Got Protein?™ Kit

Catalog Number
166-2900EDU

Testing protein content of common foods using the Quick Start™ Bradford Protein Assay

explorer.bio-rad.com

BIO-RAD

Dear Educator:

This kit is designed to introduce students to proteomics, and provides the tools for them to develop their own protein-based experiments.
Based on the Bio-Rad Quick Start™ Bradford Protein Assay, the Got Protein?™ kit is an inquiry-based biophotonics lab that allows students to analyze and compare the protein content in milk, sports drinks, egg, muscle tissue, saliva, tears, or any source of soluble biologically derived material. Protein quantitation is often necessary before isolation, separation, and analysis by chromatography, electrophoresis, or western blotting. This lab integrates biology, chemistry, and physics, allowing students to develop an understanding about how the chemical, physical, and biological properties of proteins determine their structure and function. It is impossible to place biological material under a microscope and count the number of protein molecules per unit volume the way we can count the number of cells. Therefore, something measurable that is proportional to the concentration of the substance of interest must be identified.

Beer's law states that when a solute absorbs light of a particular wavelength, the absorbance is directly proportional to the concentration of the solute in solution. The measurement most commonly used in protein assays is the absorbance of light. However, proteins do not absorb sufficient light to assay -— by themselves.

The Bradford method is based on the color development formed when the dye Coomassie Blue G-250 binds to protein. The unique chemical properties of the dye allow it to interact with the side chains, or R-groups, of specific amino acids. There is a correlation between the amount of blue color and the amount of protein in the sample: the more protein, the more intense the blue color. The simplicity of the assay allows the results to be measured qualitatively by eye, or quantitatively with a spectrophotometer. In this lab, students use absorbance values from a set of protein samples with known concentrations to create a standard curve on linear graph paper. Protein concentrations of their test samples can then be extrapolated by hand or plotted using a graphing utility such as Microsoft Excel. Students also learn to use a spectrophotometer, micropipet, and computer, which are all invaluable tools in modern bioscience research.

This curriculum was developed in collaboration with Barbara Denny from Miramonte High School in Orinda, Ca. We'd like to express our thanks for her invaluable guidance and contribution to this curriculum.

Ron Mardigian
Division Marketing Manager
Biotechnology Explorer Program

Create context. Reinforce learning. Stay current.
New scientific discoveries and technologies create more content for you to teach, but not more time. Biotechnology Explorer kits help you teach more effectively by integrating multiple core content subjects into a single lab. Connect concepts with techniques and put them into context with real-world scenarios.

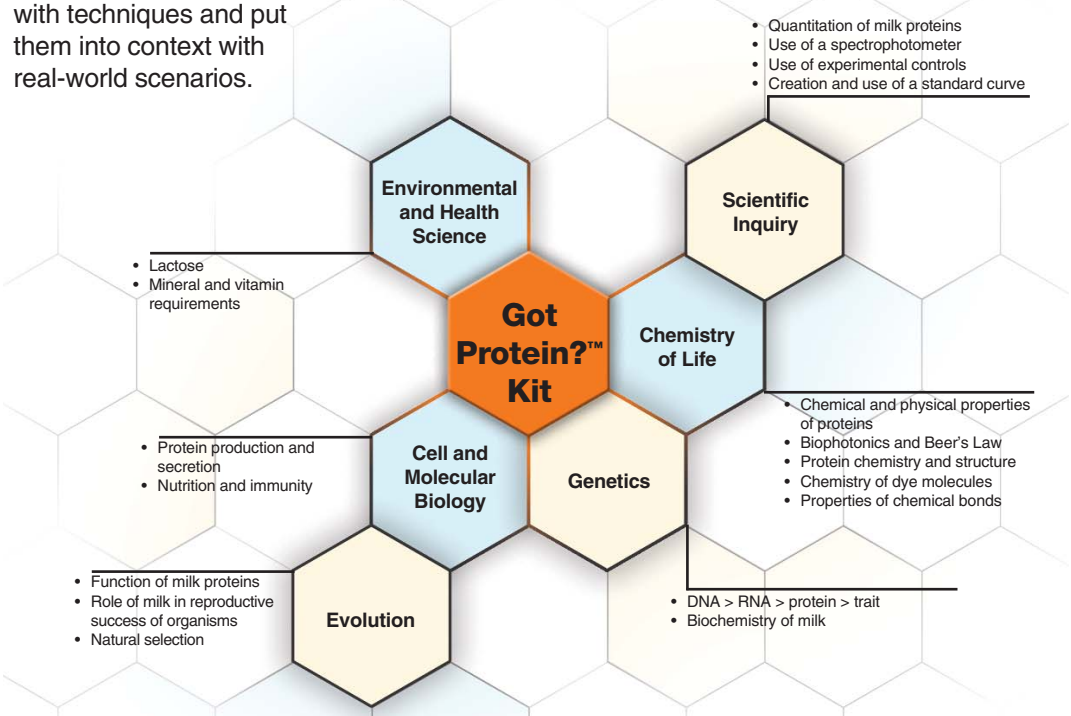


Table of Contents

Introduction

This kit is designed for testing the protein content of common foods or any source of biologically derived material. Protein quantitation is often necessary before processing protein samples for isolation, separation, and analysis by chromatographic, electrophoretic, and immunochemical methods.

To study an enzyme in the lab, you must account for two properties of the enzyme: the total amount of protein present and the total amount of activity. Over time many enzymes lose their activity, even though the total protein present in a sample may remain constant. This lab activity allows students to accurately quantitate the amount of total protein in a sample independent of other properties such as activity or conformation.

