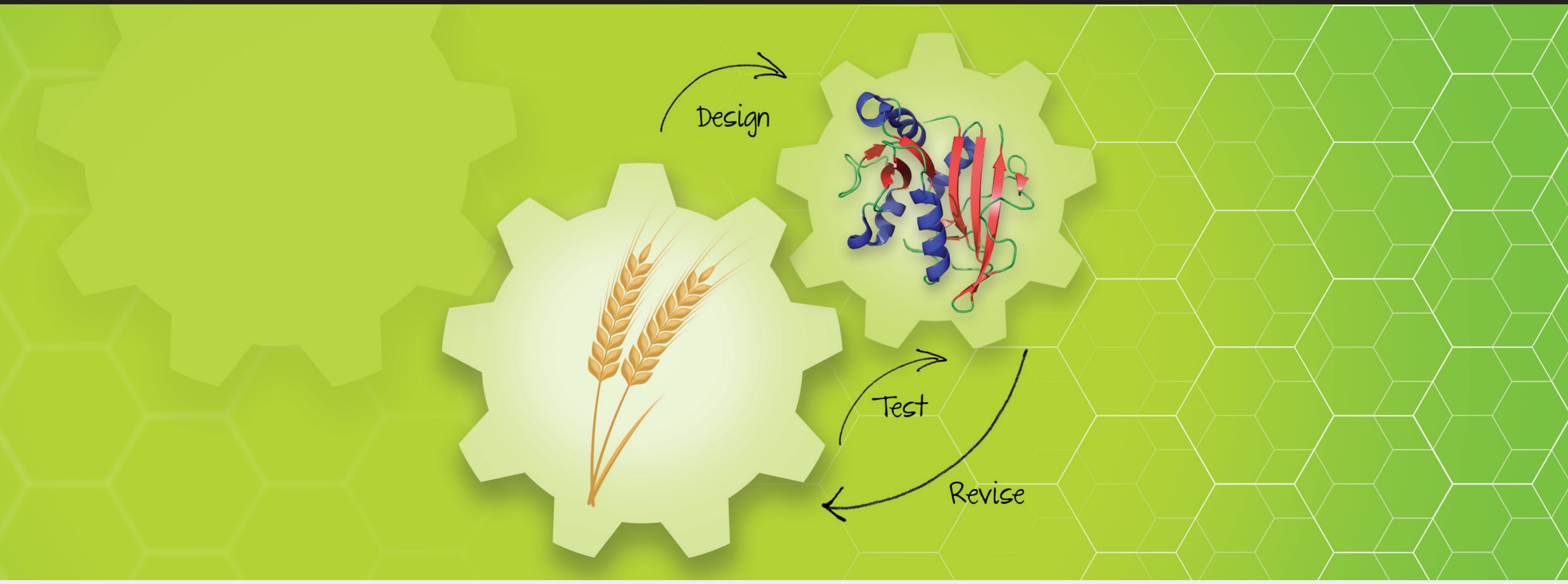
BIO-RAD Explorer



Engineering Solutions for Global Health Kit

Catalog #17005278EDU

Instructor's Guide

Note: Duplication of any part of this document is permitted for classroom use only.

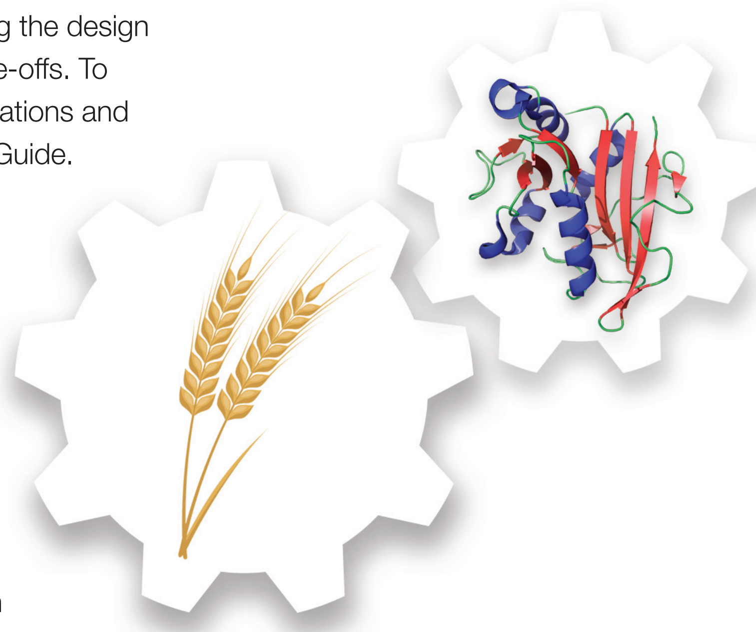
For technical services, call your local Bio-Rad office, or in the U.S., call 1-800-424-6723.

BIO-RAD

Dear Instructor

Thank you for fostering curiosity in future scientists and citizens as they prepare for an exciting future in which they will be required to use creative and critical thinking, problem solving, and effective communication and collaboration.

This curriculum guides students through the practices of defining a global problem, designing solutions, and engaging in argument from evidence. They will work with the engineering ideas of defining engineering problems, developing possible solutions, and optimizing the design solution by considering constraints and trade-offs. To facilitate your role with your students, explanations and lesson plans are included in the Instructor's Guide.



The Engineering Solutions for Global Health Kit presents students with the challenge of world hunger. Students define the problem of world hunger with respect to protein-energy undernutrition (PEU). With a colorimetric assay that uses Bradford reagent and a set of protein standards, students test the protein content of a set of liquids being considered for inclusion in a treatment proposal for PEU. Students then consider constraints and outcomes as they develop an initial treatment proposal, revise it, and construct a final treatment proposal in the form of an evidence-based argument.

We strive to continually improve our curriculum and products, and your input is extremely important to us. We welcome your stories, comments, and suggestions.

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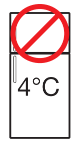
Contents

Kit Storage

When you receive the Engineering Solutions for Global Health Kit:

1. Put the Bradford 1x Dye Reagent and Protein Standard II (lyophilized BSA) in the fridge (4°C). DO NOT FREEZE.



2. Visit bio-rad.com/worldhunger to download the Student Guide.

Note: Do not discard the packing material until you have all of the kit components, as they may be shipped in separate boxes.

Safety Guidelines

Bradford reagent contains phosphoric acid. Wear appropriate Personal Protective Equipment (PPE) (including gloves) at all times when handling Bradford reagent. Read and be familiar with the Safety Data Sheet (SDS) for this activity, which is available from Bio-Rad by calling 1-800-4BIORAD (424-6723) in the U.S. Visit bio-rad.com for further information on reagents in this kit. The reagents and other materials used in the activity should be disposed of in accordance with local regulations.

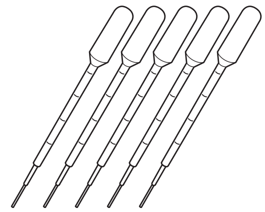
Kit Components and Ordering Information

Each kit contains materials to outfit 8 student workstations.

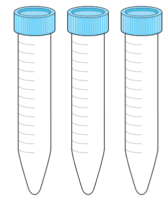
Kit Components	Quantity
Quick Start Bradford 1x Dye Reagent	1 L
Protein Standard II (lyophilized BSA)	1
Disposable Plastic Transfer Pipets, nonsterile	500
Conical Centrifuge Tubes, 15 ml	100
Instructor's Guide	1
Answer Guide	1

Required Materials (not included in this kit)	Quantity
Distilled water	1 L
150 ml beaker	16
250 ml graduated cylinder	1
Tube rack, or cups/beakers to hold 15 ml conical tubes	8
Protein liquids, at least four types (see sample protein liquids in Advance Preparation)	At least 1 ml of each

Ordering Information	
5000205EDU	**Quick Start Bradford 1x Dye Reagent**
5000007EDU	**Protein Standard II** (lyophilized BSA)
1660480EDU	**Disposable Plastic Transfer Pipets**, pkg of 500 nonsterile
1660475EDU	**Conical Centrifuge Tubes**, pkg of 50, 15 ml
1660483EDU	**Tube racks**, pkg of 5, 15 ml (optional)
1660484EDU	**Tube racks**, pkg of 5, 50 ml (optional)

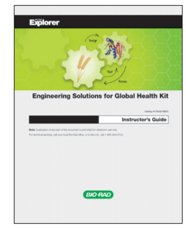


Disposable plastic transfer pipets



Conical centrifuge tubes

Quick Start Bradford 1x Dye Reagent and Protein Standard II (lyophilized BSA)



Instructor's Answer Guide

Instructor's Guide

Kit Activity Overview

Activity 1

Understanding Protein-Energy Undernutrition

Defining a problem

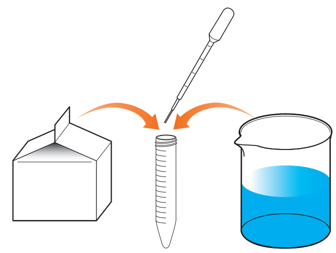
Students use prior knowledge to ask questions and generate ideas about data on global nutrition and summarize the problem of protein-energy undernutrition (PEU).

