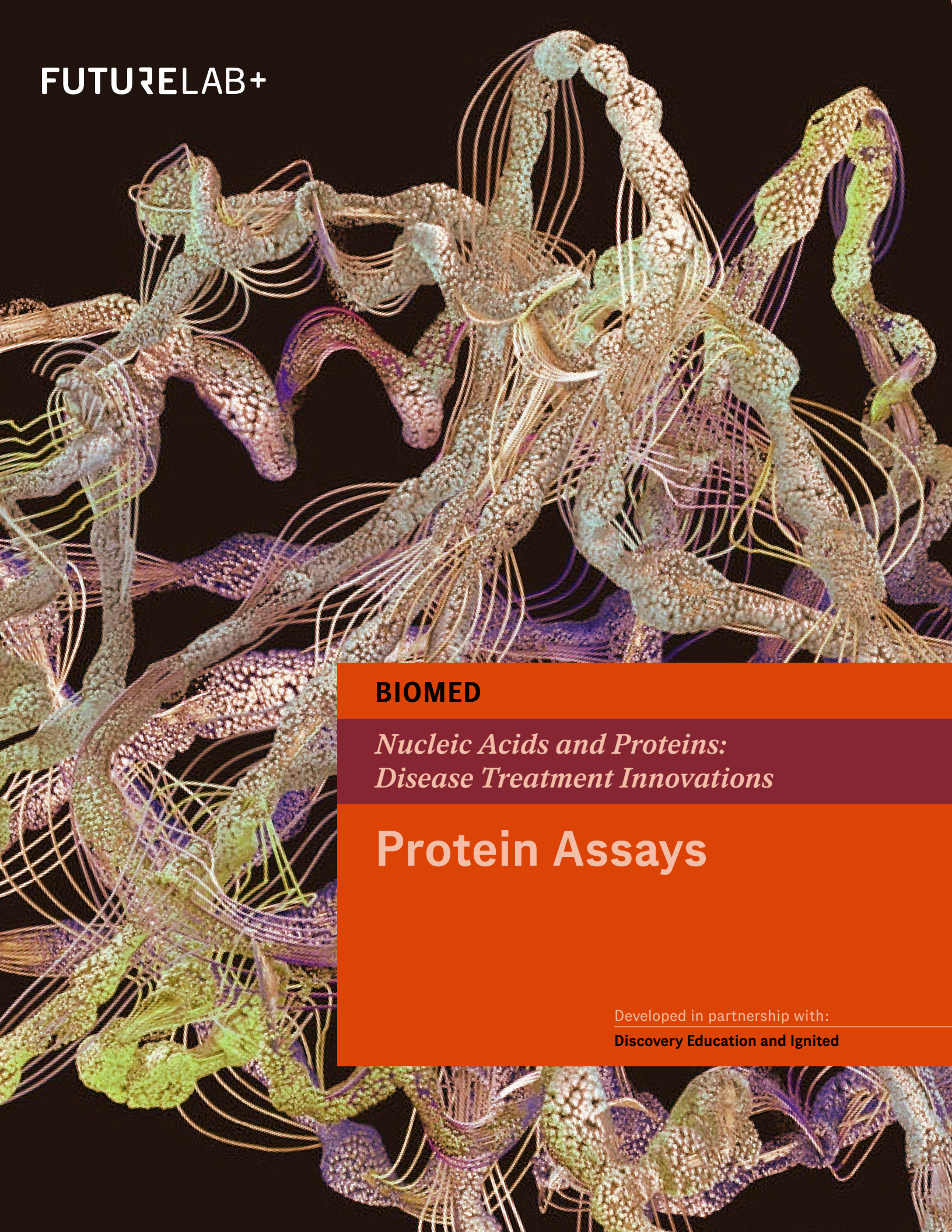


FUTURELAB+



BIOMED

*Nucleic Acids and Proteins:
Disease Treatment Innovations*

Protein Assays

Developed in partnership with:
Discovery Education and Ignited

In this Lesson Plan:

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The image shows a biochemical analyzer in a science laboratory.

Cover Image

This is an illustration of a protein.

This document is separated into two sections, For Teachers [T] and Student Resources [S], which can be printed independently.

Select the appropriate printer icon above to print either section in its entirety.

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Protein Assays

DRIVING QUESTION

How are protein assays used to make accurate medical and clinical diagnoses?

OVERVIEW

Protein assays represent a variety of tests that are used to determine the amount or concentration of either a specific protein or groups of different proteins. There are several types of protein assays in use today. Proteins, which are one of the four major macromolecules needed to sustain life, are essential to the correct functions of the cells and tissues of all living organisms. Because of this, protein assays are fundamental to biological research and clinical diagnoses.

ACTIVITY DURATION

Five class sessions
(45 minutes each)

ESSENTIAL QUESTIONS

Why do medical professionals need to determine the quantity and identity of proteins in a sample?

How do some protein assays detect specific proteins while other assays do not?

How do Biuret protein assays and ELISAs differ?