The biotechnology connection with this assay includes learning the instrumentation procedures for the spectrophotometer and the micropipet – both of which are invaluable tools in modern research. The Quick Start Bradford protein assay is a quick, simple, and inexpensive laboratory procedure to determine the protein content of a sample. The assay is based on the color development formed when the dye, Coomassie Blue G-250, binds to protein. The simplicity of the assay allows the results to be read directly in cuvettes or test tubes or, if available, with a spectrophotometer.

It is impossible to place biological material under a microscope and count the number of molecules per unit volume the way we can count the number of cells per unit volume. We must find something that we can measure that is proportional to the concentration of the substance of interest. The measurement most commonly used in protein assays is absorbance of light. Beer's Law tells us that if a solute absorbs light of a particular wavelength, the absorbance is directly proportional to the concentration of that solute in solution. A device called a spectrophotometer is used to measure, display, and/or record absorbance in quantifiable units. Often the solute by itself does not absorb light so as to allow for a practical assay. We may have to employ one or more reagents to produce colored compounds in proportion to the concentration of an unknown.

In this lab, students will create a standard curve on linear graph paper using absorbance data from a set of protein samples with known concentrations. Students will then determine the protein concentrations of unknown samples by two methods: first, by visually comparing their unknowns to a set of known protein standards; second, by using a spectrophotometer to read the specific absorbance of their samples and comparing their absorbance to the standard curve created at the beginning of the lesson.

The Human Genome Project has completed the task of sequencing all human genes. Far from closing a book, this body of work has opened up a whole new field, proteomics, which asks a far more important question — What do our genes do? Genes encode proteins and proteins determine an organism's form, function, and phenotype. Proteomics is the study of the location, function, structure, quantity, and interaction of proteins with each other and their environment.

Introduce students to the world of proteomics, and then let them use this kit as a tool to develop their own simple, protein-based experiments.

Target Audience

Grade Level: Middle School, High School, and College

Disciplines: Biology, AP Biology, Chemistry, and Biotechnology

Goals:

1. To acquaint students with a simple test for protein quantitation.
2. To introduce students to the basics of spectrophotometry.
3. To introduce students to the use of a spectrophotometer.
4. To introduce students to biophotonics.
5. To teach students how to prepare dilutions for analysis.
6. To teach students how to construct a standard curve.
7. To teach students how to utilize spreadsheet software to organize, display, and analyze data.
8. To teach students how to use a simple protein assay and a spectrophotometer to design their own experiments.
9. To expand students' understanding of protein chemistry.

Curriculum Fit

Biology/Life Sciences

Cell Biology
Genetics

Chemistry

Atomic and Molecular Structure
Chemical Bonds
Acids and Bases
Solutions
Organic Chemistry and Biochemistry

Investigation and Experimentation

Scientific progress: asking meaningful questions and conducting careful investigations.

SAFETY ISSUES

1x dye reagent contains phosphoric acid, and so appropriate safety wear (i.e. gloves, protective eyewear, and labcoats) should be worn at all times when handling this reagent. For further information, please refer to the Material Safety Data Sheets (MSDS) available from Bio-Rad (at (800) 4BIORAD in the US), or at **www.bio-rad.com**. The reagent and other materials used in this experiment should be disposed of in accordance to local regulations.

Kit Inventory Checklist

This section lists equipment and reagents necessary to conduct protein quantitation in your classroom or teaching laboratory. Each kit contains materials for 80 workstations. We recommend that students be teamed up – two to four students per workstation. Please use the checklist below to confirm inventory.

Kit Components	Number/Kit	(✔)
166-2900EDU, Got Protein? Kit includes:		
Quick Start Bradford protein assay kit 4, includes 1x dye reagent (1 L), bovine γ-globulin standard set (2 sets of 7 standards, 0.125–2.0 mg/ml, 2 ml)	1 kit	❒
10x phosphate buffered saline (PBS), 100 ml	1 bottle	❒
1.5 ml semimicro cuvettes, 100	1 pack	❒
Got Protein? instruction manual	1	❒

Required Accessories	Number/Kit	(✔)
100–1,000 µl adjustable-volume micropipet (166-0508EDU)	1	❒
2–20 µl adjustable-volume micropipet (166-0506EDU)	8	❒
100–1,000 µl pipet tips (223-9350EDU)	1 box	❒
2–20 µl pipet tips (223-9347EDU)	1 box	❒
1.5 ml microtubes (223-9480EDU)	1 bag	❒
SmartSpec™ Plus spectrophotometer (170-2525EDU)	1	❒
Distilled water	100 ml	❒
15 ml capped tubes	8	❒
Milk samples (suggestions: low fat, fat free, soy, baby formula)	10 ml	❒

Refills available separately

Quick Start Bradford protein assay kit 4, includes 1x dye reagent (1 L), bovine γ-globulin standard set (2 sets of 7 standards, 0.125–2.0 mg/ml, 2 ml), 1 kit (500-0204EDU)

10x phosphate buffered saline (PBS), 100 ml, 1 bottle (166-2403EDU)

1.5 ml semimicro cuvettes, 100, 1 pack (223-9955EDU)

Instructor's Manual Background

Biophotonics

Biophotonics describes the technology that focuses on the interaction of biological materials with light and other forms of radiant energy whose quantum unit is the photon. Radiation is energy that comes from a source and can travel through material or space. In Figure 1, the electromagnetic spectrum of light is illustrated, showing the colors associated with the wavelengths of visible light.

