

Experiments with Protein: The Bradford Assay

OVERVIEW

In the Preliminary Activity, your students will use a spectrophotometer and the Bradford protein assay to determine the protein content of nonfat milk. For more thorough background information, see the Background for Instructors section of the Instruction Manual accompanying the Got Protein? Kit (available from Bio-Rad Laboratories, Inc., #166-2900EDU). A student handout for the Open Inquiry version of the Preliminary Activity can be found at the end of this investigation. A Guided Inquiry version can be found on the CD accompanying this book.

During the subsequent Inquiry Process, your students will first find out more about protein using the course textbook, other available books, and the Internet. They will then generate and investigate researchable questions. (In the Guided Inquiry approach, students will plan and conduct investigations of the researchable question(s) assigned by you.)

LEARNING OUTCOMES

In this inquiry investigation, students will

- Identify variables, design and perform the investigation, collect data, analyze data, draw a conclusion, and formulate a knowledge claim based on evidence from the investigation.
- Create a standard protein curve using the Bradford protein assay.
- Determine the protein concentration of milk.
- Determine the protein concentration of other foods.

THE INQUIRY PROCESS

Suggested Time to Complete the Investigation

See page xiii in the Doing Inquiry Investigations section for more information on carrying out each phase of an inquiry investigation.

	Inquiry Phase	Open Inquiry	Guided Inquiry
I	Preliminary Activity	40 minutes	40 minutes
II	Generating Researchable Questions (Omitted in Guided Inquiry Approach)	10 minutes	0 minutes
III	Planning	10 minutes	10 minutes
IV	Carrying Out the Plan	40 minutes	40 minutes
V	Organizing the Data	10 minutes	10 minutes
VI	Communicating the Results	10 minutes	10 minutes
VII	Conclusion	5 minutes	5 minutes

MATERIALS

Make the following materials available for student use. Items in bold are needed for the Preliminary Activity.

data-collection program
SpectroVis Plus spectrophotometer*
eight 1.5 mL cuvettes with lids
20–200 μ L micropipet**
100–1000 μ L micropipet**
200 μ L micropipet tips (1 box)
1000 μ L micropipet tips (1 box)
two 1.5 mL microtubes

15 mL centrifuge tube
Quick Start™ Bradford Dye Reagent
Phosphate buffered saline (PBS)
Bovine γ -globulin standard set
nonfat milk
lint-free tissue
 others as requested by students

* Other Vernier-compatible spectrophotometers can be used (e.g., Vernier Spectrometer, Ocean Optics Red Tide)

** Appropriate graduated transfer pipets (1 and 5 mL) may be substituted.

I Preliminary Activity

This inquiry begins with an activity to reinforce prior knowledge of the use of Vernier data-collection technology and to introduce a method for determining protein concentration.

Sample Results

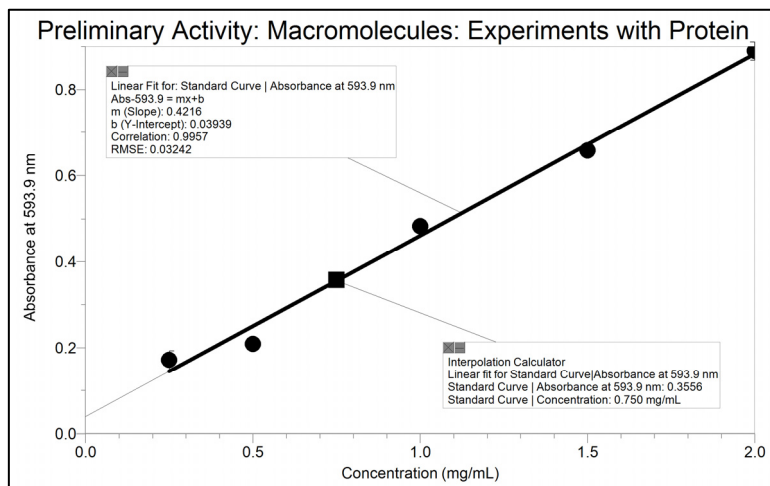


Figure 1 Using the Bradford protein assay to determine protein content of nonfat milk

Answers to the Questions

1. Determine the actual protein concentration of your milk sample. Remember that you diluted your original milk by a factor of 50 before conducting the Bradford protein assay.