OBJECTIVES

Students will be able to:

Obtain, evaluate, and communicate background information about the structure and function of proteins and the applications of protein assays.

Describe how ELISA is used to detect the presence of a specific protein in a sample.

Create a model of ELISA.

Pedagogical Framing

Instructional materials are designed to meet national education and industry standards to focus on in-demand skills needed across the full product development life cycle—from molecule to medicine—which will also expose students and educators to the breadth of education and career pathways across biotechnology.

Through this collection, educators are equipped with strategies to engage students from diverse racial, ethnic, and cultural groups, providing them with quality, equitable, and liberating educational experiences that validate and affirm student identity.

Units are designed to be problem-based and focus on workforce skill development to empower students with the knowledge and tools to be the change in reducing health disparities in communities.



SOCIAL-EMOTIONAL LEARNING

In this lesson, students analyze how the duties of an immunologist can have an effect on various communities. They will use the social-emotional learning skills of social awareness and self-awareness to participate in discussions on race, ethnicity, and socioeconomic status. This lesson also provides opportunities for students to practice self-management skills, such as persevering in the face of setbacks and frustrations, while pursuing their research. Some students will have had personal experience with disease, and carry that experience with them into sensitive discussions. This requires all discussion participants to demonstrate empathy and practice social awareness.

CULTURALLY AND LINGUISTICALLY RESPONSIVE INSTRUCTION

Instruction in this lesson provides for many small group interactions. This allows for culturally and linguistically diverse students to receive peer support and scaffolding during the lesson. The content included has implications for many communities that are historically underserved and how the use of certain assays under certain conditions may have different outcomes on various cultures. The lesson provides information about what an immunologist does and this allows students to more easily consider this as a career path.

ADVANCING INCLUSIVE RESEARCH

Diagnostic tests are important tools for analyzing a patient's health and identifying diseases. Tests range in accessibility and affordability. For many

people, protein assays can be done during a routine doctor's visit. For those without affordable care, these tests may be out of reach and serious health conditions may continue undiagnosed. Many manufacturers, non-governmental organizations (NGOs), and medical facilities are working to get these diagnostic tests to the communities that need them most.

COMPUTATIONAL THINKING PRACTICES

As students continue their learning on diagnostic tests, they utilize the computational thinking strategies of collecting data, analyzing data, finding patterns, and building models. Students simulate the role of doctors performing a urinalysis on a patient in order to detect kidney disease. In order to do this, they must conduct an experiment in order to collect data, analyze what they collected, build a model curve of the data, and find patterns in order to assess whether or not the patient has an elevated amount of proteins in their urine.

CONNECTIONS TO THE PRODUCT LIFE CYCLE

Protein assays exist in the **discovery** phase of the product life cycle, as they are used to aid in the discovery of effective treatments and therapies. Diagnostic tests, such as the urinalysis, allow scientists to measure a patient's biomarkers (such as the quantity of albumin in their urine) in order to make predictions about how their bodies are managing treatment or disease.

Have you ever wondered...

How are specific protein assays chosen?

Choosing the most effective protein assay depends on the compatibility of the method with the specific proteins to be tested. It is essential to use the method that will require the least amount of prerequisite treatment or manipulation of samples because that may interfere with the efficacy of the assay.

How does the use of protein assays highlight inequities and medical disparities in certain communities?

The availability, or lack thereof, of more effective protein assays in healthcare facilities in poorer, less equipped communities may mean missed diagnoses of dangerous chronic and infectious diseases. This, in turn, may increase their morbidity and mortality in those communities. In addition, the lack of representative patient populations in the testing phase of protein assays may affect their efficacy as well as create a barrier to the development of personalized healthcare, more accurate diagnoses, and optimal treatment for all patients.

MAKE CONNECTIONS!

How does this connect to the larger unit storyline?

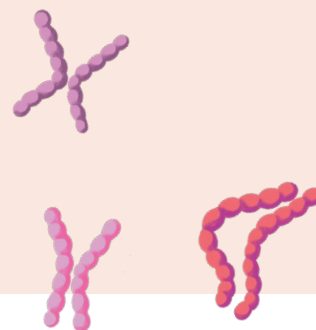
The development of protein assays is crucial in the creation and delivery of new treatments and pharmaceutical drugs. The investigative process of how medicines work on cells and different parts of the body would be infinitely more difficult to accomplish without the advent and use of protein assays.

How does this connect to careers?

Immunologists use their skills in biotechnology and basic chemistry to study issues in biological research and health care. Utilizing a good attention span and the ability to keep accurate and detailed records, immunologists hold some of the most important careers in the biomedical field.

How does this connect to our world?

Protein assays connect to our world through the advancements and development of biomedical research procedures and techniques. The ability to analyze and characterize proteins is crucial in drug design and treatments that have saved countless lives.



Day 1

Procedure

LEARNING OUTCOMES

Students will be able to:

Obtain, evaluate, and communicate background information about the structure and function of proteins and the applications of protein assays.