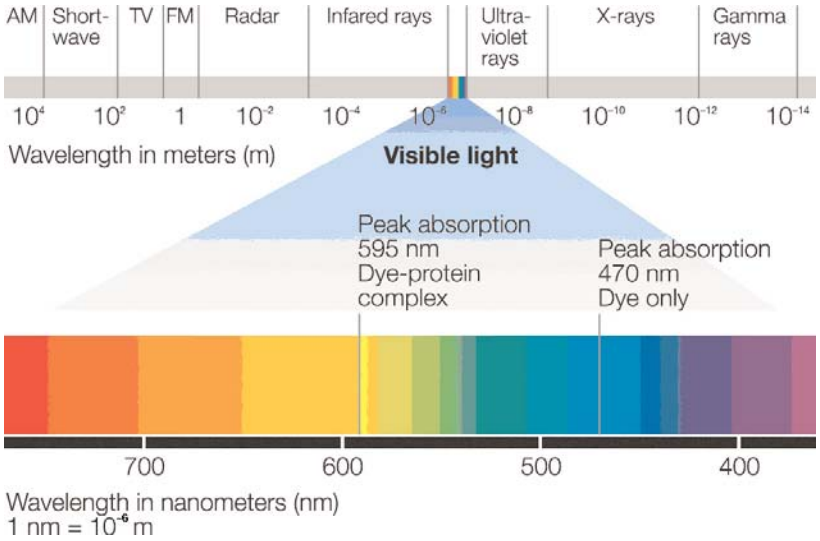


Fig. 1. The electromagnetic spectrum.

The human body is made up of different tissues and cells. Tissues and cells are composed of different biomolecules (DNA, proteins, lipids, and carbohydrates). Light can interact with biomolecules in several different ways: reflection, absorption, transmission, and light scattering. The Bradford assay is based on the absorption of light as a function of wavelength. As light passes through a material, light energy is absorbed, and each material absorbs light at a specific wavelength. The removal of these wavelengths from visible light gives the material its color. Thus the removal of the yellow wavelengths of light by the protein-dye complex at 595 nm makes the protein-dye complex blue, while the dye alone (without protein) absorbs blue light at 470 nm making the dye a reddish-brown color.

Nearly all biophotonic applications involve a light source that is passed through a target material and a detection sensor that reads the light emission from the material. A spectrophotometer has a light source that generates specific wavelengths. The light path passes through the cuvette, is absorbed by the material in the cuvette, and is read by a detector. In the Bradford assay, the peak absorbance of unprotonated Coomassie G-250 dye is at 595 nm, and the spectrophotometer is set to read at 595 nm. Colorimetric assays use standard curves created by measuring the absorbances of solutions of known concentration to determine the concentration of unknown samples.

There are several colorimetric methods for determining the total protein content of a sample: biuret, Lowry and Bradford. The biuret is the oldest method and is commonly used in high school labs to detect the presence of a protein. It involves two reactions; a chelation and a redox reaction. It is the least sensitive of the three methods. The Lowry method

involves two redox reactions. It is more sensitive than the biuret assay; however, the Lowry assay is affected by interference from many common laboratory reagents and chemicals. The Bradford protein assay is the most sensitive of the three. The Bradford assay uses a dye, Coomassie Brilliant Blue G-250, which was first described by M. Bradford in 1976. This assay takes advantage of the chemical properties of the dye and the dyes ability to interact with the side chains, or R-groups, of specific amino acids.

Coomassie G-250 exists in multiple forms. As part of the Bradford solution, the dye exists in its cationic state and takes on a reddish-brown color. The peak absorption of the dye in this state is 470 nm. When the dye binds to and interacts with amino acids, the dye is converted to a stable unprotonated blue form, and the absorption maximum shifts from 470 nm to 595 nm. This stable blue form of the dye is easily observed and quantified in a spectrophotometer. There is a correlation to the amount of blue color and the amount of protein in the sample. The more protein, the more intense the blue color. By using a dilution series of known proteins, one can generate a spectrophotometric standard curve. The curve can then be used to estimate the quantity of protein in an unknown sample, based upon the intensity of blue. The Bradford assay is simple, highly sensitive, and relatively unaffected by many common laboratory reagents and chemicals.

The exact chemical interactions or binding properties of Coomassie G-250 dye are illustrated in Figure 2. The dye binds to proteins using three types of interactions. The primary interaction of the dye with proteins occurs through arginine, a very basic amino acid, which interacts with the negatively charged sulfate groups through electrostatic interactions. Other weaker dye-protein interactions include the interaction of the aromatic rings of Coomassie G-250 dye with the aromatic rings of amino acids, such as tryptophan, through electron stacking interactions. Finally, the dye also weakly interacts with polar amino acids that have hydrophobic R-groups, such as the aromatic ring of tyrosine. The binding of the protein to the dye converts the dye to a stable, unprotonated, blue form. The intensity of the blue color indicates the level of protein in a sample. The more intense the blue color, the more protein present in the sample.

The Bradford assay is easy to perform and involves four main steps:

- Preparation of a dilution series of known protein standards and preparation of unknowns
- Addition of Bradford dye (brown, cationic form) and incubation for >5 minutes (not to exceed 60 minutes)
- Binding of dye to protein, resulting in color change to the blue, unprotonated dye form and quantitative reading of the absorption at A_{595} in a spectrophotometer
- Compilation of the data into a standard curve and unknown protein concentration determination

