

Protein Standard Curve and Protein Assay of Milk and Protein Drinks

In this lab you will determine the amount of protein in different types of milk and in protein drink products. In order to determine the amount of protein present in an unknown sample one first needs some way to compare the unknown to known protein values. This may be accomplished by setting up a protein standard curve. You will take known amounts of a protein, in this case bovine serum albumin (BSA), and do a standard colorimetric test known as a total protein assay. When the total protein reagent (TPR) is added to a solution containing protein, a chemical reaction occurs that turns the solution blue. The more protein present, the deeper blue the resultant product, and thus the higher the absorbance reading in the spectrophotometer. By graphing the relationship between these absorbance readings and the known amounts of protein assayed you will have a standard curve. To determine the protein content of your unknown solution you will do the protein assay on it, take an absorbance reading, and plug that number into the line equation of your protein standard curve. By knowing the volume of unknown you assayed you then can find the concentration of protein in the unknown solution.

Procedure

A. The Protein Standard Curve

1. To obtain data for your standard curve set up six test tubes with the amounts of protein and deionized water listed in the table below (tubes # 1-6). The protein being used as the standard is bovine serum albumin (BSA) at a concentration of 5 mg/ml.
2. To determine the protein content of your unknown set up 2 tubes (# 7-8 below) each with 1.0 ml of the unknown and 1.0 ml of dH₂O. You are doing this in duplicate to check your accuracy.

Test Tube #	ml BSA (5 mg/ml)	ml unknown	ml dH ₂ O	mg protein	Absorbance @ 540 nm
1	0.0	---	2.0	0	
2	0.1	---	1.9	0.5	
3	0.3	---	1.7	1.5	
4	0.5	---	1.5	2.5	
5	0.7	---	1.3	3.5	
6	1.0	---	1.0	5.0	
7	---	1.0	1.0	?	
8	---	1.0	1.0	?	

3. Add 3.0 ml of Total Protein Reagent (TPR) to each of the test tubes. Mix and wait 10 minutes.
4. Set the wavelength on the spectrophotometer to 540 nm.
5. Pour the contents of test tube #1 into a cuvette. This is your blank because it has every component of the reaction mixture but protein. Put the blank into the spectrophotometer and set to zero absorbance.
6. Take the blank out. Sequentially take the readings for tubes 2-6 and then your unknown samples. Make sure you record your data in the table above.
7. Plot your protein standard curve – absorbance on the y-axis and protein content (mg) on the x-axis for tubes # 1-6. Determine the equation of the line.
8. To determine the protein content (x) of the unknown plug your absorbance value (y) for the unknown into the equation of the line.
9. Determine the protein concentration of your unknown. The concentration of protein in the unknown would be the protein content/volume of unknown assayed (in this case 1 ml).

B. Determining the Amount of Protein in a Protein Drink or Milk

Now that you have a protein standard curve you may determine the amount of protein in just about anything. We will give you a protein drink and different types of milk and you will determine the amount of protein in a sample of those drinks and then the protein concentration.

1. You want the total volume of your sample to be 2.0 ml. Because you don't know the protein concentration of the drink you are testing you might want to assay different volumes so you get an absorbance that is on your standard curve - not too high or too low. You also may need to dilute the sample before you assay as well. So, you could assay 1.0 ml of protein drink and 1.0 ml dH₂O, or 0.1 ml of

protein drink and 1.9 ml dH₂O, or 0.5 ml of a 1/10 dilution of the protein drink with 1.5 ml of dH₂O, etc. For the milk samples you might want to dilute your milk with equal volumes of dH₂O (for example: 1 ml milk with 1 ml dH₂O) or dilute the milk 1/10 or 1/100 and then assay 0.1 ml of that. The table below gives an example of how you might set the samples up.

Test Tube	dH ₂ O (ml)	protein drink (ml)	diluted milk (diluted with dH ₂ O) (ml)	Absorbance @ 540 nm
1	2.0	---	----	
2	1.9	0.1	----	
3	1.9	----	0.1	

2. Add 3.0 ml of Total Protein Reagent (TPR) to each of the test tubes. Mix and wait 10 minutes.
3. Read at 540 nm, being sure to blank the spectrophotometer (tube #1).
4. Determine the protein content of your samples from the standard curve. Determine the protein concentration of the drinks.

C. Sample Problems using your Standard Curve

1. Calculate the protein concentration in mg per ml of the following :
 - A stock solution of soybean extract was diluted 1/20.
 - To perform a protein assay using TPR, 0.4 ml of this stock was brought up to 2 ml with dH₂O and then the TPR was added.
 - The absorbance of this solution at 540 nm was 0.18.
2. Calculate the protein concentration in mg per ml of the following :
 - 0.2 ml of a solution of giraffe white blood cells was brought up to 2 ml with dH₂O and then TPR was added.
 - The absorbance at 540 nm was 0.01.
3. Calculate the protein concentration in mg per ml of the following:
 - A stock solution of goose liver was diluted 1/40.
 - To perform a protein assay using TPR, 0.8 ml of this stock was brought up to 2 ml with dH₂O and then the TPR was added.
 - The absorbance of this solution at 540 nm was 0.24

