
4. Pipet 200 ul of Coomassie blue into each of the “initial” tubes. Be careful not to ingest it or get the solution on your skin as the staining solution is corrosive. Mix well by pipetting up and down multiple times in each well or gently tapping the tube.
5. Allow tubes to sit for a minute to allow sufficient reaction of the color with the protein.
6. Now we will dilute these samples five-fold. Take 200 ul from each “initial” tube and place it in their respective “diluted” tubes. Pipet 800 ul of distilled water into each “diluted” tube.
7. In your designated microplate column, pipet 200 ul of the “diluted” samples into the well, going down the column.
8. In each of the last three wells, pipet 200 ul of your unknown protein solution. These are the replicate trials and you will use the average of these values.
9. Place the plate carefully in the microplate reader and record absorbance values at 630 nm.

Data Analysis:

1. Graph the absorbance values versus protein concentrations of the four standard solutions.
2. Find the line-of-best-fit and record the equation.
3. Average the absorbances of your unknown protein sample. Use the equation to determine the concentration of protein in your sample.

Discussion Questions:

1. How does Coomassie stain bind to proteins?
2. What disrupts the 3D structure of a protein? Why?
3. Describe the four levels of protein structure.

For the Teacher:

Prep:

1. Make 1 mg/ml (1000 ug/ml) and 3 mg/ml (3000 ug/ml) stock solutions of whey protein.
2. Make solutions of known whey concentrations for students to generate a standard curve, using the table provided. Multiply the volumes according to how many students/groups you have.

Desired Concentration (ug/ml)	Volume desired (ml)	Concentration stock to be used (ug/ml)	Stock volume (ml)	Water volume (ml)
0	1	0	0	1
100	1	1000	0.1	0.9
500	1	1000	0.5	0.5
1000	1	1000	1	0
1500	1	3000	0.5	0.5

3. Each group will be given an "unknown" protein solution to determine concentration using their generated standard curve. You can just give them an unlabeled tube with one of the concentrations used for the curve generation or you can make truly unknown solutions by diluting the standards appropriately. Keep in mind that the Coomassie Blue test works best in a range from 100-1500 ug/ml.

Each group will need a full 1.5 ml tube of staining solution. The Coomassie blue solution needs to be stored at 4°C. Allow it to warm to room temperature before the experiment.

Product reorder list (VWR product numbers):

- Shallow well microplate (350 ul): (89237-504)
- Single Channel pipettors (100 ul fixed, 83009-768 ; 200 ul fixed, 83009-770)
- Pipet tips, pack of 1000 (200 ul): (53508-783)
- Microcentrifuge tubes, 1.7 ml, natural, pack of 500 : (87003-294)
- Coomassie Blue (95043-424)
    - MSDS: http://www.sciencelab.com/msds.php?msdsId=9925778
- Microtube rack: (20901-675)

Clean-up:

1. Collect Coomassie blue in a hazardous waste container. Dispose according to risk management policies.
2. Whey protein solutions can be disposed down the sink.