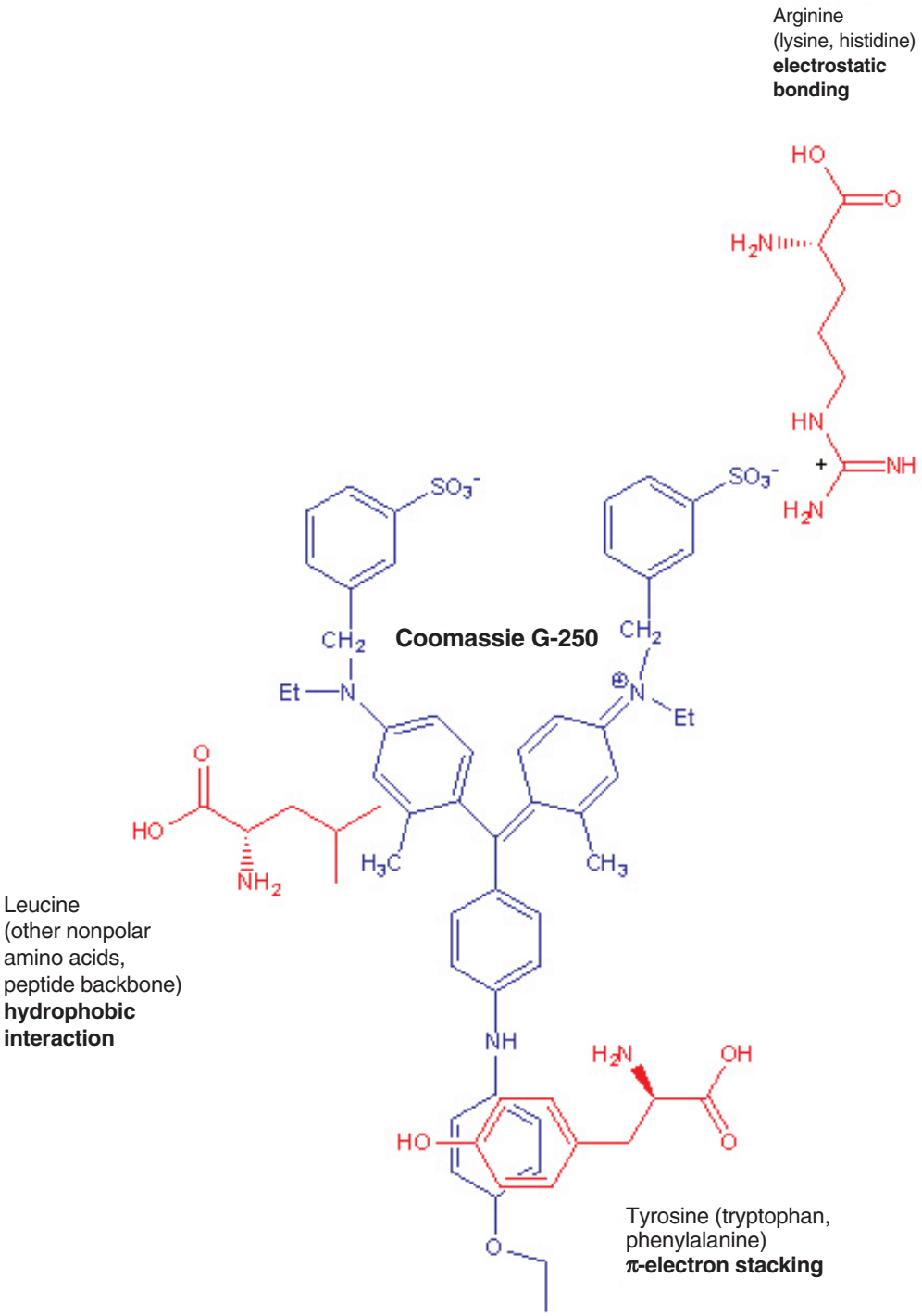


Fig. 2. Coomassie G-250 interactions with amino acid residues.

In this laboratory exercise, the Bradford assay is used to quantitate the amount of protein in different types of milk samples. Casein is the most abundant form of protein in milk and the amino acid composition of the protein is shown in Figure 3. Casein contains a total of 224 amino acids, with a molecular mass of 24,967 daltons. Casein contains 13 amino acids which strongly react with Coomassie dye: 4 arginines (R), 1 tryptophan (W), 4 tyrosines (Y), and 4 histidines (H). These dye-binding amino acids are shown as bold text in the sequence. Because the Coomassie dye molecule is much larger than a typical amino acid (854 daltons for Coomassie, compared to the average of 110 daltons per amino acid), it is quite easy to visualize how a few Coomassie dye molecules can bind and "coat" a typical protein in solution. This binding or coating of proteins is the principle behind the Bradford assay.

```
  1    MKVLILACLVALALARELEELNVPGEIVESLSSSEESITRINKKIEKFQSEEQQQTEDEL
 61    QDKIHPFAQTQSLVYPFPGPIPNSLPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPK
121    QKEMPFPKYPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSL
181    SQSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPIIV
```

Fig. 3. Amino acid composition of casein.

In this lab students will use absorbance data from a set of protein samples with known concentrations to create a standard curve on linear graph paper. Protein concentrations of their unknown samples can then be calculated. Students may also plot their data using a graphing utility such as Microsoft Excel. They can then use Excel to determine the correlation coefficient (R^2 value). The closer the correlation coefficient is to 1.00, the better the fit of the standard curve, and the better the estimate of concentration. Figure 4 illustrates a representative standard curve that can be generated in this exercise. In this figure, the raw absorbance data was plotted (absorbance vs. concentration), and a best-fit curve was generated. The high R^2 value depicted for this curve ($R^2 = 0.98$) illustrates the strong linearity of these data. Correlation coefficients of >0.9 reflect data which exhibit a high degree of linearity and can be used to accurately estimate unknown values. To generate the standard curve, the measured absorbance of each standard in the curve is plotted against the known protein concentration. The resulting standard curve can be used to estimate the concentration of an unknown protein based upon its measured absorbance value. Alternatively, if a spectrophotometer is not available, students can compare their unknown samples qualitatively to a dilution series to determine the protein concentrations.