Answers will vary. $0.750 \text{ mg/mL} \times 50 = 37.5 \text{ mg/mL}$

2. Obtain the published protein value for the milk sample from your instructor. This value is typically found on the nutrition label of the milk container. Convert the published value to mg/mL of protein.

The observed protein values should be within 3 mg/mL of the published protein concentrations. One reason why they may not match exactly is that the published values represent the average protein concentration from multiple tests.

3. List three protein-containing liquids whose protein concentration might be determined using this protocol.

Some protein-containing liquids whose protein concentration might be determined with this protocol include nonfat milk, low-fat milk, whole milk, soy milk, rice milk, soy sauce, whipping cream, yogurt, half and half, high protein shake, bean protein, casein protein, egg protein, and whey protein.

4. List at least one researchable question concerning protein concentration.

Answers will vary. See the Researchable Questions list below for some possible answers.

II Generating Researchable Questions

Note: Researchable questions are assigned by the instructor in the Guided Inquiry approach. See page xiii in the Doing Inquiry Investigations section for a list of suggestions for generating researchable questions. Some possible researchable questions for this investigation are listed below:

Recommended for Open Inquiry or Guided Inquiry (sample results provided)

- How do the protein contents of various milk products compare?
- How do the protein contents of various “milks” (non-dairy and cow milks) compare?
- How do the protein contents of various high protein shakes compare?

Recommended for Open Inquiry or Guided Inquiry (sample results not provided)

- What is the protein concentration in an instructor-prepared unknown?
- How do the protein contents of nonfat, low-fat, and whole milk compare?
- How do the protein contents of casein protein, egg protein, and whey protein drinks compare?
- How do the protein contents of bean protein, hemp protein, and pea protein drinks compare?

Recommended for Advanced Students (sample results not provided)

- Can the protocol be modified to determine the protein content of solid foods?
- How do results using a nonfat milk protein standard curve compare to those using a bovine γ -globulin standard curve?

There are many more possible researchable questions. Students should choose a researchable question that addresses the learning outcomes of your specific standards. Be sure to emphasize experimental control and variables. (Instructors using the Guided Inquiry approach select the researchable questions to be investigated by their students. We encourage you to assign multiple researchable questions because this strategy enhances student interaction and learning during phases IV–VII.)

III Planning

During this phase students should formulate a hypothesis, determine the experimental design and setup, and write a method they will use to collect data. The plan should list laboratory safety concerns and specify how they will be addressed during the investigation. Circulate among the student groups asking questions and making helpful suggestions.

IV Carrying Out the Plan

During this phase, students use their plan to carry out the investigation and collect data. Circulate among the student groups asking questions and making helpful suggestions.

V Organizing the Data

See page xv in the Doing Inquiry Investigations section for suggestions concerning how students can organize their data for their inquiry presentations.

VI Communicating the Results

See page xv in the Doing Inquiry Investigations section for a list of inquiry-presentation strategies.

VII Conclusion

Using your notes recorded during the Communicating the Results phase, summarize the group results for the experiment and tell how they will fit into the upcoming instruction.

VIII Assessment

See page xv in the Doing Inquiry Investigations section for ideas on assessment strategies.

SAMPLE RESULTS

Student results will vary depending on experimental design.