COMPUTATIONAL THINKING IN ACTION

In this exercise, students discuss how the computational thinking strategy of analyzing data helps medical professionals assess the health of patients.



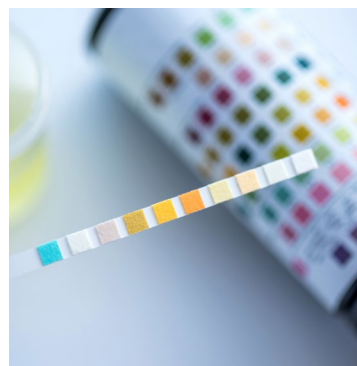
Whole Group (15 minutes)

- 1 Use a virtual discussion board or have students turn to their elbow partner to share about a time they or a family member had a urine analysis or had blood drawn for analysis. Ask students:
 - a. How did you or someone you know discover that analysis was needed?
 - b. What were the doctors looking for?
- 2 Share with students that doctors often examine urine and blood for the presence of proteins.
 - a. Review with students the importance of proteins in the cells and body.
 - i. Proteins are one of four groups of biological macromolecules. The other groups are carbohydrates, lipids, and nucleic acids.
 - ii. Proteins are made of monomers called amino acids and contain the elements carbon, hydrogen, oxygen, nitrogen, and sometimes sulfur.
 - iii. Proteins are large, complex molecules that carry out important roles in the cells and the body:
 - Keratin and collagen are structural proteins that make up skin, hair, and nails
 - Myosin and actin are contractile proteins found in muscles.
 - Enzymes are proteins that speed up chemical reactions.
 - Antibodies are proteins that help the immune system recognize and attack invaders.
 - Hemoglobin is a protein that transports oxygen through the blood.
 - Albumin is a protein that carries hormones, vitamins, and other proteins in your blood. It also helps regulate the water balance of your blood.

Continues next page >

Day 1

Continued



Procedure

- b. Share with students that the concentration of specific proteins in specific parts of the body is critical to their function.
 - i. Remind students that protein concentrations are regulated by negative feedback loops.
 - ii. Share with students that too much or too little of a protein in a particular part of the body may indicate an imbalance or problem.
 - iii. Share with students that the presence of foreign proteins in a sample from a patient may indicate infection or poisoning.
- 3 Ask students to pay attention to why this immunologist conducts protein assays as they watch the following video: *Allergist and Clinical Immunologist Monica Lawrence, MD*.

Individual Work (20 minutes)

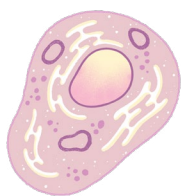
Encourage students to use the internet to gain background knowledge about how protein assays are used in medicine. This also may be an opportunity to visit the campus library and engage your school librarian in guiding students to research this topic. The teacher may choose to have students investigate one or more of these applications depending on time available and student ability. *Jigsaw* is a good strategy for having students research and share what they find with the class.

- pregnancy tests
- HIV testing
- cancer screening
- drug testing
- COVID testing
- detecting blood doping

Whole Group (10 minutes)

Encourage students to share what they learned by completing a *3-2-1 Bridge* style Exit Ticket or Journal entry:

- a. Identify three facts that they learned.
- b. Describe two areas that they are interested in learning more about.
- c. Ask one question they still have.



Day 2

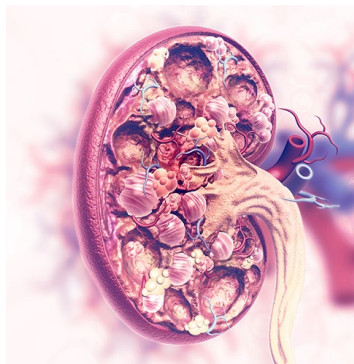
Procedure

LEARNING OUTCOMES

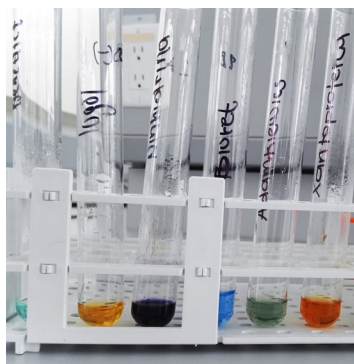
Students will be able to:

Describe how the Biuret test is used to **detect** the presence of protein in a sample.

Obtain, evaluate, and communicate information about conditions and diseases that cause kidney disease and how these conditions and diseases often affect different sub-populations more than others.



The image shows a 3D illustration of kidney disease.