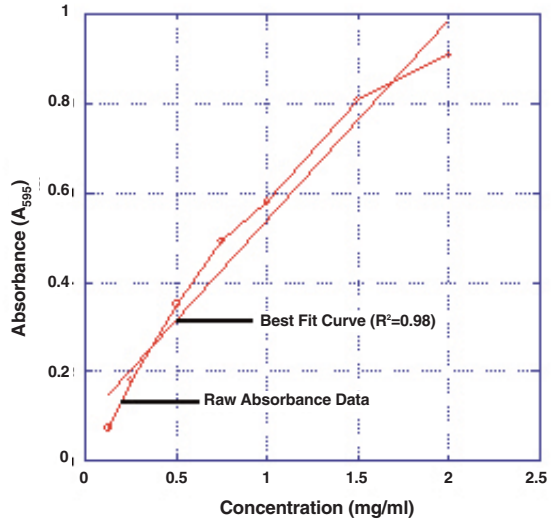


Fig. 4. Standard curve showing absorbance plotted against concentration.

Instructor's Advance Preparation

This protocol is designed for 80 workstations of 4 students. Each group will prepare a set of standards, a blank, and 2 milk samples (can be a blind test or known samples). The instructor will provide quantitative data for a set of standards and each student group will prepare a set of standards and qualitatively compare the standards to their two test milk samples. Quantitative data from the milk samples can be compared to the standard curve that will be provided by the instructor (or generated by individual student groups, if time allows). The setup time for this exercise requires a short period of time on two consecutive days.

Day 1 **Estimated preparation time: one hour**
Read through protocol.
Obtain milk samples (see suggested milk samples in materials section).

Day 2 **Estimated preparation time: one hour**
Aliquot materials for student workstations (30 min).
Make standard dilutions, prepare standard curve, read absorbances on SmartSpec Plus Spectrophotometer, and generate standard curve.

Reagent preparation for student workstations and instructor standard curve.

1. Aliquot 200 µl into 8 microtubes for each type of milk sample to be tested. Label test milk samples "Sample A" and "Sample B".
2. Provide 10 cuvettes for each group (1 blank, 7 standards, and 2 milk samples).

   Note: If you do not have a spectrophotometer and are going to perform the lab in a qualitative fashion, 10 small test tubes can be substituted for the cuvettes.
3. Remove the 1x Quick Start Bradford dye reagent from 4°C storage and invert several times to mix.
   - Label 9 disposable 15 ml capped tubes "1x dye reagent"
   - Aliquot 12 ml of the 1x dye reagent into each disposable 15 ml capped tube
4. Prepare 10 ml 1x PBS buffer.
   - Mix 1 ml of 10x PBS buffer and 9 ml distilled water in a 15 ml capped tube
   - Aliquot 500 µl of 1x PBS into 9 microtubes labeled "1x PBS" (to be used for blanks and student milk sample dilutions)

5. Prepare 9 sets of standards, 1 set per workstation and 1 set for instructor standard curve.
    - Label seven microtubes #1–7, and aliquot 25 µl of each standard into the appropriately labeled tubes as shown below

Tube Label	Standard (mg/ml)
1	0.125
2	0.250
3	0.500
4	0.750
5	1.000
6	1.500
7	2.000

6. Prepare the standard curve samples for the instructor standard curve.

    Note: Be careful not to touch the optically clear sides of the cuvettes. Any fingerprints will interfere with the light path.

    Label 8 cuvettes (blank, #1–7) and prepare standards.

    - Mix 1x dye reagent by inverting before pipeting
    - Pipet 1 ml of 1x dye reagent into 8 cuvettes
    - Pipet 20 µl of 1x PBS into blank cuvette
    - Pipet 20 µl of each standard into the corresponding cuvette
    - Mix samples by pipeting up and down or by covering with Parafilm and inverting 3 times
    - Read absorbance of samples on spectrophotometer within 1 hour

Setting up the SmartSpec™ Plus Spectrophotometer

Operation of the SmartSpec Plus is easy and intuitive. Brief instructions have been provided throughout this procedure. For more detailed information, please refer to the SmartSpec Plus Spectrophotometer instruction manual. Text in boxes is the text seen in the SmartSpec Plus Spectrophotometer data window. The cuvettes must be inserted in the correct orientation to obtain a proper reading. Refer to Figure 5 as a guide for loading cuvettes.

Fig. 5. Proper placement of a cuvette in the SmartSpec Plus.

1. Turn the SmartSpec Plus on and press the "Protein" button.
2. Choose the type of assay by pressing the "Select" button.

Select assay: Bradford

Select "Bradford", and press "Enter".

3. Turn background subtraction off.

Do you want to subtract background reading? ***NO***

Select "***NO***", and press "Enter".

4. Setting up for a new Standard Curve.

Do you want to make a New STANDARD curve? ***YES***

Select "***YES***", and press "Enter".

5. Program the method for the number of replicates (for this lab, one).

Enter number of blank REPLICATES (1–9) 1

Select "1" and press "Enter".

6. Place cuvette with the PBS blank into chamber with smooth side facing light path as shown in Fig. 5.

Insert Blank #1/1 and press <Read Blank>

Press "Read Blank".

7. Enter the number of standards to be used in generating the standard curve (7).

Enter the number of STANDARDS (2–9):

Using the numeric keypad, press "7", and press "Enter".

8. Set the concentration units (mg/ml).

Select concentration units: mg/ml

Select "mg/ml", and press "Enter".

9. Enter the number of replicates to be read (no replicates for this lab).

Are any STANDARDS to be read in replicate? ***NO***

Select "***NO***", and press "Enter".

10. Enter the concentration for the first standard (0.125 mg/ml).

Enter concentration of STANDARD #1:_____mg/ml

Using the numeric keypad enter the concentration of std #1. Enter "0.125", and press "Enter".

11. Read the absorbance value for standard #1.

Insert STANDARD #1 and PRESS <Read Sample>

Place the cuvette in chamber. Close the sample compartment door, and press "Read Sample".