Activity 2

Investigating Protein Content in Food

Investigating a solution to a problem

Students learn about the Bradford assay. Students discuss how the Bradford assay can be used as a tool to develop a treatment proposal to address PEU. Students conduct an investigation to test liquids for possible inclusion in the treatment proposal.



Activity 3

Designing a Treatment Proposal for Protein-Energy Undernutrition

Designing, testing, and revising a solution to a problem

Students use the evidence from their research and investigation to design an initial treatment proposal. They consider constraints and outcomes from testing, and revise their initial proposal. Students write a final treatment proposal in the form of an evidence-based argument.

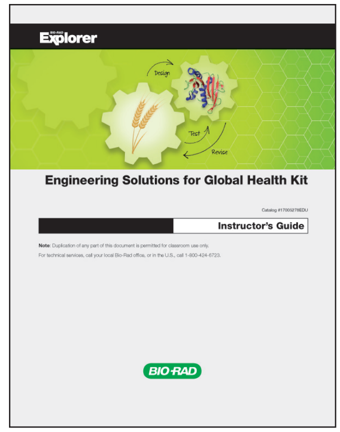
Post-investigation questions

Optional post-investigation questions guide students to apply their understanding from the investigation.

Roadmap to the Instructor's and Student Guides

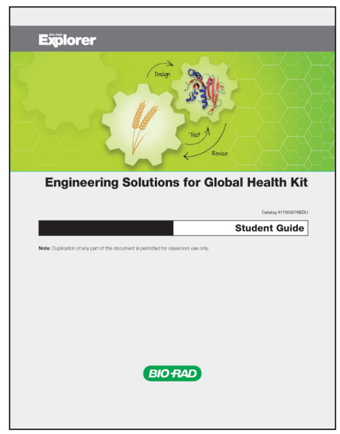
The **Instructor's Guide** is written to provide you, the instructor, with sufficient background information and planning tools for you to facilitate the student learning experience. It is designed to be used with or without the Student Guide and provides all the information and lesson details necessary to complete the activities. The Instructor's Guide includes:

- An **overview of the kit** with information about how the kit activities have been designed to support student learning and questions to help you guide your students
- An overview of the **science and engineering practices, engineering ideas, and crosscutting concepts** that are supported by the activities
- **Timeline and Advance Preparation Instructions**
- **Lesson plans** with step-by-step classroom instructions that can be used to prepare for instruction or used in class during the activity
- **Appendices** with student materials, supplemental activities, and optional student background information



Instructor's Guide

The **Student Guide** includes a framework of questions, prompts, and charts to guide student thinking. In-depth instructions and background are intentionally omitted or found only in the Instructor's Guide to foster students' use of prior knowledge and sense-making.



Student Guide

The **Instructor's Answer Guide** includes examples of how students may respond to the prompts and questions presented in the Student Guide. However, most of the prompts in the Student Guide do not have "correct" answers and are provided as a space for your students to record and develop their ideas and understanding.

Instructor's Answer Guide

Curriculum Fit and Inquiry Support

Curriculum Fit and Topic/Concept Connections

The Engineering Solutions for Global Health Kit focuses on the following science and engineering practices:

- Defining problems
- Designing solutions
- Using mathematics and computational thinking
- Engaging in argument from evidence

Students will work on the practice of defining problems when they use prior knowledge to ask questions and generate ideas about data on global nutrition, and summarize the problem of PEU. Students will practice designing solutions as they use the evidence from their research and investigation to design an initial treatment proposal. Students will engage in the practice of argument from evidence as they write a final treatment proposal.

The kit focuses on the following engineering ideas:

- Defining engineering problems
- Developing possible solutions
- Optimizing the design solution

Students will practice defining engineering problems as they consider constraints and the possible trade-offs of the constraints for their treatment proposal to address the global issue of PEU. Students will develop a treatment proposal solution that takes into account the constraints they considered such as cost, safety, and reliability.

The kit incorporates the following science crosscutting concept:

- Cause and Effect

Students will practice using the concept of cause and effect as it relates to nutrition. A deficiency in nutrients such as protein can cause physical effects including stunting and wasting. Depending on their design and effectiveness, nutrition treatments can lead to patient outcomes of improvement, no improvement, or death. Students will also think about the concept of cause and effect during the Bradford assay. Bradford is a reagent that appears brown in color. The binding of certain amino acids to the reagent causes a blue color change.

The kit also provides the opportunity for students to make interdisciplinary connections to:

- Geography/history
- English Language Arts

Visit bio-rad.com/worldhunger for more information about curriculum standards.

Connections to Prior Knowledge

The Engineering Solutions for Global Health Kit builds conceptually on the topics and ideas of photosynthesis and cellular respiration. During a unit on photosynthesis and cellular respiration, students learn that plants and algae produce glucose through photosynthesis. Glucose is a form of stored chemical energy. Animals consume the glucose produced by plants. Bio-Rad Explorer has a kit that addresses these ideas, the Photosynthesis and Cellular Respiration Kit for General Biology (12005534EDU).

The idea below can follow those of photosynthesis and cellular respiration:

- Organization of Matter and Energy in Organisms: Sugar molecules are made up of hydrogen, carbon, and oxygen; their hydrocarbon backbones are used to make amino acids and other carbon-based molecules that can be assembled into larger molecules, such as proteins

If you choose to use the Photosynthesis and Cellular Respiration Kit for General Biology, before starting the Engineering Solutions for Global Health Kit, consider a transition from photosynthesis and cellular respiration by asking students, what makes up the food we eat? What happens if we eat too much or not enough food? (See Appendix A for more information to support this teaching transition.)

Tell students they will be learning more about proteins in food as they define a problem, then investigate and create a solution to an issue related to world hunger in the Engineering Solutions for Global Health Kit.

A Focus on Engineering

In the Engineering Solutions for Global Health Kit, students use a problem solving process to define the problem of undernutrition in world hunger and develop a solution in the form of a treatment proposal. The process engages students in the engineering practice of problem solving, including creative thinking and communication.

The kit focuses on the following phases of engineering and problem solving: define, test, design, and revise. In the define phase, students make observations and ask questions as they explore data in order to define a problem. Then students use a technological tool to gather evidence about the protein content of liquids to design an initial treatment proposal and consider constraints in their design. Students then simulate a testing process and use the outcomes of the testing to revise their solution. Students propose a final solution to the problem in the form of a scientific argument.

You may find it useful to prompt students with the questions on the following page in order to engage them in each phase. These questions can be used with your students at any point in the curriculum and may guide your thinking as you support students' learning about the application of the Bradford assay to help solve a problem they have defined. These are open-ended prompts with no correct answers. Specific answers are not provided in order to foster the authentic development of your students' thinking using their diverse ideas and prior knowledge in your classroom discourse.

Engineering Phase	Suggested Questions and Prompts to Support Student Learning and Discussion	Kit Specific Applications
Define	**Making observations and asking questions to define a problem** What do you see? What do you know about what you see? What patterns do you observe? What do you wonder about what you see or the patterns you observe? Why do you think those patterns exist?	Exploring data and images about nutrition (See References on page 27)
	Useful references for investigating world hunger and defining the problem of PEU: • Food security in the U.S. Interactive Charts and Highlights • Global Nutrition Report • The State of Food Security and Nutrition in the World 2017 • Levels and Trends in Child Malnutrition	
Test and Design	**Testing and designing solutions to a problem** How can the Bradford assay be helpful for developing a protein-energy undernutrition treatment proposal?	Testing protein liquids with Bradford reagent, designing an initial treatment proposal, and considering constraints
Revise	**Revising solutions to a problem** What are the outcomes of the testing of your initial treatment proposal? What changes or improvements can you make to the design based on the testing?	Testing and refining an initial proposal

Background for Instructors

World Hunger and Undernutrition

Hunger is a global issue, and the trends indicate it will only become more challenging. The World Health Organization (WHO) estimates that there are over 800 million people globally who are chronically undernourished, meaning their caloric (dietary energy) intake is insufficient to meet minimum energy requirements. In the United States, over 40 million Americans live in food-insecure homes. The members of a food-insecure home are uncertain of having or unable to secure enough food to meet their needs during some time of the year.

Proper nutrition is essential for human health, and consists of a diet made up of protein, carbohydrates, and fat. Protein-energy undernutrition (PEU) is a primary diagnosis of undernourishment in children. Two indicators of PEU are stunting and wasting. Stunting is the failure to grow physically and cognitively as a result of recurrent undernourishment. Wasting is the effect of rapid weight loss over a short period of time or the failure to gain weight. Globally, over 100 million children under the age of five experience stunted growth and over 50 million experience wasting.

In these activities, students will define the problem of PEU. Students will use a Bradford assay to quantify the protein content of liquids for possible inclusion in a treatment proposal. Students create an initial treatment proposal, then consider constraints and revise their proposal based on outcomes. They will create a final evidence-based treatment proposal for PEU.

Tips & Tricks

Protein-energy undernutrition (PEU) is a specific set of conditions characterized by deficient calorie and/or protein intake.