Whole Group (15 minutes)

- 1 Share with students that some protein assays detect the presence of any protein while other protein assays detect the presence of a specific protein. In addition, protein assays measure the concentration of the protein they detect.
- 2 Encourage students to think of situations in which the presence of protein and the presence of a specific protein would be needed. Encourage students to think about why doctors might want to know the concentration of protein present. Use an equitable calling strategy to have students share their ideas with each other or the class.
- 3 Introduce the Biuret test to the class. Use a model to show students that Biuret detects protein by changing color when it binds to peptide bonds between amino acids.
- 4 Share with students that the kidneys filter waste out of the blood while retaining substances your body needs, such as protein. Share with students that protein should not be detected in the urine and its presence in the urine may indicate a temporary problem, such as dehydration, stress, or strenuous exercise, or a more serious condition involving kidney disease. Share with students that kidney disease can be caused by a variety of other diseases and conditions that often disproportionately affect people of color. Introduce students to [Kidney Disease PSA Assignment](#) in which they create a public service announcement (PSA) in a format of their choice to educate a population of their choice about one disease or condition that may lead to kidney disease. Students may choose from, but are not limited to:
 - diabetes
 - endocarditis
 - heart disease
 - high blood pressure
 - Hodgkin's lymphoma
 - lupus
 - rheumatoid arthritis
 - sickle cell anemia

Their public service announcement may be delivered as:

 - a poster
 - a magazine advertisement
 - an infographic
 - a radio advertisement
 - a television commercial
 - TikTok or social media video

Continues next page >

Day 2

Continued

Procedure

-
- 5 Introduce students to the laboratory experiment they will carry out alongside the project: *Biuret Protein Assay Serial Dilution and Standard Curve*. In the lab, they will use Biuret to test samples of known protein concentration and to establish a standard curve. They will then use the standard curve to analyze urine samples from patients.

Teacher Note > *In case only one spectrophotometer is available, students can work on their projects while they wait for other students to collect lab data. Teachers may decide to have the same student groups conduct the lab and the project or have students conduct the lab in groups and the project individually.*

Small Group or Individual Work (25 minutes)

- 1 Encourage students to choose the disease or condition for their public service announcement and begin their research.
-
- 2 Students should their focus their research on the following:
- causes of the disease or condition
 - prevalence of the disease or condition and, especially if it affects a certain population disproportionately
 - how the disease or condition causes protein to be in the urine
 - other symptoms of the disease or condition
 - treatments for the disease or condition
-
- 3 Remind students that later, they will decide how to educate their target population about the disease or condition.

Whole Group (5 minutes)

You may assign the lab for students to read as homework.

Day 3

LEARNING OUTCOMES

Students will be able to:

Establish a serial dilution and a standard curve with appropriate laboratory materials and techniques.

Evaluate a patient sample using the standard curve.

COMPUTATIONAL THINKING IN ACTION

As students establish a serial dilution and standard curve, they are using the computational thinking strategies of collecting data, analyzing data, and building models to make predictions about whether or not their patient has kidney disease.

Procedure

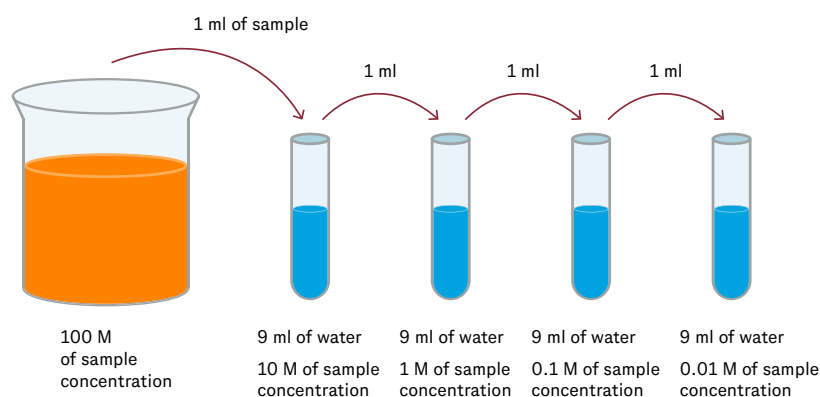
Whole Group (10 minutes)

- 1 Discuss lab with students. Pay particular attention to lab safety.
- 2 Remind students that when they have down time in the lab, they can continue to work on their projects.

Small Group (30 minutes)

- 1 Students will set up a serial dilution beginning with a stock solution of 60 mg of albumin per 50 mL of water.

Serial Dilution



- 2 Students will place 10 mL of each dilution to individual cuvettes, add 10 drops of Biuret solution, and mix carefully.
- 3 Students will use the spectrophotometer to measure the absorbance of each dilution. If no spectrophotometer is available, students can rank the dilutions from darkest to lightest and assign a numeric score.
- 4 Students will use the known protein concentrations and their absorbances to create a standard curve.
- 5 Students will obtain a 24-hour urine collection from a patient and test it in the same way they tested their dilutions. Students will use the standard curve to determine the amount of albumin in the sample. Knowing that more than 30 mg of albumin in the urine indicates kidney disease, students will determine the health status of their patient.

Continues next page >

Day 3

Continued

Procedure

Small Group or Whole Group (5 minutes)

1 Wrap up the day's learning by using one of the strategies below:

- a. **Small Group:** Use the *Silent Appointment* strategy to have students share what they learned with each other.