12. Repeat for standards 2–7. **Note**: If a number is entered incorrectly, press “cancel”.

Cuvette	Blank	1	2	3	4	5	6	7
Concentration	0	0.125	0.250	0.500	0.750	1.000	1.500	2.000

13. Assuming the standard curve data was entered correctly, prompt the spectophotometer not to modify.

Do you wish to modify the standard curve? ***NO***

Select "***NO***" and press "Enter".

14. At this time, choose not to view the data from the standard curve.

Want to view info on new STD CURVE now? ***NO***

Select "***NO***" and press "Enter".

15. At this time, save the data from the standard curve. The prompt from "**NO**" to "**YES**" can be changed by pressing the "Select" key.

Save standard curve? ***YES***

Select "***YES***" and press "Enter".

16. Provide a name for the standard curve.

Save as Std curve # _____ Name (8 char)

Using numeric keypad, enter a "#" and press "Enter".

Press the **Alpha** key located next to the key pad. Enter information, and press "Enter".

17. Instruct the spectophotometer not to read in replicate.

Are any samples to be read in replicate? ***No***

Select "***NO***" and press "Enter".

18. Press the left arrow to exit the assay.

19. Record data and post for students.

Print full report? ***NO***

Select "***YES***" and press "Enter".

20. The SmartSpec Plus will print out the data. You are now ready to proceed to the student portion of the activity.

21. From the absorbance data printout, enter the A_{595} data into the table, and provide a copy of the data for each student group. If time allows and student groups will be generating their own data, this table can be used as a comparison and discussion point as to why different standard curves may have slightly different absorbance values.

Sample	**A_{595}**	**Concentration (mg/ml)**
#1		0.125
#2		0.250
#3		0.500
#4		0.750
#5		1.000
#6		1.500
#7		2.000

22. Prepare the SmartSpec Plus for the student lab. Select "Protein".

23. Choose the type of assay by pressing the "Select" button.

Select Assay: Bradford

Select "Bradford", and press "Enter".

24. Turn background subtraction off.

Enter concentration of STANDARD #1:_____mg/ml

Select "***NO***", and press "Enter".

25. Recall the appropriate standard curve by name.

Do you want to make a new STANDARD curve? ***NO***

Select "***No***", and press "Enter".

26. Recall the standard curve by name.

Do you want to recall a stored Std curve? ***YES***

Select "**YES**" and press "Enter".

27. The latest stored standard curve should appear in the text box.

Select curve: Bradford Std Curve #X, your name

Press "Enter" to recall this curve.

28. Because the data have already been printed, elect to not view the info on the curve.

Want to view info on STD Curve #X now? ***NO***

Select "**NO**" and press "Enter".

29. Enter the number of replicates to be read (no replicates for this lab).

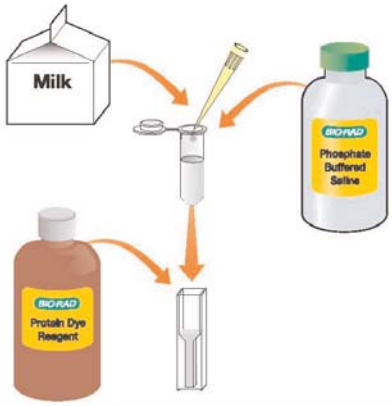
Are any samples to be read in replicate? ***NO***

Select "**NO**" and press "Enter". The SmartSpec Plus Spectrophotometer is now ready to be used by the student groups.

Got Protein?™ Kit – Quick Guide

Prepare test samples for spectral analysis

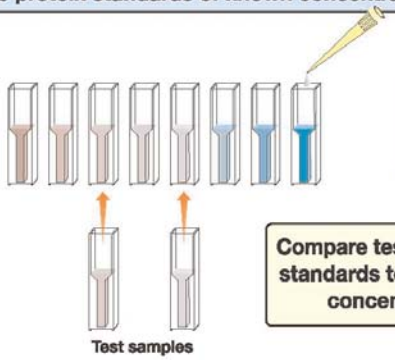
Dilute test samples of unknown protein concentration 1:50 in phosphate buffered saline



Add 20 µl diluted test samples and 1 ml protein dye reagent to cuvettes

Prepare protein standards of known concentration

Add 20 µl of a series of protein standards of known concentration to cuvettes

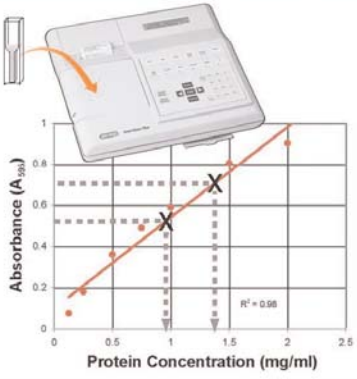


Add 1 ml protein dye reagent to each cuvette

Compare test samples to protein standards to estimate unknown concentrations by eye

Read protein standards and test samples in spectrophotometer

Generate standard curve from protein standards' absorbance data



Determine protein concentrations of test samples from the standard curve

Compare test samples' true protein concentration to published product labels

LAB 1

QUICK GUIDE

Student Manual

Starting the Lab

Materials and reagents required at the workstations prior to beginning the exercise. There should be a separate common workstation and individual student workstations.