Comparing the Protein Content of Milk Products

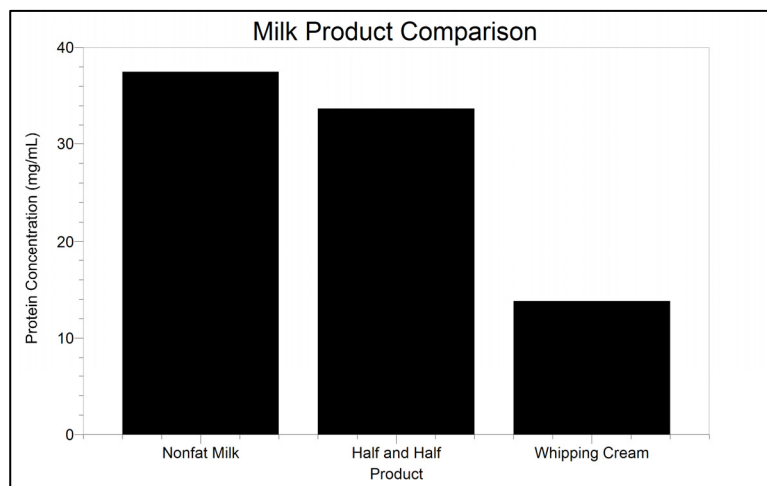


Figure 2 *Protein concentrations of milk products*

Table 1: Protein Content of Various Milk Products			
Sample	Protein conc. (mg/mL)	Actual protein conc. (mg/mL)	Conc. on label (mg/mL)
Nonfat milk	0.750	37.5	38.0
Half and Half	0.673	33.7	33.3
Whipping cream	0.276	13.8	0

This investigation addresses the question, “How do the protein contents of various milk products compare?” These data were collected using the procedure from the Preliminary Activity.

Of the milk products tested, nonfat milk had the highest protein concentration.

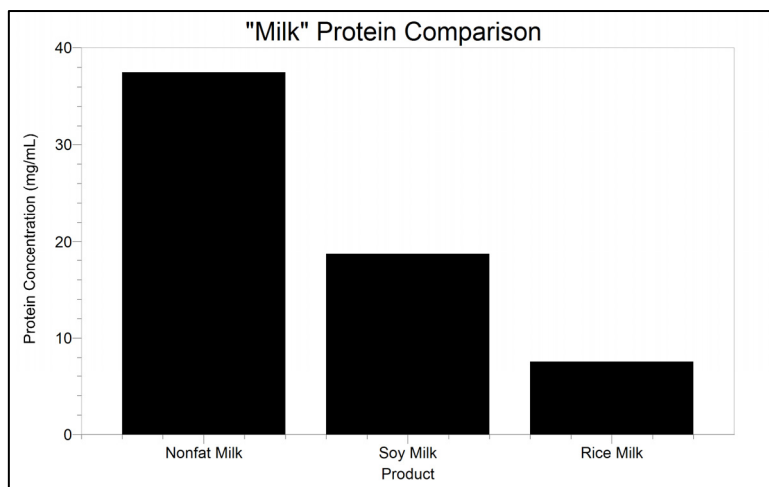
Comparing the Protein Content of Various “Milks”

Figure 3 *Protein concentrations of nonfat milk, soy milk, and rice milk*

Table 2: Protein Content of Nonfat Milk, Soy Milk, and Rice Milk			
Sample	Protein conc. (mg/mL)	Actual protein conc. (mg/mL)	Conc. on label (mg/mL)
Nonfat milk	0.750	37.5	38.0
Soy milk	0.374	18.7	25.0
Rice milk	0.152	7.6	8.3

This investigation addresses the question, “How do the protein contents of various “milks” compare?” These data were collected using the procedure from the Preliminary Activity.

Of the products tested, nonfat milk had the highest protein concentration. The measured protein concentrations were generally of the magnitude indicated on the product label.