Silent Appointment: Students make an appointment with a partner using only nonverbal communication without talking. Then, they meet with their partners to discuss their learning. When they are finished listening and sharing, they return immediately to their seats and should be ready to share what they heard from their partners, if called upon.

- b. **Whole Group:** Use *Pick a Stick* to ask students what they learned from their partners.

Pick-a-Stick: To show what they know, only the student whose stick is picked shares his or her thoughts with the class. All are ready to share using turn-taking.



Day 4

Procedure

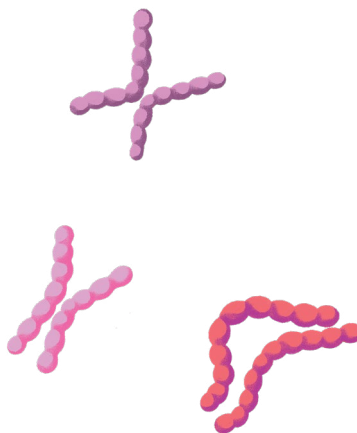
LEARNING OUTCOMES

Students will be able to:

Obtain, evaluate, and communicate information about conditions that cause kidney disease and how they can often affect different sub-populations more than others.

Individual Work (45 minutes)

Students continue to work on their lab and their PSA. Invite students to use their **Design Journal** to capture how content learned in this lesson connects to the culminating project.



Day 5

LEARNING OUTCOMES

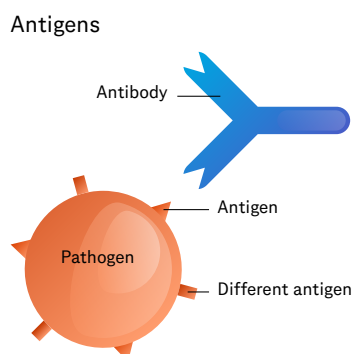
Students will be able to:

Describe how ELISA is used to detect the presence of a specific protein in a sample.

Create a model of ELISA or construct an explanation of ELISA.

COMPUTATIONAL THINKING IN ACTION

The ELISA assay is a tool that scientists use to be able to zero in on a particular antigen within the body. The ELISA assay is an example of how doctors use the computational thinking strategy of abstraction to make diagnoses.



Procedure

Whole Group (15 minutes)

- 1 Remind students that some protein assays, such as the Biuret test, measure the concentration of protein by non-specific means while other protein assays, such as ELISA, measure the concentration of a specific protein.
- 2 Give each student a shape marked “antigen” or a complementary shape marked “antibody.” Allow students to move about the classroom to find the student with the complementary shape and then sit together.
- 3 Explain to students that antigens are molecules produced by pathogens or are parts of the pathogen themselves. Explain to students that antibodies are proteins the immune system makes to recognize pathogens and aid in their destruction. Ask student pairs to discuss the relationship between an antigen and an antibody. Use the *Pick a Stick* strategy to hear from two pairs. Explain to students that ELISA uses the relationship between an antigen and an antibody.
- 4 As a class, watch the University of Michigan video *Enzyme-Linked Immunosorbent Assay (ELISA)*. The teacher will review the history and details of an ELISA test, and then facilitate a class discussion about the uses of ELISA in biomedical testing. Talking points should include:
 - ELISA is used to detect the presence of antigens, such as a toxin or an unknown substance, in a certain sample.
 - Immunofluorescence, which is the basis of the ELISA test, was successfully used to identify antigens in tissue samples in 1941.
 - The modern ELISA test, which uses antibodies to detect the presence of viruses or hormones, was first developed in 1971.
 - ELISA tests have been used as an identifying tool for everything from pregnancy tests to forensic science to HIV and COVID19.
- 5 Have students *Brainstorm* how the use of ELISA testing has affected life in underserved communities either positively and negatively. The talking points from #1, should provide sufficient probing points to lead students to brainstorm constructively on the uses of ELISA testing and its effect on BIPOC communities.

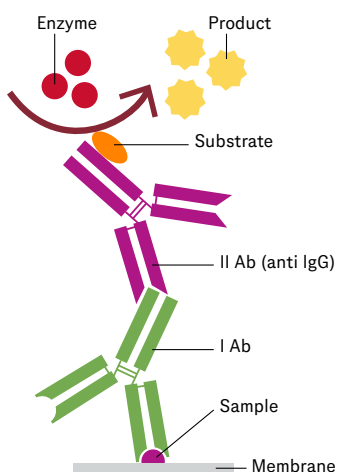
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Day 5

Continued

INDUSTRY AND CAREER CONNECTIONS

Immunologists and lab technicians are only a small number of workers in the bioscience, healthcare, and law enforcement industries who use ELISA testing.



Procedure

Small Group (25 minutes)

- 1 Distribute the *Interactive ELISA Assay Virtual Lab*.
- 2 In groups of two or three, students will log onto the interactive *ELISA Activity*.
- 3 Students will complete all steps of the virtual lab to diagnose a disease based on the immune response.

