Bradford Test for Protein

Introduction

In your previous biology classes you should have determined the presence or absence of various organic molecules with specific testing reagents such as Benedicts (tests for reducing sugars) or Lugol's Iodine (tests for starch) or even Biuret (tests for proteins). These reagents were qualitative in nature. They gave a color change but that was only used to determine if they were present or absent. There are methods that can be used to determine their concentration. Qualitative measures determine if they are there or not, by color or some other quality. Quantitative measures determine the amount or quantity present.

Quantitative estimation of the total protein content of a sample is frequently necessary in cell physiological and biochemical studies. Several methods of determining the total protein content of a sample have been developed and widely used during this century. One of the simplest and most sensitive is the "Bradford" assay, which was introduced in the mid-1970s. This assay is based on the binding specificity of the dye Coomassie Brilliant Blue-G250 for protein molecules but not for other cellular constituents. This organic dye binds specifically to tyrosine side chains.

The binding of the dye to protein shifts the peak absorbance of the dye. Unbound Coomassie Blue absorbs light maximally at a wavelength of 465 nm, while the absorption maximum is at 595 nm when the dye is bound to protein. The absorbance of light by the dye-protein complex at 595 nm is proportional to the amount of protein bound (over a limited range); i.e. there is a linear relationship between absorbance and the total protein concentration of the sample over a narrow range.(source: http://www.acad.carleton.edu/curricular/BIOL/resources/rlink/lab1p4.html)

So the main idea is to have a series of proteins that you know the concentration of protein and mix them with Bradford reagent. Once you determine the absorbance with a series of known proteins, you can graph protein concentration vs. absorbance. By mixing unknown proteins with the same reagent, you can use the graph to work backwards from absorbance and determine protein concentration (see Figure 1).

Procedure

Note: do all determinations in duplicate by working in collaboration with another group.

Construction of Standard Curve

1. To determine the concentration of protein in an unknown solution it is first necessary to make a control standard and create a standard curve using a series of

known protein concentrations. We will be using BSA or Bovine Serum Albumin (cow blood protein) or Bovine Gamma Globulin.

2. You will be making a series of dilute solutions. Using the formula C1V1=C2V2 make 100 ul of each of the following BSA dilutions. .2, .4, .6, .8 mg/ml(ug/ul) Use 1 X Phosphate Buffered Saline to do the dilutions. (This may be done ahead of time by using Quick Start Standards from Bio-Rad)
3. Add 50 µl of known BGG protein solution to the cuvette.
4. Add 2.5 ml of Bradford Reagent to each cuvette. Mix well and let sit 5 minutes.
5. Set Spectrophotometer to 595nm and let warm up for a min of 15 minutes. Set Transmittance to 100 using a cuvette that has 50 ul of 1X PBS and 2.5ml of Bradford reagent. This is the negative control and is used to make the machine ignore the glass and the unbound Bradford reagent.
6. Remove the negative control tube and add your .2mg/ml tube. Press the mode button and switch to read absorbance at 595 nm. Record the absorbance.
7. Take out the known sample and repeat by adding the blank and read each control sample.

Testing Unknown Solutions:

1. Dilute the milk sample 1/50 by adding 1 µl of milk to 49 µl 1x PBS.
2. Take 50 µl of the diluted milk sample and mix with 2.5 ml of the Bradford reagent and let sit 5 min.
3. Use the blank again as in steps 5 and then place your milk tubes in as you did in steps six and seven above.

Determination of Unknown Protein Concentration in Milk.

Graph the absorbance vs. protein concentration. Use Excel and make sure to display the correlation (R2) and the equation of the line. It will look something like this.

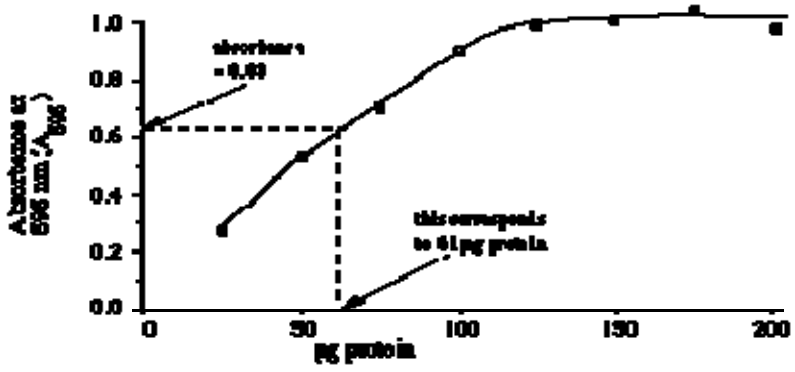

Figure: 1

Sample Graph and use to determine protein concentration after getting absorbance of unknown.