Comparing the Protein Content of High Protein Shakes

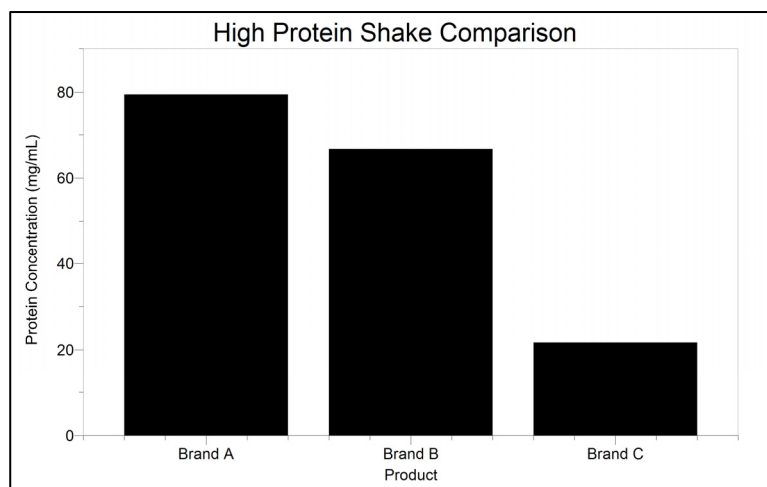


Figure 4 Protein concentrations of three high protein shakes

Table 3: Protein Content of Various High Protein Shakes			
Sample	Protein conc. (mg/mL)	Actual protein conc. (mg/mL)	Conc. on label (mg/mL)
Brand A	1.590	79.5	75.0
Brand B	1.341	67.1	66.6
Brand C	0.434	21.7	37.5

This investigation addresses the question, “How do the protein contents of various high protein shakes compare?” These data were collected using the procedure from the Preliminary Activity.

For the high protein shakes tested, the range of actual protein concentration determined was 21.7–79.5 mg/mL. Once again, there was a good correspondence between actual protein concentration and protein concentration specified on the product labels.

TIPS

- The instructions provided assume that you have purchased the “Got Protein? Kit” from Bio-Rad Laboratories, Inc. (explorer.bio-rad.com, Catalog # 166-2900EDU).
Additional cuvette lids are available from Vernier (order code: CUV-LID).
- To prepare the Bradford Reagent:
 - Remove the Quick Start Bradford reagent from proper storage (4°C) just before use.
 - Invert the bottle several times and then pour 20 mL of reagent into a beaker on ice. This should be enough for 1–2 groups.
 - You can also choose to aliquot the reagent into 15 mL centrifuge tubes. In this case, give a centrifuge tube of Bradford reagent to each group when the lab starts.

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3. To prepare the 1x PBS (phosphate buffered saline) solution:
 - a. Remove the 10x PBS solution from proper storage (4°C).
 - b. Add 10 mL of 10x PBS solution to 90 mL distilled water.
 - c. Store in a capped container at 4°C. Phosphate buffer can be stored for a week at this temperature.
4. To prepare the protein standards:
 - a. The protein standards come in seven pre-mixed and labeled microtubes.
 - b. To avoid contamination, aliquot 40 µL of each standard into a separate microtube.
 - c. Provide each group with a full set of protein standard aliquots.
5. The Preliminary Activity directs students to use the Interpolation Calculator feature of the software to determine the concentration. If you wish to include more mathematics in this investigation, your students could calculate the concentration using the equation for the linear fit

$$y = mx + b$$

where x is concentration, y is absorbance, m is the slope, and b is the y -intercept.

For example, suppose the data-collection program displays the following information for the linear fit: $y = 0.3556$, $m = 0.4216$, $b = 0.03939$, and that the concentration units are mg/mL. Upon substituting into the equation $y = mx + b$ you obtain the following solution.

$$0.3556 = 0.4216x + 0.03939$$

$$x = 0.750 \text{ mg/mL}$$

Multiplying by 50 due to the 50x dilution gives the following concentration.

$$0.750 \text{ mg/mL} \times 50 = 37.5 \text{ mg/mL}$$

6. HAZARD ALERT:

Quick Start Bradford Dye Reagent: Contains methanol and phosphoric acid. Hazard code Xn, Harmful. Eye irritant; may be harmful if inhaled, toxic by ingestion, harmful if absorbed through skin. Wear gloves and eye protection.

The hazard information reference is: Bio-Rad Laboratories Inc, MSDS for product 5000205, 800-424-6723, www.bio-rad.com.

7. More information about the sensor used in this Investigation, as well as tips for optimal performance, can be found in the sensor's user manual available for download from the Vernier web site, www.vernier.com/sensors.
8. The plans that your students submit for approval should list laboratory safety concerns, including chemical safety concerns, and specify how they will address these safety concerns during their investigations.

This investigation was adapted from Bio-Rad Laboratory's Got Protein? Kit. Text and figures are used with permission.