Individual Work (5 minutes)

As an Exit Ticket, ask students to make a sketch or write a two or three sentence explanation that demonstrates how ELISA uses the relationship between antigens and antibodies to detect the presence of a specific protein in a sample.

Teacher Note > *A sixth day will be needed if students are to share the projects with each other.*

National Standards

**Next
Generation
Science
Standards**

Science and Engineering Practices

Planning and Carrying Out Investigations

Select appropriate tools to collect, record, analyze, and evaluate data.

Crosscutting Concepts

Systems and System Models

Models (e.g., physical, mathematical, computer models) can be used to simulate systems and interactions—including energy, matter, and information flows—within and between systems at different scales.

Patterns

Different patterns may be observed at each of the scales at which a system is studied and can provide evidence for causality in explanations of phenomena.

**Career and
Technical
Education
(CTE)**

A3.0

Demonstrate competencies in the fundamentals of molecular cell biology, including deoxyribonucleic acid (DNA) and proteins and standard techniques for their purification and manipulation.

A3.4

Employ standard protein techniques, including antibody production, enzyme assays, spectrophotometry, gel electrophoresis, chromatography, and document and evaluate results.

Biuret Protein Assay Serial Dilution and Standard Curve**ANSWER KEY****Do not share with students**

1. Complete the serial dilutions chart of the unknown and standard samples below ranging in concentration from 0 to 10 mg/mL so that the final volume in each assay tube is 3.0 mL.

Tube	Albumin Protein Solution concentration (mg/mL)	Deionized water (dH ₂ O) in mL	Albumin Protein Solution amount (mL)	Biuret Reagent amount (mL)	Total volume in Tube (mL)
1	0	1.0 mL	0 mL	2.0 mL	3.0 mL
2	1	0.9 mL	0.1 mL	2.0 mL	3.0 mL
3	2	0.8 mL	0.2 mL	2.0 mL	3.0 mL
4	3	0.7 mL	0.3 mL	2.0 mL	3.0 mL
5	4	0.6 mL	0.4 mL	2.0 mL	3.0 mL
6	5	0.5 mL	0.5 mL	2.0 mL	3.0 mL
7	6	0.4 mL	0.6 mL	2.0 mL	3.0 mL
8	7	0.3 mL	0.7 mL	2.0 mL	3.0 mL
9	8	0.2 mL	0.8 mL	2.0 mL	3.0 mL
10	9	0.1 mL	0.9 mL	2.0 mL	3.0 mL
11	9.5	0.05 mL	0.95 mL	2.0 mL	3.0 mL
12	10	0.00 mL	1.0 mL	2.0 mL	3.0 mL

← For your reference

Continues next page >

Biuret Protein Assay Serial Dilution and Standard Curve**ANSWER KEY****Do not share with students***Continued*

2. Describe what you think the series of tubes will resemble in a tube rack after all of the solutions are made?

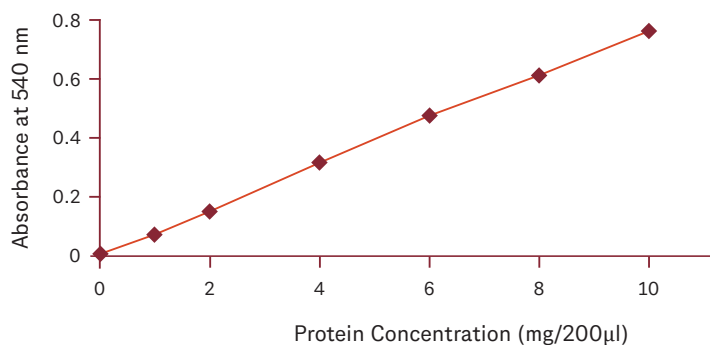
The twelve tubes will have progressively darker colors. Tube 1, which is the reference tube, should be clear since there is no protein (therefore no peptide bonds present). In Tubes 2 to 10, there should be increasingly darker shades of violet. Tube 10 should be the darkest because it has the greatest amount of protein solution.

- 3a. Using the data collected in the chart below, construct a standard curve graph.

Concentration (mg/mL)	Standard Absorbance	Sample Absorbance
0	0.00	0.00
1	0.01	0.02
2	0.04	0.09
3	0.07	0.20
4	0.09	0.26
5	0.10	0.31
6	0.12	0.39
7	0.15	0.46
8	0.20	0.59
9	0.24	0.73
9.5	0.35	0.97
10	0.44	1.36

- 3b. Construct a standard curve graph below.

Student constructed standard curve should resemble something like below:



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Biuret Protein Assay Serial Dilution and Standard Curve

Background

The Biuret test is a general test for compounds having a peptide bond. Biuret is a compound formed by heating urea to 180°C. When Biuret is treated with dilute copper sulfate in alkaline condition, a purple-colored compound is formed. This is the basis of the Biuret test widely used for the identification of proteins and amino acids.