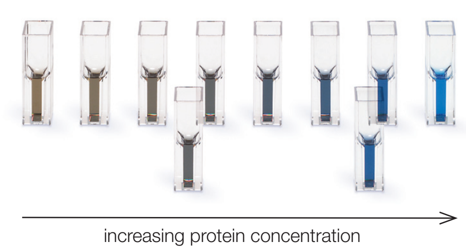
Biochemistry of Hunger

Animals eat food in order to obtain energy in the form of glucose. During metabolic processes, glucose gets broken down into chemical energy called adenosine triphosphate (ATP). There are three metabolic states: well fed, fasting, and starvation. The table below summarizes the basic biochemistry of each state.

Well fed	Fasting	Starvation
• Plenty of glucose • Excess glucose is stored as glycogen in the liver	• Glucose is used up in one day • Gluconeogenesis in the liver converts amino acids, fats, and lactate into glucose	**First three days:** • Muscle is broken down for amino acids to produce glucose in liver through gluconeogenesis **After 3 days:** • Severe muscle wasting • Ketone bodies are made from the breakdown of fatty acids to provide energy for the brain and heart

Bradford Assay

The Bradford assay is a colorimetric method for detecting the presence and concentration of total protein content in a sample. This assay cannot distinguish between types of proteins because it reacts with particular amino acids that are found in nearly all proteins. Bradford reagent appears brown and then turns blue in the presence of protein. It can be used qualitatively (brown or blue color) to detect whether a sample has protein or not. Alternatively, a spectrum of colors from brown to gray to blue can be formed with standards that have a known range of protein concentrations. The protein concentration of a sample can be determined by comparing its color (with Bradford added) to the spectrum of colors of the standards with a known range of protein concentrations.



Example Preparation Schedule

Use this example schedule as a reference for planning the activities and advance prep over 6 days. Refer to Advance Preparation Instructions on page 11 for preparation details.

	Before the activity	Activity
Monday	• Decide if student questioning will focus on global and/or local data, including food insecurity in the United States • Collate resources (paper and/or digital) for data, images, and other information about world hunger to drive student questioning	Activity 1, Understanding Protein-Energy Undernutrition
Tuesday	No prep	Activity 2, Investigating Protein Content in Food, first day: Using scientific data to design an evidence-based solution
Wednesday	• (Optional) Cue the Using a Transfer Pipet video (See References on page 27 for link) • Copy and cut the Recommended Daily Allowance (RDA) cards (see Appendix C). Cut the chart into single cards that can either be assigned or randomly distributed to each group or distribute the RDA requirement in chart form and tell each group to mark the requirement(s) they are assigned. • Purchase protein liquids students decided to test, or assign students to bring them **Note:** Decide if students will test a distinct range of concentrations or if it is acceptable for some of the liquids to have similar concentrations (see the example liquids suggested in Advance Preparation) **Note:** Do not discard the food labels and cost information. Remind students to bring the labels and cost information for liquids they provide	Activity 2, Investigating Protein Content in Food, second day: Developing a solution
Thursday	• Prepare the liquid samples if teacher-prepared • Record the price per volume in ml of protein liquids • Prepare the BSA standards • Set up workstations	Activity 2, Investigating Protein Content in Food, third day: Conducting the investigation
Friday	• Read "Scientific Practice for Activity 3: Engaging in Evidence-Based Argumentation" • Copy and cut the Constraints cards (see Appendix C). Cut the chart into single cards or distribute the Constraints in chart form and tell each group to mark the constraints they are assigned • Copy the Patient Outcomes Chart (See Appendix C) for each student workstation	Activity 3, Designing a Treatment Proposal for Protein-Energy Undernutrition, first day: Designing and testing an initial treatment proposal
Monday	No prep	Activity 3, Designing a Treatment Proposal for Protein Undernutrition, second day: Revising and finalizing an initial treatment proposal

Advance Preparation Instructions

The materials are for eight workstations of four students.

Required Materials (not included in this kit)	**Quantity**
Distilled water	1 L
150 ml beaker	16
250 ml graduated cylinder	1
Tube rack, or cups/beakers to hold 15 ml conical tubes	8
Protein liquids, at least four types, (see example protein liquids below)	At least 1 ml of each

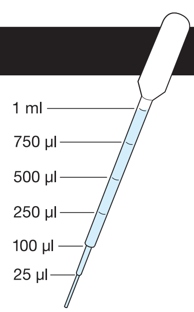
Example Protein Liquids	**Approximate protein concentration**
Almond beverage	1 g/240 ml (or ~1 cup)
Whole cow's milk	8 g/240 ml (or ~1 cup)
Nonfat dry milk (reconstituted)	8 g/240 ml (or ~1 cup)
Nonfat cow's milk	9 g/240 ml (or ~1 cup)
Protein drink	20 g/350 ml (or~1½ cups)

Optional Materials (not included in this kit)	**Quantity**
50-ml beaker for rinsing plastic pipets (if reusing)	9

Tips & *Tricks*

All calculations should be done using metric units.

The volumes for the protein liquids should be recorded in ml.



Prior to Activity 2, Day Three:

Resuspend the freeze-dried BSA

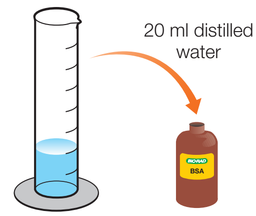
1. Add 20 ml distilled water directly to the amber vial. Recap the vial with the rubber stopper and invert gently to mix.

   Refer to the label on the vial and copy the final concentration of the BSA solution provided, ___________ mg/ml.

   Note: The lyophilized BSA is very powdery. Be sure to gently pour the water into the vial to prevent the BSA from clouding up and out of the vial.

   Note: Store the rehydrated BSA at 4°C for up to 4 months. However, for longer than 2 months, store at –20°C for up to 1 year.

   Each vial of BSA has a specified range of concentration of 1.2–1.6 mg/ml. For the purposes of the BSA standards in this activity, and in order to streamline the preparation of the standards, use an approximation of the concentration of BSA in the vial as **1.6 mg/ml**.



Tips & Tricks

If you wish to follow the exact concentration of BSA given on the vial, modify step 1 in "Prepare the four BSA stock solutions" and label the tubes accordingly:

S1: Use the concentration provided on the BSA bottle.

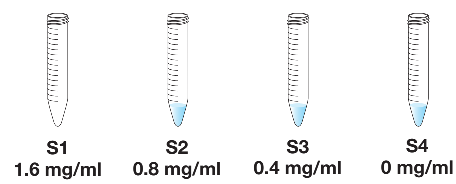
S2: Divide the concentration of S1 by 2.

S3: Divide the concentration of S2 by 2.

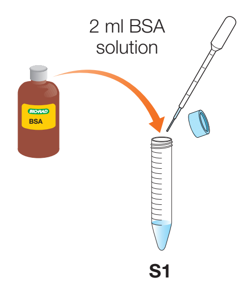
Proceed as written.

Prepare the four BSA standard stock solutions

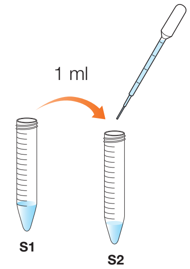
1. Label four 15-ml tubes **S1: 1.6 mg/ml**, **S2: 0.8 mg/ml**, **S3: 0.4 mg/ml**, and **S4: 0 mg/ml**. Add 1 ml distilled water to tubes **S2**, **S3**, and **S4** (do not add distilled water to **S1**).



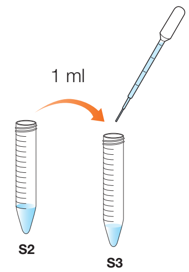
2. Label a clean plastic pipet **BSA** and use it to add 2 ml of the BSA solution from the bottle to tube **S1**.



3. Use the **BSA** plastic pipet to take 1 ml of solution from tube **S1** and add it to tube **S2**. Cap the tube and invert to mix.



4. Use the **BSA** plastic pipet to take 1 ml of solution from tube **S2** and add it to tube **S3**. Cap the tube and invert to mix.

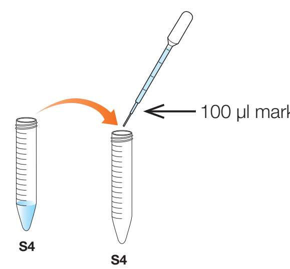


Prepare eight sets of BSA standards for student workstations

1. (Optional) Label a 50 ml beaker **Rinse** and fill it approximately half full with distilled water for rinsing pipets.

2. Label eight 15 ml tubes **S4: 0 mg/ml**, eight tubes **S3: 0.4 mg/ml**, eight tubes **S2: 0.8 mg/ml,** and eight tubes **S1: 1.6 mg/ml**.

3. Use a clean plastic pipet to add 100 µl of stock **S4** to each student tube **S4**. **Note:** Be sure to maintain pressure on the pipet bulb once the liquid has been drawn up to avoid the small volume from being drawn into the shaft or bulb.



Tips & *Tricks*

If the small volume of liquid gets drawn into the pipet shaft or bulb:

- Gently tap the pipet tip on the pipet packaging or other nonporous surface and re-draw any liquid
- If tapping does not work, expel as much liquid form the pipet as possible and start over

4. Using the same plastic pipet and rinsing each time, repeat Step 3 for student tubes **S3**, **S2**, and **S1**. To rinse, gently squeeze the bulb then place the pipet in a beaker of distilled water for rinsing, release the bulb to draw water up into the pipet, and squeeze the bulb to expel the water.