Common workstation

Material	Quantity
Spectrophotometer	1
Absorbance data for standard curve	1

Student workstation

Material	Quantity
1x Bradford dye reagent (12 ml)	1
Microtubes with protein standards	7
Test milk samples	2
1x PBS (500 µl)	1
Microtubes for making dilutions	4
100–1,000 µl adjustable-volume micropipet	1
2–20 µl adjustable-volume micropipet	1
100–1,000 µl pipet tips	1 box
2–20 µl pipet tips	1 box
Cuvettes (or test tube substitutes)	10
Milk carton with nutrition information	1
Parafilm (small pieces to seal cuvettes)	10

Student Laboratory Activity

1. Prepare a 1:50 dilution of the milk samples using 1x PBS.
   - Label 2 microtubes
     Sample A
     Sample B
   - Pipet 196 µl PBS into the labeled microtubes
   - Add 4 µl of milk into corresponding tube, and invert to mix
2. Label cuvettes as follows:

Label	Standard (mg/ml)
blank	1x PBS
1	0.125
2	0.250
3	0.500
4	0.750
5	1.000
6	1.500
7	2.000
A	Sample A
B	Sample B

3. Invert dye reagent to mix.
    - Add 1 ml of dye reagent to each cuvette
    - Add 20 µl 1x PBS to the cuvette labeled 'blank'
    - Using a fresh tip for each sample, pipet 20 µl of each standard into the appropriate cuvette
    - Using a fresh tip for each sample, pipet 20 µl of each diluted milk sample into the appropriate cuvette
4. Cover each cuvette with parafilm.
    - Invert each cuvette 3x to mix
5. Incubate at room temperature for a period of at least 5 minutes (but not to exceed 60 minutes).
6. Visually compare the color of your unknown samples against the standards of known concentrations. A representative set of standards and a typical color spectrum are shown in Figure 6. Using the palette of standards, try to qualitatively determine to which known standard your unknown sample corresponds.
    - Examine the color of the first unknown
    - Compare it to Std. #1
    - Is it lighter or darker?
    - Compare it to Std. #2
    - Is it lighter or darker, etc.
    - Record your observations in Table 1 below. It is now time to proceed to the quantitative evaluation of the samples in the SmartSpec Plus Spectrophotometer

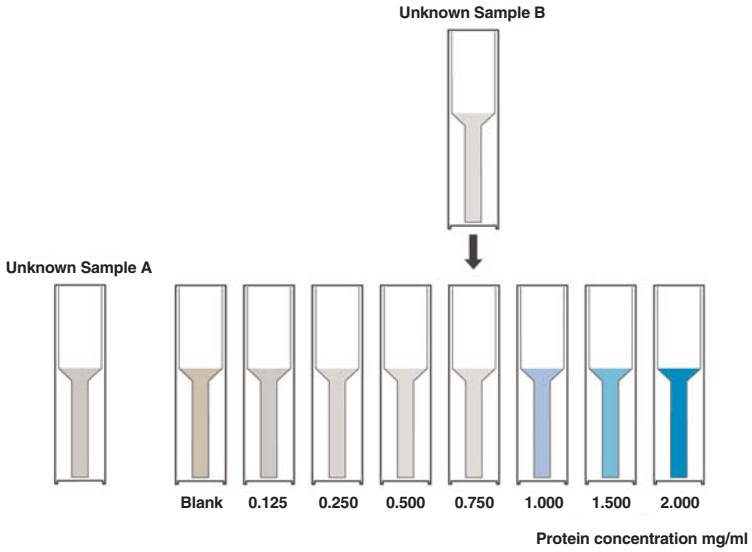


Fig. 6. A qualitative view of a Bradford standard curve

Table 1. Unknown protein concentrations

Sample	Estimated Protein Concentration (mg/ml)
Sample A	
Sample B	

Quantitative Reading of Samples in the SmartSpec Plus Spectrophotometer

1. Read samples in spectrophotometer.

Ready to read absorbance <Exit Assay > = Options

Place blank cuvette containing PBS in spectrophotometer chamber, ensuring that the spectrophotometer light path will pass through the solution in the cuvette. Close door and press "Read Blank". The value for the blank will appear on the screen.

A_{595} = 0.000 < > = continue

Press

2. Read unknown samples in spectrophotometer.

Ready to read absorbance <Exit Assay > = Options

Place test Sample A in cuvette chamber. Close door, press "Read Sample".
Read absorbance and concentration value, and press "Enter".

3. Place the next sample into cuvette chamber.

Ready to read absorbance <Exit Assay > = Options

Press "Read Sample", record data, and press "Enter".

4. Select the "Print" button to print the report. Select option #3 by pressing the numeric "3" button.

1 = Print Unprinted Samp#3 2 = Select #3 = Full Report

Select "3", and the SmartSpec Plus Spectrophotometer will print report.

5. Enter the absorbance data and the concentration values for the unknown samples in Table 2 below.

Table 2. Spectrophotometric Data for Unknown Samples.

Sample	A_{595}	Protein Concentration (mg/ml)
Sample A		
Sample B		

6. Compare your quantitative spectrophotometer determined concentrations to your qualitative estimates (entered in Table 2), and enter the data in Table 3.

Table 3. Estimated vs. Quantitated Protein Concentrations

Sample	Estimated Protein Concentration, (mg/ml) (from Table 1)	Quantitated Spectrophotometer Protein Concentration (mg/ml) (from Table 2)
Sample A		
Sample B		

Data and Analysis:

1. Record the standard curve absorbance data from the spectrophotometer report at the common workstation in Table 4.

Table 4. Standard Curve Absorbance Values

Sample	A_{595}	Concentration (mg/ml)
Std. #1		**0.125**
Std. #2		**0.250**
Std. #3		**0.500**
Std. #4		**0.750**
Std. #5		**1.000**
Std. #6		**1.500**
Std. #7		**2.000**

2. Create a standard curve by plotting the A_{595} values of the known standards (from step 1) on the y-axis versus the concentrations in mg/ml on the x-axis. Plot the data points on linear graph paper, and draw a line of best fit.
3. Read the concentration of the unknown samples by reading across from the absorbance of the unknown samples until you intersect with the standard curve and then read the concentration. Record these data in Table 5 below.
4. Adjust the final concentration of the unknown samples determined in step 4 by multiplying the concentration by the dilution factor used.