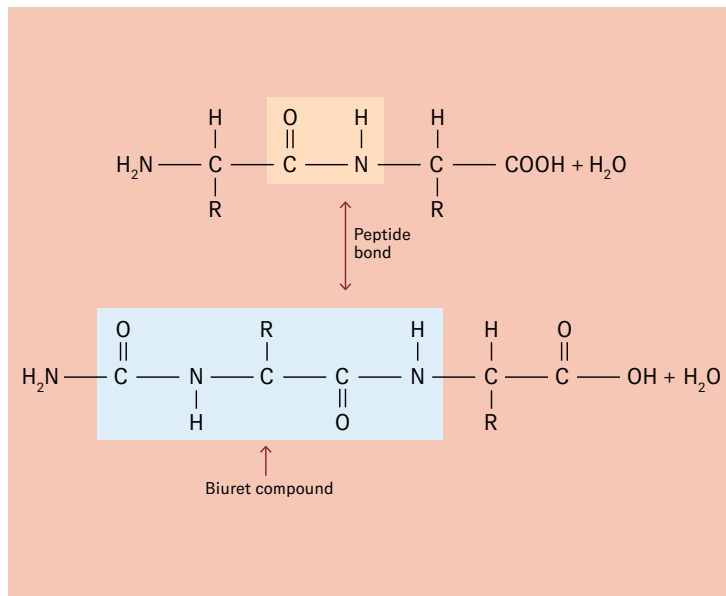
Serial dilutions involve a sequence of dilutions used to make a more concentrated solution into a less concentrated, but more usable solution. It is a stepwise process that is commonly performed in either a two-fold or ten-fold manner.

Directions

Complete the serial dilutions chart of the unknown and standard samples below ranging in concentration from 0 to 10 mg/mL so that the final volume in each assay tube is 3.0 mL. The presence of peptide bonds in the Biuret assay, causes the solution in the tube to turn violet. The shade of violet gets darker with increasing concentrations of peptide bonds.

Continues next page >

Chemical Reaction in the Biuret test



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Biuret Protein Assay Serial Dilution and Standard Curve

Continued

- Complete the serial dilutions chart of the unknown and standard samples below ranging in concentration from 0 to 10 mg/mL so that the final volume in each assay tube is 3.0 mL.

Tube	Albumin Protein Solution concentration (mg/mL)	Deionized water (dH ₂ O) in mL	Albumin Protein Solution amount (mL)	Biuret Reagent amount (mL)	Total volume in Tube (mL)
1	0	1.0 mL	0 mL	2.0 mL	3.0 mL
2	1	0.9 mL	0.1 mL	2.0 mL	3.0 mL
3					3.0 mL
4		0.7 mL			
5					
6					
7					
8					
9					
10					
11	9.5	0.05 mL	0.95 mL		
12		0.00 mL	1.0 mL	2.0 mL	3.0 mL

← For your reference

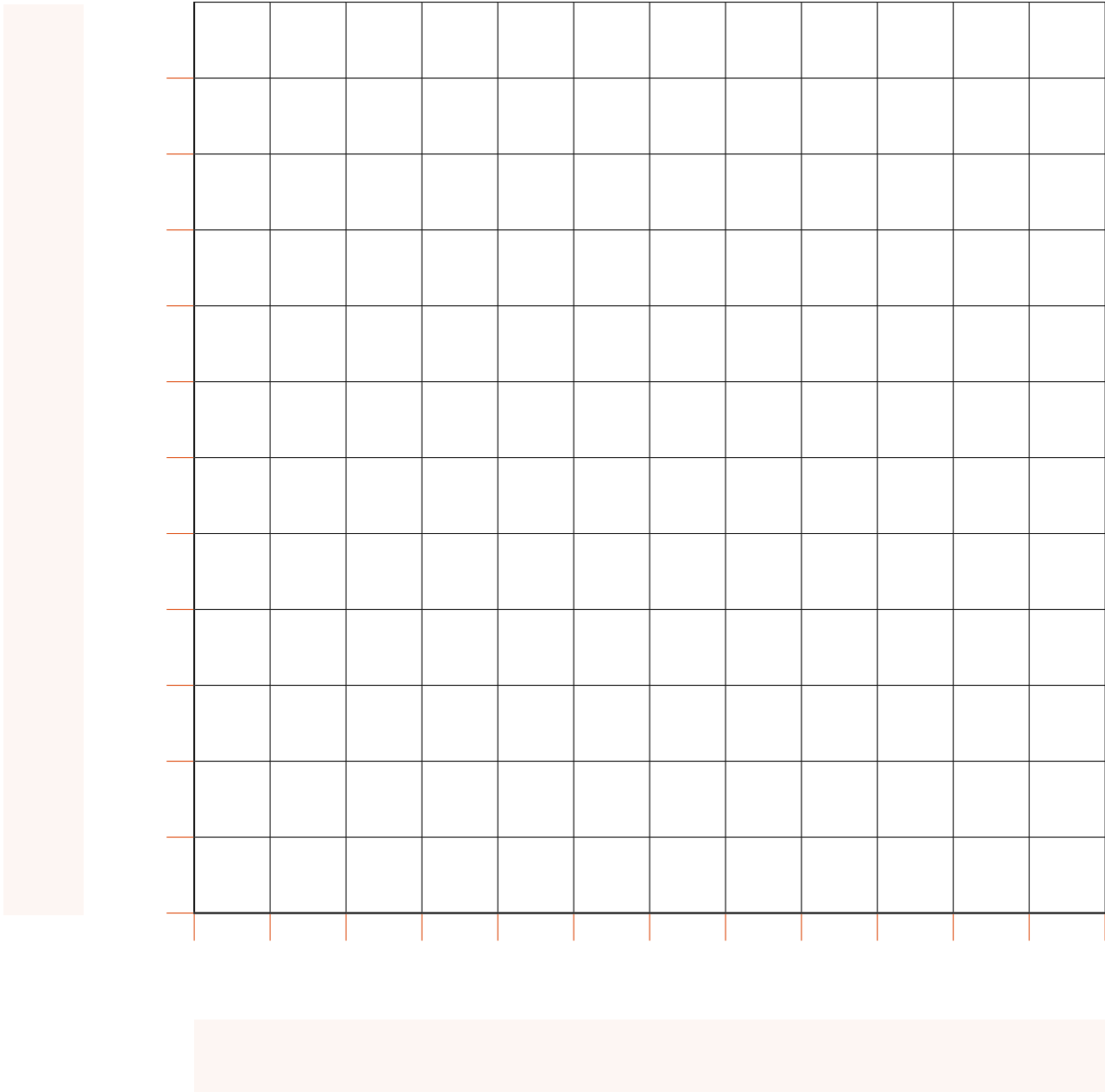
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Biuret Protein Assay Serial Dilution and Standard Curve