Prepare eight sets of sample protein liquids. Alternatively, you may wish to have students bring and prepare their own samples. Instruct students accordingly.

1. (Optional) Label eight 50 ml beakers **Rinse** and fill each beaker approximately half full with distilled water for students to rinse pipets.

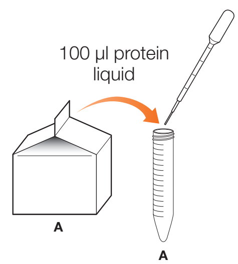
2. Label eight 15 ml tubes **A**, eight tubes **B**, eight tubes **C**, and eight tubes **D**. Label the protein liquid containers A, B, C, and D and record the protein liquid contents for each.

Tips & Tricks

Consider covering the nutritional information on the protein liquid containers so students can't read the protein content from the containers during their investigation.

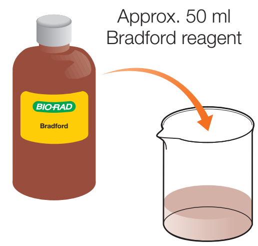
Sample	Protein liquid contents
A	
B	
C	
D	

3. Use a new pipet to transfer 100 µl of protein liquid A into each tube **A**. Clean the pipet by rinsing in water at least two times. Repeat for protein liquids **B**, **C**, and **D**.



Prepare Bradford reagent

1. Label eight 150 ml beakers **Bradford**. Add approximately 50 ml of Bradford reagent into each beaker. **Note**: It is important for the beaker containing the Bradford reagent to be very well rinsed as some detergents can interfere with the Bradford assay.



Activity 1

Understanding Protein-Energy Undernutrition

Lesson overview

Defining a problem (45 min)

- Students generate questions and ideas about data on protein-energy undernutrition (PEU)
- Students discuss and summarize the concept of PEU

Learning outcomes

- Students engage in asking questions
- Students understand that PEU applies to a specific set of conditions characterized by deficient calories and/or protein

Classroom preparation

- Split the class into groups of four students
- Decide if you want student questioning to focus on global and/or local data, including food insecurity in the United States
- Collate resources (paper and/or digital) for data, images, and other information about world hunger to drive student questioning

Tips & Tricks

While this kit focuses on protein-energy undernutrition, the problem of global nutrition includes malnutrition, defined by both undernutrition and obesity. Depending on the goals of your class, you may wish to have a discussion about this distinction with your students.

Defining a problem (45 min)
Observing indicator color change

Goal: Students use prior knowledge and ask questions about data on undernourishment.

1. ***Display data about global nutrition.*** Provide students with the driving question, "How can we engineer a treatment for world hunger?" Suggestions for data can be found in the References on page 27.
   Note: Resources and data may change over time. Have students record as many observations and questions as they can about the data you provide and encourage them to use their prior knowledge. Record students' questions and ideas on the board during a class discussion.

2. ***Have students write a summary of the problem of protein-energy undernutrition (PEU).*** This is an opportunity for students to investigate the crosscutting concept of Cause and Effect by investigating the social, environmental, economic, and biological causes of PEU and their physical effects on people.

   Provide students any additional background on the problem of PEU that will be necessary for them to write a complete and accurate summary of the problem. Consider giving them time to research the problem on their own. For example, students could investigate people who are affected with symptoms of PEU, where they live, and the social, economic, and environmental factors involved in PEU.

Tips & *Tricks*

Interdisciplinary opportunity: geography/history
Students can research which regions are affected by PEU, climate, history, and other social, economic, and environmental issues that have contributed to undernutrition. Also consider any local connections to the problem.

Activity 2

Investigating Protein Content in Food

Lesson Overview

First day: scientific data to design an evidence-based solution (45 min)

- Students learn about the Bradford assay
- Students use their understanding about PEU to consider and generate initial ideas to address the question, "How can we design a treatment proposal to address PEU?"
- Students discuss what data is sufficient in order to make evidence-based decisions about protein liquids to include in a treatment proposal

Second day: developing a solution (45 min)

- Students brainstorm and choose a set of protein liquids to test using the Bradford assay
- Students are given product requirements
- Students watch a video to learn the technique for using plastic pipets (optional)

Third day: conducting the investigation (45 min)

- Students prepare the standards
- Students conduct the Bradford assay with the protein liquids they chose on the second day
- Students analyze the data they collected using the Bradford assay

Learning outcomes

- Students understand how to make evidence-based observations from an investigative phenomenon
- Students use the Bradford assay to test protein liquids to include in a treatment proposal design
- Students use computational thinking to consider product requirements and apply known standards to data collected in an investigation

Classroom preparation

- Split the class into groups of four students
- (Optional) Cue the Using a Transfer Pipet video (see References on page 27 for a link)
- Copy and cut the recommended daily allowance (RDA) cards. See Example Preparation Schedule instructions for details and alternatives
- Purchase protein liquids or assign students to bring them (see Advance Preparation instructions for example protein liquids)
- Prepare the protein liquid samples if you decide they will be teacher-prepared
- Record the price per volume in ml of each protein liquid
- Prepare the BSA standards
- Set up workstations

Student Workstations items	Quantity per workstation
Set of BSA standards (S1 to S4)	1
Set of sample protein liquids (A to D)	1
Beaker of distilled water	1
Beaker of Bradford reagent	1
Plastic pipets	16
Marking pen	1
Beaker of water for rinsing (optional)	1

First Day (45 min): Using scientific data to design an evidence-based solution

Goal: students learn about the Bradford assay and discuss what data are sufficient for making an evidence-based decision about protein liquids to include in a treatment proposal.

3. ***Ask students what information or data are needed in order to design a treatment proposal to address protein-energy undernutrition.***

   Begin by asking students what information or data they need about food in order to develop a treatment proposal for PEU. Likely answers will include the protein content in the food. Ask them where they can go to find the protein content in food. Students will likely suggest nutrition labels. Tell them they can't be sure how the information provided on the label was gathered, and some foods might not have nutrition labels at all. Tell them they will be using a scientific process to measure the relative concentration of protein in the food so they have evidence to use for their treatment proposal.

   Briefly discuss with and/or have students read about what the Bradford assay is and how it is used (see Background on page 9 and Appendix B). This is also the starting point for students to think about the crosscutting concept of Cause and Effect as it relates to the interaction of amino acids with the Bradford reagent and associated color change. Ask students to discuss in groups how the Bradford assay could be used as a tool to gather information for designing a treatment proposal. Ask students to share their ideas with the class.

4. ***Discuss with students what data from the Bradford assay are sufficient in order to make evidence-based decisions about foods to include in a treatment proposal.***

   Ask students, "If Bradford reagent measures the relative concentration of protein in a sample, how do we know what that concentration is?" Guide them to the idea that there must be a known comparison or standard.

   Explain that scientists use a set of standards to compare the color of the sample because one standard will only match one relative concentration. If a sample has a different concentration than the standard, you can't estimate its relative concentration with only one comparison. In this investigation students will be comparing test samples to four protein standards to estimate the unknown concentration by eye.

Second Day (45 min): Developing a solution

Goal: students brainstorm foods to test using the Bradford assay and are given recommended daily allowance (RDA) of protein for patients.

5. ***Invite students to brainstorm a set of foods to test using the Bradford assay.***
   Review with students that the Bradford assay is a tool that can be used to test for the presence and relative concentration of protein in a sample. Review the problem of protein-energy undernutrition and associated symptoms. Ask student groups to:
   - Brainstorm a set of foods to test for possible inclusion in a treatment proposal to address the problem of PEU
   - Choose the top four protein liquids to investigate from their brainstorm
   - Write a rationale for why they are proposing them

6. ***Provide students with recommended daily allowance (RDA) requirements.***
   Distribute to each group at least one RDA Requirement card (see Appendix C), which describes the treatment requirements for a group of patients in terms of RDA of protein and a given weight. Tell students to calculate and record the RDA for their patients. Tell them to keep these requirements in mind as they test their four protein liquids using the Bradford assay.

7. ***(Optional) Have students watch a video to learn the technique for using a transfer pipet.***
   See References for a link to the video, "Using a Transfer Pipet." Alternatively, students can watch the video for homework outside of class.

Tips & Tricks

White, clear, or gray liquids work best with the Bradford reagent.

Tips & Tricks

All calculations should be done using metric units.
The RDA of protein for healthy children and adults is a minimum of:

0.8 g protein/kg of body weight = 800mg/kg or

0.36 g/lb of body weight = 360 mg/lb

Tips & Tricks

You may wish to set aside time for students to practice using the transfer pipet with various volumes of a liquid such as water colored with food dye.

Third Day (45 min): Conducting the investigation

Goal: students prepare standards and use the Bradford assay to investigate the protein content of a set of protein liquids.

8. ***Have students prepare and observe the standards.***
Discuss the set of standards students will be using to test their samples. Each group will add Bradford reagent to the set of prepared standard dilutions. Ask students to record their observations.