   For example, milk diluted 1:50 gives a reading of 0.224 absorbance units, which gives a concentration of ***M*** mg/ml. The final concentration of milk is ***M*** x 50 =_____mg/ml

5. Determine the final concentration of the unknown samples and record in Table 5 below.

Table 5. Final Concentration of Unknown Samples

Sample	A_{595}	Concentration Read from Standard Curve (mg/ml)	Dilution Factor	Final Concentration (mg/ml)
Sample A				
Sample B				

6. Compare your results to your estimates in Table 1, and enter the data in Table 5.
7. Find the protein content information for your milk samples on the milk carton label (look under 'Nutrition Facts'). Convert this information into mg/ml of protein.
8. Compare the values from your own standard curve with the protein content stated on the food label.

Table 6. Comparing Measured Protein Concentrations to the Values Found on Food Labels.

Sample	Bradford Assay (mg/ml) Final Concentration (from Table 5)	Food Label (mg/ml)
Sample A		
Sample B		

9. Why might your values be different from those of the manufacturer?

Extensions

1. Plot a standard curve using Microsoft Excel. Add a trend line and determine the correlation coefficient (R^2 value) and equation for the trend line. The closer the correlation coefficient is to 1.00, the better the fit to the standard curve, and the better the estimate of the concentration. Use the equation to calculate the concentrations of your unknown samples and compare to the concentration determined from your hand-plotted standard curve.
2. Have students design their own experiments using the Bradford assay. Students could assay the protein in saliva or tears, or try other food samples.
3. Students can also assay the protein content of eggs. Separate egg yolk and white into two clean beakers. Determine the mass in grams of both egg white and yolk.
    - 1 gram of egg = approximately 1 ml
    - Dilute egg 100x with PBS
4. If you would like to expand your lab curriculum in proteomics, here are some other labs that may be of interest:
    - Comparative proteomics kit I: protein profiler (166-2700EDU)
    - Comparative proteomics kit II: western blot module (166-2800EDU)
    - Green fluorescent protein chromatography kit (166-0005EDU)
    - Secrets of the Rainforest™ kit (166-0006EDU)
    - Size exclusion chromatography kit (166-0008EDU)
    - ELISA Immuno Explorer™ kit (166-2400EDU)
5. Analyze the proteins in different milk samples by performing SDS-PAGE electrophoresis.
    - After quantitating the protein content in milk, dilute the protein samples to 1 mg/ml in Laemmli buffer. Electrophorese 20 µg, 10 µg, and 5 µg of total protein on a gel, and try to pick out the main protein bands in the different samples. In the gel in Figure 7, four different types of milk samples were analyzed: fat free, low fat, whole, and soy milk.
    - The most abundant protein in milk samples from bovines is casein, which migrates at ~25 and 28 kD (denoted with an "*" in Figure 7). Because casein is highly allergenic, an alternative milk source for those individuals allergic to casein is soy milk, which is derived from plant sources. Note that soy milk does not contain the prominent casein band, but instead contains several proteins unique to soy (denoted with “S”).

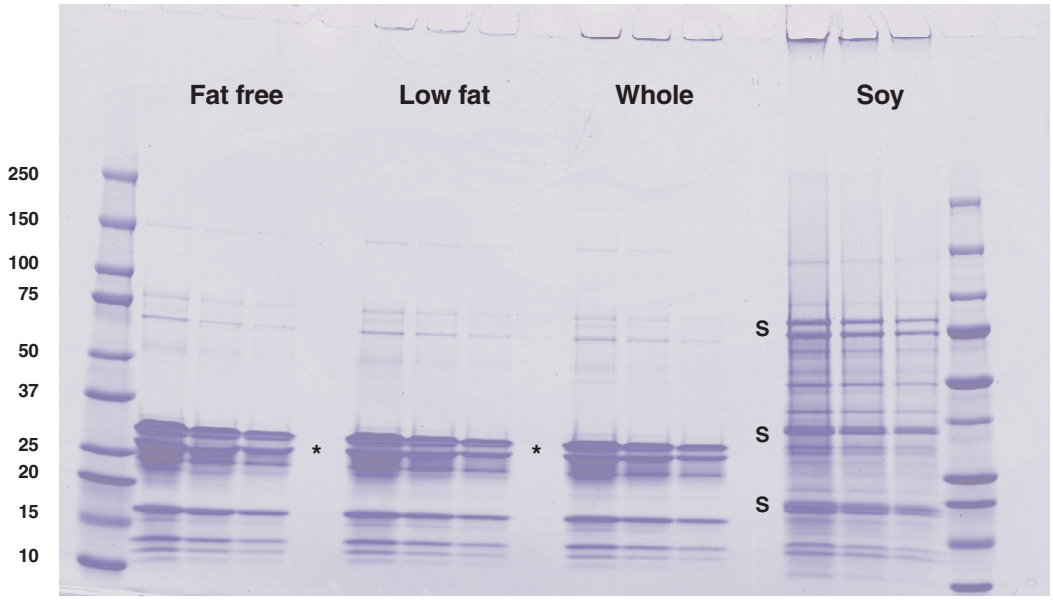


Fig. 7. Electrophoretic analysis of proteins from milk samples.

FAQs

1. Is the Bradford 1x dye reagent light sensitive?
   - The reagent is not light sensitive.
2. How long can the samples sit before being read?
   - The samples can be read up to one hour after preparation.
3. Do bubbles on the surface of the sample affect the reading?
   - No. The light path passes through the lower portion of the cuvette.
4. Do fingerprints on the cuvette affect the reading?
   - Yes. Grasp the upper part of the cuvette, as shown in the photo in Figure 5. Do not touch the optically clear face of the cuvette. Bio-Rad cuvettes have ridged sides for handling.

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