Continued

3b. Using the data collected in the chart on the previous page, construct a standard curve graph below.



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Kidney Disease PSA Assignment

Overview

The kidneys filter waste out of the blood while retaining substances the body needs, such as protein. Protein should not be detected in the urine. Its presence in the urine may indicate a temporary problem, such as dehydration, stress, or strenuous exercise, or a more serious condition involving kidney disease. Kidney disease can be caused by a variety of diseases and conditions that often disproportionately affect people of color.

Directions

Your task is to create a public service announcement (PSA) in a format of your choice to educate a population of your choice about one disease or condition that may lead to kidney disease.

You may choose from, but are not limited to:

diabetes	endocarditis
heart disease	high blood pressure
Hodgkin's lymphoma	lupus
rheumatoid arthritis	sickle cell anemia

Your PSA may be delivered as:

a poster	a magazine advertisement
an infographic	a radio advertisement
a television commercial	TikTok video

Your research should focus on the following:

1. causes of the disease or condition
2. prevalence of the disease or condition and, especially if it affects a certain population disproportionately
3. how the disease or condition causes protein to be in the urine
4. other symptoms of the disease or condition
5. treatments for the disease or condition

Remember that the goal is to educate your target population about the disease or condition.

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Kidney Disease PSA Rubric

Score	4	3	2	1
Required Elements	PSA includes all required elements as well as additional information.	All required elements are included in the PSA.	Most of the required elements are included in the PSA.	Several required elements are missing.
Description of Test	Demonstrates a full grasp of the topic, presenting complete and accurate information.	Demonstrates understanding of the topic; presents accurate information.	Lacks important details and/or presents inaccurate information.	Does not have a grasp of information, many statements are incorrect and unsupported.
Overall Appearance	Project appears professional and polished, no obvious errors.	Effort made toward appearance, minimal errors, attention to detail.	Some effort made toward appearance, errors present but not distracting, some attention to detail.	Project appearance is sloppy, lacks attention to detail, and has numerous mistakes.
Target Audience	The combination of creativity, disease information, and call to action are very effective in delivering a strong message to the target audience.	The combination of creativity, disease information, and call to action delivers a clear message to the audience.	The intent of the PSA is understood, but is only somewhat engaging and has little motivational value.	The message is not clear in the PSA; it does not engage or reach the audience.
Final Score				

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Interactive ELISA Assay Virtual Lab

Directions

Access [The Virtual Immunology Lab](#) using a device connected to the internet, and conduct the lab.

1. Once at the home page, read the information under the “Diagnosis” and “Background” tabs (feel free to take notes in your notebook to prevent having to toggle back and forth to these tabs once you have started the virtual lab).
2. After pressing the “Notebook” tab, press “click here” to start the lab. If you need to pause, you will be able to resume the lab by pressing the “resume” button.
3. Glossary terms are found by pressing the “Glossary” tab.
4. Using the “Help” tab may answer some questions about this lab should they present themselves.
5. After finishing your virtual lab, work with a partner to complete the following reflection as an Exit Ticket:
 - What did you like about today’s virtual lab?
 - What would you do to improve or change it?
 - Were there opportunities in this virtual lab to incorporate the use of the ELISA test in the cycle of product development of drugs and therapeutics? Explain.



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