> **Tips & Tricks**
>
> To save time, students can prepare their protein samples during the 5 minute wait period after Bradford reagent is added to the standards.

9. ***Have students investigate the set of liquid samples they chose.***
Consider having each student in a group test one sample so there are four samples per group. Additional samples can be tested if time and materials permit. Have students record the name of the protein liquid in the Contents column of the data table. Conceal the nutrition label information.

10. ***Have students analyze the data they collected using the Bradford assay.***
Have students compare their samples to the standards and record the data. Explain that that the data they collected was for a 1:50 dilution of 100 µl of the original liquid, meaning the diluted solution is 50 times more dilute than the original protein liquid from the container. This dilution was necessary in order to get usable visual data from the Bradford test. Instruct students to multiply the concentration of their 1:50 diluted protein liquid by 50 in order to calculate the concentration (in mg/ml) of protein in the original protein liquid.

    Hold a discussion about class results. Ask each group to share their results. If students have differing results for the same protein liquid, ask them why they might be different. Likely answers will include contamination, improper dilution, mixing up samples, etc. Decide how the class will come to consensus. Students can average the results for a given protein liquid or throw out results that are obvious outliers.

> **Tips & Tricks**
>
> If accuracy is an important skill you want students to practice they can re-test samples for which there are discrepancies.

Scientific Practice for Activity 3: Engaging in Evidence-Based Argumentation

Engaging in argument from evidence is a process by which evidence is used to discuss and reach an agreement about the most acceptable scientific explanation for a phenomenon or design solution to a problem.

In argumentation, students compare and evaluate competing ideas and processes when considering a scientific phenomenon or testing a solution to a problem.

See the Instructor's Answer Guide for Activity 3 for a sample claim, evidence, and reasoning and a sample response to the capstone question below.

- **Sentence stems/frames:**

  Claim:
  The problem I/we are trying to solve is ______________________________

  The product requirements I/we were given for the solution are _________
  ____

  I argue ____ (claim)

  Evidence:
  The evidence that supports this claim is ____________________________
  ____

  Reasoning:
  The strength of the evidence to support this claim is _________________
  (low/medium/high) because _______________________________________

  The decision is based on ____

  Others might argue __________________________________ (counterclaim)

- **Capstone question**
  (use as a formative or summative assessment):

Write a claim with evidence for the question, "How can we design a protein recovery plan for an endurance athlete (for example, a runner) or strength athlete (for example, a power lifter) who weighs 150 pounds (68kg)?" Write a counterclaim to your claim.

Activity 3

Designing a Treatment Proposal for Protein-Energy Undernutrition

Lesson Overview

First day: designing and testing an initial treatment proposal (45 min)

- Students design an initial treatment proposal
- Students consider the RDA requirement and constraints, and revise their initial treatment proposal
- Students consider outcomes for their initial treatment proposal

Second day: revising and finalizing an initial treatment proposal (45 min)

- Students consider additional constraints and revise their initial treatment proposal
- Students consider additional outcomes for their initial treatment proposal
- Students construct an evidence-based argument for their final treatment proposal for PEU

Learning outcomes

- Students design, test, and revise an initial proposal for a treatment
- Students construct and engage in an evidence-based scientific argument
- Students understand that protein-energy undernutrition (PEU) applies to a specific set of conditions characterized by deficient calories and/or protein

Classroom preparation

- Read, Scientific Practice for Activity 3: Engaging in Evidence-Based Argumentation (see page 21) to prepare support for students in writing an evidence-based treatment proposal
- Copy and cut the Constraints cards. See Example Preparation for instructions and alternatives
- Copy the Patient Outcomes chart for each student workstation

First Day (45 minutes): Designing and testing an initial treatment proposal

Goal: students design an initial treatment proposal and revise it based on a constraint and outcomes.

11. ***Have students develop an initial treatment proposal.***
Ask students to work as a group to discuss and record the criteria they will include in the development of an initial proposal. They should consider:

- Information about PEU from Activity 1
- The product requirement(s) they were given
- The evidence they collected from the protein liquids they tested

Ask students to review the product requirements they were given. Ask them to use their analysis of the data from the Bradford assay and work with their group to determine how best to meet the product requirements.

Have students create an initial treatment proposal in the form of a drawing and/or a written plan. They should include:

- Description of RDA requirement
- Type and volume of protein liquid
- Any information learned from researching PEU that factors into the creation of the initial proposal (for example, in what part of the world do the patients live? What social, economic, and environmental factors are involved?)

Tips & Tricks

Consider having students focus on one particular country or part of the world that was researched in Activity 1 to provide specific context for the elements of their design.

Tips & Tricks

You may wish to have students participate in a gallery walk for peer feedback. Provide a focus prompt such as:

- What ideas did you see included in other initial proposals that you did not include?
- What is one question that you have that would clarify your understanding of the initial proposal?

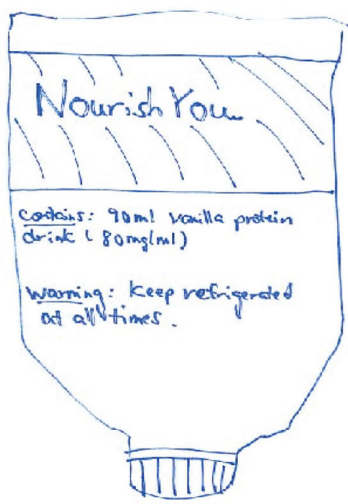
After the gallery walk, ask students to reflect on how to revise/improve their proposal.

Tips & Tricks

You may wish to have students build a physical prototype of the initial treatment they propose including:

- The protein liquid
- Packaging of treatment dose (provide various empty containers and plastics)
- Label with treatment dose
- Shipping packaging and instructions
- Any other features students think are important to include

Student prototypes may look something like this:



12. *Have students consider a constraint and revise their initial proposal.*

In a real-world engineering process, students would potentially test their initial treatment in humans. In this activity, students will test the effectiveness of the initial proposal product and constraints using simulated outcomes. Students will begin the process with their RDA requirement and selected protein liquid, and one constraint.

Direct students to the first row of the Initial Proposal Validation chart (example partial chart below) on page 11 of the Student Manual. Tell them to record in the second column (Description) the calculated RDA requirement, who it is for, and the proposed protein liquid. In the third column (Product or User), students should record "Product" since the RDA requirement is on the product side.

Next, give each group one constraint (condition needed or desired) from the Constraints cards (see Appendix C). Some constraints are on the product or production side of the treatment and some are on the user side and can only be evaluated after "use" of the treatment. Direct students to the second row (Constraint 1) of the Initial Proposal Validation chart and tell them to describe their constraint 1 in the second column (Description) of the chart. In the third column, students should record the type of constraint card they were given, Product or User. Ask them to consider the constraint along with the criteria of their initial treatment proposal to revise and improve their initial proposal.

Tips & Tricks

To emphasize the iterative process of this stage of engineering, after considering constraints and if time and materials permit, students may do more testing with the Bradford reagent.

13. *Instructor validates students' RDA requirement and constraint.*

Use the Initial Proposal Validation chart (see Appendix C and example partial chart below) to give each group a value for their RDA requirement and proposed protein liquid based on the rubric in the chart. The value reflects the degree to which students met the requirement with their calculations and protein selection and simulates a process called validation. Ask them to mark or circle on their chart the value you give them.

Tips & Tricks

Note that the values for the simulated testing/outcomes are not intended to "grade" students' reasoning. If you wish to assess students' reasoning, consider assessing their initial treatment proposal.

Criteria	Description	Product or User	Value = 1	Value = 2	Value = 3	Outcome
RDA requirement			Incorrect calculation of RDA, and incorrect calculation of protein concentration of protein liquid, and inaccurate/ inappropriate selection of protein liquid	Either incorrect calculation of RDA OR incorrect calculation of protein concentration of protein liquid OR inaccurate/ inappropriate selection of protein liquid	Correct calculation of RDA, and correct calculation of protein concentration of protein liquid, and accurate/appropriate selection of protein liquid	
Constraint 1						
Constraint 2						

Use the Initial Proposal Validation chart to give each group a value for their constraint in the same procedure that was used for the RDA requirement. For each constraint card that is labeled "Product" the outcome should match the validation value that was given. For each constraint card that is labeled "User" the outcome should be chosen randomly because a good outcome depends on an unknown; for example, how well the user follows the directions, etc. Ask them to mark or circle the value you give them in the Constraint 1 row on their Initial Proposal Validation chart. Tell students this is the validation of their constraint.

14. Have students determine outcomes of their RDA requirement and one constraint.

This is an opportunity for students to think about the crosscutting concept of cause and effect as it relates to the treatment (cause) and its outcomes (effect). Ask students to match the value they were given for validation of the RDA requirement and proposed protein liquid to an outcome on the Patient Outcomes chart (see Appendix C and example below). An outcome is dire for a value of 1 on the validation chart, mediocre for a value of 2, or good for a value of 3. Ask students to briefly describe the outcome in the last column of the Initial Proposal Validation chart in the RDA requirement row.

Criteria	Description
RDA requirement	**Dire**: Some patients had to be hospitalized **Mediocre**: Some patients did not improve **Good**: Some patients gained weight

1 = Dire outcome
2 = Mediocre outcome
3 = Good outcome

Ask students to match the value they were given for validation of the constraint to an outcome on the Patient Outcomes chart. Ask them to briefly describe the outcome in the last column of the Initial Proposal Validation chart in the Constraint 1 row.

15. Have students revise their initial design.

Ask students to discuss the outcomes for their RDA requirement, proposed protein liquid, and constraint and decide how they will revise their initial treatment proposal. Tell them to make their revisions directly on their initial proposal.

Second Day (45 minutes): Revising and finalizing an initial treatment proposal

Goal: students consider more constraints, revise their initial treatment proposal, and construct an evidence-based argument for their final treatment proposal.

16. ***Engage students in the iterative engineering process with more constraints.***
Give students a second constraint. Repeat the process of using the Initial Proposal Validation chart to validate students' incorporation of the constraints. Ask students to determine the patient outcomes with the values they were given using the Patient Outcomes chart and add to the revisions of their initial treatment proposal accordingly. Optionally, give students a third constraint or as many as are appropriate for class objectives.

17. ***Have students construct an evidence-based argument for their final treatment proposal to address PEU.***
When students have completed the iterative test/refine cycles for their treatment, instruct them to write a final evidence-based treatment proposal, individually or as a group, which should include:

- Claim of the treatment being proposed
- Description of the product requirement and constraints
- Evidence from the investigation with the Bradford assay
- Outcomes from the testing
- Reasoning for how strong the evidence is in supporting the claim
- Reasonable counterargument

Tips & Tricks

To further emphasize the iterative process of this stage of engineering, after the testing and if time and materials permit, students may do more testing with the Bradford reagent.

Tips & Tricks

If you assign the constraints, consider the following:

- Give students a user constraint if their first constraint was a product constraint and vice versa
- Distribute the less challenging constraints first. The constraints for cost and mixing more than one protein liquid are the most challenging, followed by the constraints for volume. All other constraints are less challenging

References

Global Nutrition Report

https://www.globalnutritionreport.org/the-report/

This report outlines five core areas of development connected to nutrition that run through the Sustainable Development Goals (SDGs): sustainable food production, infrastructure, health systems, equity and inclusion, and peace and stability. Be sure to check for the most recently published report. Accessed December 3, 2018.

Academy of Nutrition and Dietetics
Protein and the Athlete – How Much Do You Need?

https://www.eatright.org/fitness/sports-and-performance/fueling-your-workout/protein-and-the-athlete

This article summarizes the role of protein and how much is needed in an athlete's diet. Accessed December 3, 2018.

Food Security in the U.S.
Access Research Atlas

https://www.ers.usda.gov/data/fooddesert

The atlas uses various measures of supermarket accessibility to provide a spatial overview of food access for low-income and other census tracts. Accessed December 3, 2018.

Food Security in the U.S. Interactive Charts and Highlights

https://www.ers.usda.gov/topics/food-nutrition-assistance/food-security-in-the-us/interactive-charts-and-highlights/

This site provides charts of trends and characteristics of food insecurity in the United States. Accessed December 3, 2018.

Levels and Trends in Child Malnutrition

https://www.who.int/nutgrowthdb/estimates/en/

This document includes charts, graphs, and maps to summarize child malnutrition and includes global and regional estimates of stunting, wasting, overweight, and severe wasting. Accessed December 3, 2018.

Nutriset

https://www.nutriset.fr/en

Nutriset is a company that researches, designs, and produces nutritional solutions for malnutrition. Accessed December 3, 2018.

Nutrition and the Post-2015 Sustainable Development Goals

https://www.unscn.org/files/Publications/Briefs_on_Nutrition/Final_Nutrition%20and_the_SDGs.pdf

This document describes how nutrition connects to the Sustainable Development Goals. Accessed December 3, 2018.

Protein-Energy Undernutrition

https://www.merckmanuals.com/professional/nutritional-disorders/undernutrition/protein-energy-undernutrition-peu

This document defines and describes protein-energy undernutrition (PEM) including symptoms, diagnosis, and treatment. Accessed December 3, 2018.

The State of Food Security and Nutrition in the World
https://www.who.int/nutrition/publications/foodsecurity/state-food-security-nutrition-2017/en/

This report outlines the progress made toward a world without hunger and malnutrition, and how nutrition and food security relate to progress on Sustainable Development Goals. Be sure to check for the most recently published report. Accessed December 3, 2018.

Sustainable Development Goals
https://www.un.org/sustainabledevelopment/sustainable-development-goals/

This site outlines the interconnected Sustainable Development Goals and the global challenges of poverty, inequality, climate, environmental degradation, prosperity, and peace and justice. Accessed December 3, 2018.

Understanding the Prevalence, Severity, and Distribution of Food Insecurity in the United States
https://www.ers.usda.gov/amber-waves/2017/september/understanding-the-prevalence-severity-and-distribution-of-food-insecurity-in-the-united-states/

This site provides descriptions, charts, and graphs of levels, prevalence, severity, and distribution of food insecurity among U.S. households. Accessed December 3, 2018.

Video: Using a Transfer Pipet
https://www.youtube.com/watch?v=J_XhKFspBo8

Accessed December 3, 2018.

Appendix A

Investigating an Anchoring Phenomenon

Consider adding the following before starting Activity 1 of the kit to provide students experiences to build on the concepts of photosynthesis and cellular respiration and more deeply explore the anchoring phenomenon of protein-energy undernutrition.

Food is made up of matter in the form of protein, carbohydrates, and fats. The glucose we consume from plants is a simple carbohydrate, which is a form of stored energy. Glucose and other carbohydrates contain carbon, hydrogen, and oxygen. Some of the carbon, hydrogen, and oxygen-containing matter in food we consume is rearranged during cellular respiration in order to assemble carbon-containing molecules such as amino acids. These carbon-based molecules are used to make larger molecules such as proteins. Proteins are essential to grow organisms and do the work to keep the organisms alive. Proteins and other carbon-based molecules are used for a variety of functions, for example to repair, replace, and grow new cells.

Part 1: Modeling what happens to food after we eat it (80 min)

Goal: students use prior knowledge to draw an individual model of what happens to food after we eat it.

1. ***Have students create an individual initial model of what happens after food is eaten.***
   Encourage students to use any prior knowledge they have about food and digestion and the molecules/organs/systems involved. Remind them that there is no correct answer at this point. The point of the model is to visually summarize their current ideas and thinking. They will have opportunities to add to and revise their thinking using the model over the next few days.

2. ***Have students create a group model of what happens after food is eaten.***
   Using one large sheet of paper, groups should compile the ideas from their individual models to construct a group model of what happens to food after it is eaten.

3. ***Have students visit other group models and give feedback.***
   Display student models around the room. Have student groups visit at least three other group models and give written feedback on sticky notes. The following are example feedback sentence stems that can help students think about and write constructive feedback:

   - I find it interesting that….
   - I am curious about…
   - This reminds me of…
   - This contradicts…
   - It does not seem logical that…
   - I have more questions about…

Part 2: Protein-energy undernutrition and biomolecules

Goal: students conduct research to investigate what happens in the body during the fed, fasting, and starvation states of metabolism, and use this understanding to explore the question, "What happens when we don't take in enough food?"

1. ***Have students revisit or ask three key questions from Part 1.***
   These questions will get them started on their research. In addition to investigating the science of the fed, fasting, and starvation states of metabolism, encourage students to investigate the social, environmental, and economic factors involved in the issue of PEU. Have students revisit their model from Part 1 and add or revise the model using any new information they obtained in their research.

2. ***Students use their model to answer the question, "What happens when we don't take in enough food?"***
   Decide how students will compile or discuss individual, group, or class ideas, depending on the class goals.

Appendix B

Reading: Bradford Reagent and Casein

Have you ever wondered why we need to eat food to survive? The human body is made up of different tissues and cells. Tissues and cells are composed of biomolecules, DNA, proteins, lipids, and carbohydrates. The food we eat is one source of the proteins, lipids, and carbohydrates our cells need. Each of these biomolecules is relatively large and made up of smaller subunits bonded together. For example, proteins are made up of smaller subunits called amino acids.

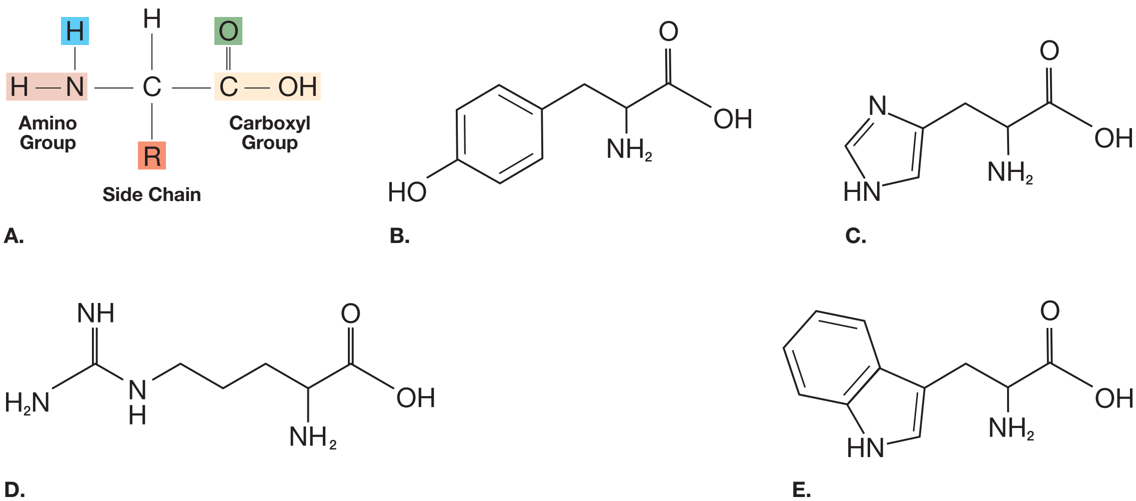


Fig. 1. Amino acid structure. A, Basic amino acid structure. Four of the 20 amino acids our bodies need for building proteins are **B**, tyrosine (Y), **C**, histidine (H), **D**, arginine (R), and **E**, tryptophan (W).

Our cells use biomolecules in order to carry out functions that support life. Protein biomolecules are essential for almost everything that an organism does. For example, proteins are used for storage, transport, movement, and defense against pathogens. Other proteins are used to speed up chemical reactions.

You will be designing a treatment proposal to address the problem of protein-energy undernutrition (PEU). One method for determining the total protein content of a sample is a procedure that uses Bradford reagent. The Bradford procedure uses a dye, Coomassie Brilliant Blue G-250. Coomassie Brilliant Blue G-250 dye exists in multiple forms. The dye in the Bradford reagent is a reddish-brown color. When the dye binds to and interacts with amino acids, the dye is converted to a blue form. The blue form of the dye is easily observed. The more protein in the sample, the more intense the blue color. The Bradford procedure cannot distinguish between types of proteins (for example, the protein in milk vs. the protein in nuts) because it reacts with particular amino acids that are found in nearly all proteins.

Fig. 2. The Bradford assay. The cuvette on the left (back) is a control with no protein, and the cuvettes to the right (back) contain increasing amounts of protein. The protein concentration can be determined by comparing unknown samples (front) to the standards.

Coomassie Brilliant Blue G-250 binds to proteins using three types of interactions with amino acids. The strongest interaction occurs with arginine (R). Other weaker dye-protein interactions include tryptophan (W), tyrosine (Y), and histidine (H).

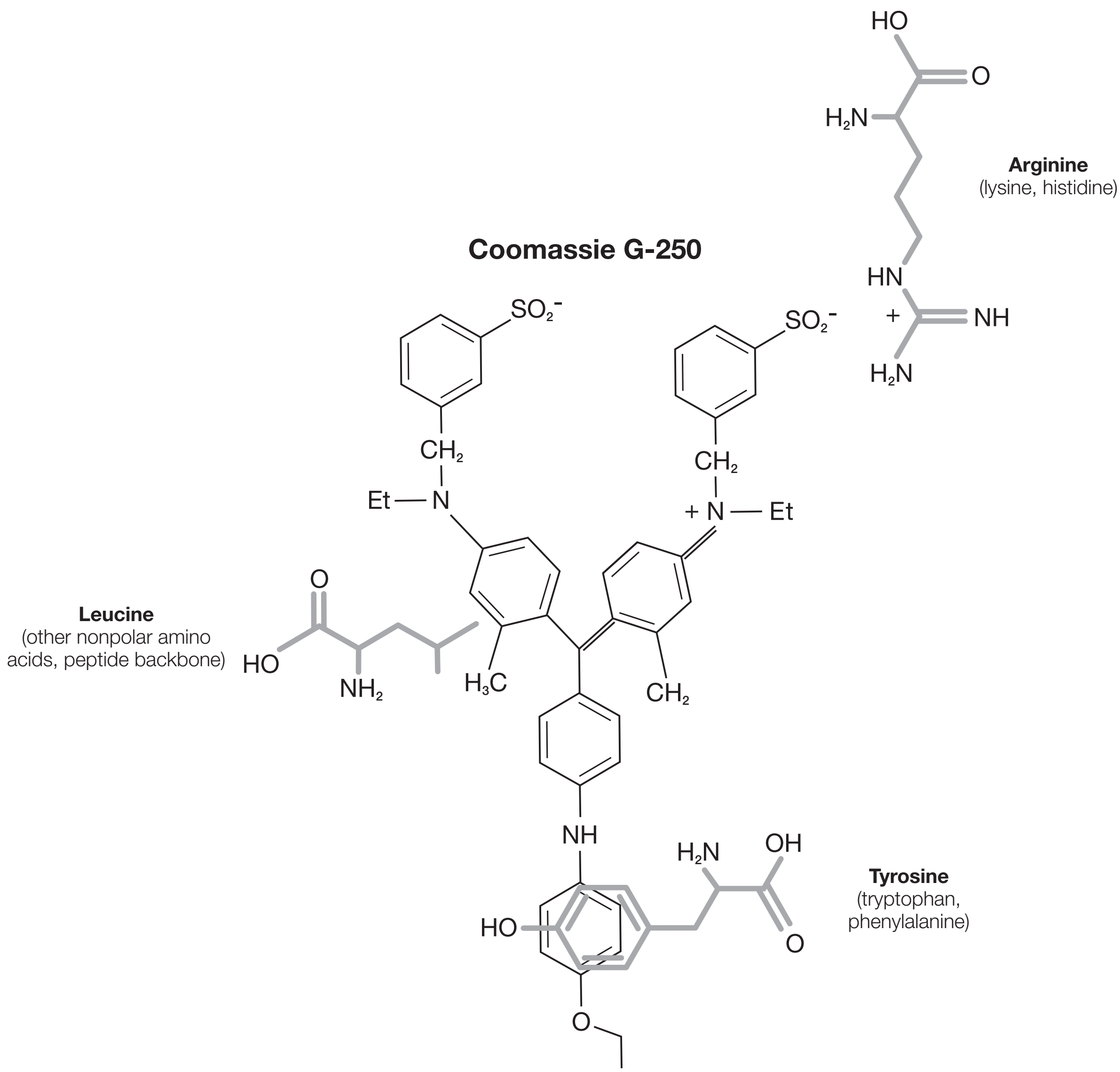


Fig. 3. Coomassie G-250 interactions with amino acid residues.

Casein is one example of a protein. Casein is the most abundant form of protein in milk. The amino acids that make up casein are shown below.

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

Fig. 4. Amino acid composition of casein. R = arginine, Y = tyrosine, H = Histidine, and W = tryptophan

Casein contains a total of 224 amino acids. Thirteen of the amino acids in casein bind strongly with Coomassie Blue G-250. These are shown in bold in the sequence.

Appendix C

Product Requirement and Constraints

RDA Requirement

Child weighing 40 pounds (18 kg)

50% of the RDA of protein for child weighing 40 pounds
RDA protein for children and adults is 0.8 g/kg body weight

RDA Requirement

Child weighing 20 pounds (9 kg)

200% of the RDA of protein for child weighing 20 pounds
RDA protein for children and adults is 0.8 g/kg body weight

RDA Requirement

Child weighing 18 pounds (8 kg)

150% of the RDA of protein for child weighing 18 pounds
RDA protein for children and adults is 0.8 g/kg body weight

RDA Requirement

Child weighing 15 pounds (7 kg)

100% of the RDA of protein for child weighing 15 pounds
RDA protein for children and adults is 0.8 g/kg body weight

RDA Requirement

Child weighing 20 pounds (9 kg)

50% of the RDA for a child weighing 20 pounds
RDA protein for children and adults is 0.8 g/kg body weight

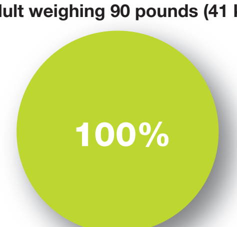
RDA Requirement

Child weighing 20 pounds (9 kg)

100% of the RDA of protein for child weighing 20 pounds
RDA protein for children and adults is 0.8 g/kg body weight

RDA Requirement

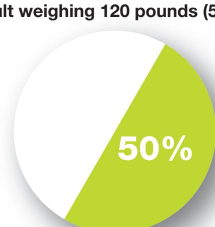
Adult weighing 90 pounds (41 kg)



100% of the RDA for an adult weighing 90 pounds
RDA protein for children and adults is 0.8 g/kg body weight

RDA Requirement

Adult weighing 120 pounds (55 kg)



50% of the RDA for an adult weighing 120 pounds
RDA protein for children and adults is 0.8 g/kg body weight

Constraints

Your product should be lactose free ________________ Product	**One dose should not exceed 50 ml** ________________ Product
Your product should contain iron and magnesium supplements ________________ Product	**Your product should cost no more than $0.50 per dose (food material only)** ________________ Product
The RDA requirement is doubled ________________ Product	**There should be more than one protein liquid in the treatment** ________________ Product
Your product should cost no more than $1 per dose (food material only) ________________ Product	**One dose of treatment should not exceed 500 ml** ________________ Product

Constraints

Product requires refrigeration during transport, storage, and after opening User	**Your product should be reconstituted in water before administering** User
Your product should be edible by someone who has trouble swallowing User	**Each treatment dose should be in packaging that keeps it from being exposed to direct sunlight** User
Your product should be administered in one dose, one time per day User	**Your product should be delivered in more than one dose per day** User
Each treatment dose should be packaged individually in plastic User	**There should be more than one protein liquid mixed together just before the treatment is administered** User

Initial Proposal Validation

Criteria	Description	Product or User	Value = 1	Value = 2	Value = 3	Outcome
RDA requirement			Incorrect calculation of RDA, and incorrect calculation of protein concentration of protein liquid, and inaccurate/ inappropriate selection of protein liquid	Either incorrect calculation of RDA OR incorrect calculation of protein concentration of protein liquid OR inaccurate/ inappropriate selection of protein liquid	Correct calculation of RDA, and correct calculation of protein concentration of protein liquid, and accurate/appropriate selection of protein liquid	
Constraint 1			Constraint was not successfully applied	Constraint was only partially applied	Constraint was successfully applied	
Constraint 2			Constraint was not successfully applied	Constraint was only partially applied	Constraint was successfully applied	
Constraint 3			Constraint was not successfully applied	Constraint was only partially applied	Constraint was successfully applied	

Patient Outcomes for cards labeled "Product"

Use the results from the rubric for each requirement or constraint (remember, constraints are either product or user constraints) to evaluate the outcomes of your treatment proposal. **Note:** The outcomes in the right column may apply to more than one type of constraint in the left column:

1 = dire outcome
2 = mediocre outcome
3 = good outcome

RDA Requirement or Constraint	Outcome
RDA requirement	**Dire**: Some patients had to be hospitalized **Mediocre**: Some patients did not improve **Good**: Some patients gained weight
Product should be lactose free	**Dire**: Some patients experienced severe vomiting and diarrhea and died **Mediocre**: Some patients experienced mild diarrhea and did not improve **Good**: Some patients gained weight
Volume of dose	**Dire**: Some patients took too little or too much of the treatment and experienced severe vomiting and diarrhea and died **Mediocre**: Some patients experienced mild diarrhea from taking too much or too little of the treatment at one time and did not improve **Good**: Some patients gained weight
Product should contain iron and magnesium	**Dire**: Some patients experienced severe fatigue/weakness (iron deficiency) or dehydration due to electrolyte (magnesium) deficiency causing extended hospitalization **Mediocre**: Some patients experienced fatigue/weakness (iron deficiency) or dehydration due to electrolyte (magnesium) deficiency, and did not improve **Good**: Some patients gained weight
Cost per dose (food material only)	**Dire**: Some patients could not begin treatment due to cost and died **Mediocre**: For some patients treatment was not long enough due to cost and they did not improve **Good**: Some patients gained weight
There should be more than one protein liquid in the treatment	**Dire**:: Some patients had to be hospitalized **Mediocre**: Some patients did not improve **Good**: Some patients gained weight

Patient Outcomes for cards labeled "User"

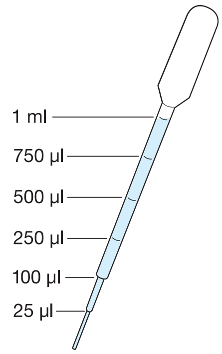
Note: These outcomes are randomly chosen or assigned because a good outcome depends on an unknown, i.e. how well the user follows the directions, etc.

1 = dire outcome
2 = mediocre outcome
3 = good outcome

Constraint	Outcome
Product requires refrigeration during transport, storage, and after opening	**Dire**: Product was not properly refrigerated during transport or product was not stored properly and patients died **Mediocre**: For some patients treatment was damaged from improper refrigeration so patients did not improve **Good**: Some patients gained weight
Packaging	**Dire**: For some patients treatment was not long enough due to loss of product from damaged packaging and patients died **Mediocre**: For some patients treatment was damaged from improper packaging so patients did not improve **Good**: Some patients gained weight
Product should be reconstituted in water before administering	**Dire**: Some patients developed a bacterial infection from water used to mix the treatment or consumed with the treatment and patients died **Mediocre**: Some patients experienced overhydration so patients did not improve **Good**: Some patients gained weight
Product should be edible by someone who has trouble swallowing	**Dire**: Some patients experienced severe dehydration causing extended hospitalization **Mediocre**: Some patients experienced dehydration, and did not improve **Good**: Some patients gained weight
Your product should be administered in one dose one time per day	**Dire**:: Some patients took too little or too much of the treatment and experienced severe vomiting and diarrhea and died **Mediocre**: Some patients experienced mild diarrhea from taking too much or too little of the treatment at one time and did not improve **Good**: Some patients gained weight
Your product should be delivered in more than one dose per day	**Dire**: Some patients took too little or too much of the treatment and experienced severe vomiting and diarrhea and died **Mediocre**: Some patients experienced mild diarrhea from taking too much or too little of the treatment at one time and did not improve **Good**: Some patients gained weight
There should be more than one protein liquid mixed together just before the treatment is administered	**Dire**: Some patients had to be hospitalized **Mediocre**: Some patients did not improve **Good**: Some patients gained weight

Appendix D

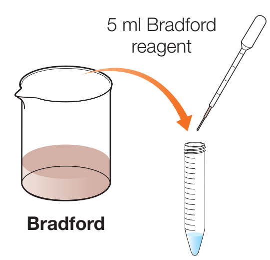
Quick Guide



Step 1: Prepare standards in Bradford reagent (one set per group)

1. *Identify the four tubes labeled **S1 1.6 mg/ml, S2 0.8 mg/ml, S3 0.4 mg/ml, and S4 0 mg/ml**. These tubes already contain 100 µl of protein standard at the indicated concentrations.*

2. *Add 5 ml of the Bradford reagent to each tube S1 through S4. Cap the tube tightly and invert gently to mix. Incubate for 5 minutes.* ➜



3. *Record your observations.*

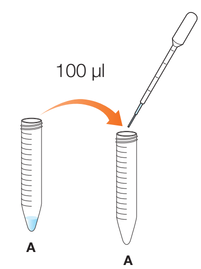
Step 2: Prepare a 1:50 dilution of protein liquid sample (four per workstation)

4. *If your protein liquids have already been prepared, locate your set of protein liquids, **A**, **B**, **C**, and **D** and skip steps 5–10.*

5. *Select a protein liquid (labeled **A, B, C**, or **D**) for each person in your group to test.*

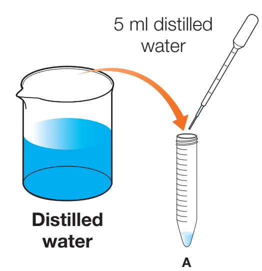
6. *Label a new tube with the same letter from the sample chosen in step 4.*

7. *Pipet 100 µl of Sample A into your tube labeled **A.** Rinse the pipet at least two times with distilled water using the beaker labeled **Rinse.** To do this, gently squeeze the bulb then place the pipet in the beaker of distilled water, release the bulb to draw water up into the pipet, and squeeze the bulb to expel the water.* ➜



8. *Using the same pipet and rinsing between samples, repeat step 7 for **B**, **C**, and **D**.*

9. *Use a clean plastic pipet to add 5 ml distilled water into tube **A.** Cap the tube tightly and invert gently to mix.* ➜

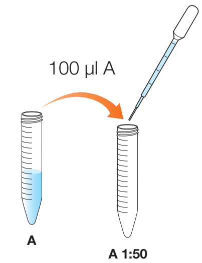


10. *Using the same pipet and rinsing each time, repeat step 9 for **B**, **C**, and **D**.*

Step 3: Test diluted protein liquid samples and compare to standards (four per group)

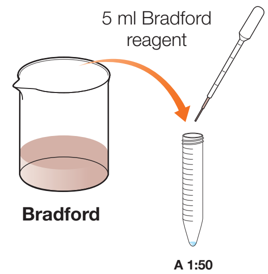
11. *Label four new tubes* ***A 1:50, B 1:50, C 1:50, and D 1:50.***

12. *Use a new pipet to add 100 µl of* ***A*** *to the* ***A 1:50*** *tube. Rinse the pipet at least two times in distilled water.*



13. *Using the same pipet and rinsing between samples, repeat step 12 for* ***B, C,*** *and* ***D.***

14. *Use a new pipet to add 5 ml of Bradford reagent to tube* ***A 1:50.*** *Repeat for samples* ***B 1:50, C 1:50,*** *and* ***D 1:50.*** *Cap the tube tightly and invert to gently mix. Incubate for 5 minutes.*



15. *Visually compare each of your protein liquids to the four standards against a white or light background.* ***Note:*** *If you do not have a tube rack, cap the tubes tightly and place them on their sides on the table.*
    a. *Record your observations of the color of each protein liquid and the standard it matches most closely.*
    b. *Record the protein concentration of the standard that most closely resembles the color of the sample 1:50 protein liquid.*
    c. *Record the estimated protein in the original protein liquid by multiplying the concentration of the 1:50 protein liquid by 50.*

BIO-RAD Explorer

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