

## TEACHER GUIDE

### A Look inside the Lab: ELISA Plate Reader

#### OVERVIEW

The VMP Next Step Science resources provide mini lessons, videos, and other materials related to the practice of science. These resources are meant to enhance the exploration of a particular topic or offer insights into the profession of scientific research.

The Next Step Science “A Look inside the Lab” series is comprised of reading passages and videos designed to introduce students to pieces of laboratory equipment and the scientists who use them. The series can be used to enhance an existing lesson or as a starting point for introducing a concept.

Each mini lesson includes:

- Short video featuring scientists from the Children’s Hospital of Philadelphia Research Institute discussing their work and how they utilize the lab equipment on a day-to-day basis
- Related reading passage
- Teacher guide
- Student worksheet

Additional VMP “A Look inside the Lab” videos and materials can be found at [vaccinemakers.org/next-step-science](http://vaccinemakers.org/next-step-science).

#### OBJECTIVES

“A Look inside the Lab” series activities are designed to:

- Introduce students to equipment commonly used in medical research laboratory settings and explore how the technology impacts and serves society
- Introduce students to scientists, science careers, and the types of investigations that scientists conduct on a day-to-day basis
- Provide an opportunity for students to read informational text about scientific topics to enhance their understanding of how science is done and consider how scientists develop possible solutions to problems

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ELISA lesson objectives:

- Students will understand the different types of ELISAs.
- Students will be able to explain how the ELISA plate reader works and why it is a useful laboratory tool.

### LESSON RESOURCES

- Lesson video, *A Look inside the Lab: ELISA Plate Reader*, <https://vimeo.com/530455118>
- Video transcript PDF: [https://vaccinemakers.org/sites/default/files/resources/Look in Lab ELISA video transcript FINAL.pdf](https://vaccinemakers.org/sites/default/files/resources/Look%20in%20Lab%20ELISA%20video%20transcript%20FINAL.pdf)
- Reading passage, *The Common Features of ELISAs and Sandwiches*, [https://vaccinemakers.org/sites/default/files/resources/Look Lab ELISA reading%20passage FINAL.pdf](https://vaccinemakers.org/sites/default/files/resources/Look%20Lab%20ELISA%20reading%20passage%20FINAL.pdf)
- Student worksheet: [https://vaccinemakers.org/sites/default/files/resources/Look Lab ELISA worksheet FINAL.pdf](https://vaccinemakers.org/sites/default/files/resources/Look%20Lab%20ELISA%20worksheet%20FINAL.pdf)
- Resources related to ELISA
  - An Introduction to the Enzyme-Linked Immunosorbent Assay – ELISA Test, article, Technology Networks, <https://www.technologynetworks.com/analysis/articles/an-introduction-to-the-enzyme-linked-immunosorbent-assay-elisa-test-350024>
  - Enzyme-Linked Immunosorbent Assay (ELISA), animation, Open.Michigan, <https://youtu.be/RRbuz3VQ100>
  - An introduction to the different types of ELISA tests, blog, Integra, <https://www.integra-biosciences.com/united-states/en/blog/article/introduction-different-types-elisa-tests>
  - ELISA Detection Strategies, article, Future Lab, <https://www.biocompare.com/Editorial-Articles/591588-ELISA-Detection-Strategies/>
  - Enzyme-Linked Immunosorbent Assay (ELISA), information page, Molecular Devices, <https://www.moleculardevices.com/applications/enzyme-linked-immunosorbent-assay-elisa#sandwichElisa>
  - Four Types of ELISA, information page, Cusabio, <https://www.cusabio.com/c-20659.html>
  - ELISA Plate Reader (Microplate Reader or Assay Reader), article, Microbe Notes, <https://microbenotes.com/elisa-plate-reader-microplate-assay-reader/>
  - Overview of ELISA, information page, ThermoFisher Scientific, <https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology->

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- [learning-center/protein-biology-resource-library/pierce-protein-methods/overview-elisa.html#:~:text=Washing%20steps%20are%20necessary%20to,or%20antigen%20from%20the%20well](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2526547/)
- The ELISA Standard Save: Calculation of sample concentrations in assays with a failed standard curve, article, The Journal of Immunological Methods, <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2526547/>
  - ELISA resources from Bio-Rad Explorer:
    - ELISA – A Complete Introduction, <https://www.bio-rad-antibodies.com/an-introduction-to-elisa.html>
    - ELISA Disease Detection Modeling, PPT, <https://www.bio-rad.com/webroot/web/pdf/lse/literature/ELISA%20Disease%20Detection%20Modeling.pdf>
    - How To Perform A Quantitative ELISA, video, <https://youtu.be/cLVKB4MPVMA>
  - Resources related to ELISA plate reader mechanism
    - Microplate readers, info page, Hözel Diagnostika, <https://www.hoelzel-biotech.com/en/hoelzel-life-science-lexicon/basics/application-basics/microplate-reader-basics.html>
    - Optics – The mechanism inside the plate reader, video, Well Explained by Tecan, <https://youtu.be/ABhUbC9AugQ>
    - Microplate Reader VS Spectrophotometer: What’s the difference? blog post, The Lab World Group, <https://www.thelabworldgroup.com/blog/microplate-reader-vs-spectrophotometers/>
    - Principle And Applications Of Microplate Reader, info page, SMACgig WORLD, <https://www.smacgigworld.com/blog/principle-and-applications-of-microplate-reader.php>
    - “Basics of Assay Equipment and Instrumentation for High Throughput Screening,” book chapter, National Library of Medicine, <https://www.ncbi.nlm.nih.gov/books/NBK92014/>

## LESSON

The lesson progression outlined below can be completed in two to four 50-minute class sessions. If the “Extension” activity is included, additional time may be required.

### Engage

Time: 10-15 minutes

Have students:

- Watch the short video, *A Look inside the Lab: ELISA Plate Reader*
- Brainstorm ways that they think ELISAs can be used based on the video content and their own ideas.

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### Explore

Time: 25-45 minutes

Have students:

- Read the passage, *The Common Features of ELISAs and Sandwiches*, which provides an overview of how ELISAs detect and measure a protein in a sample and different types of ELISAs.
- Work individually or in small groups to explore lesson resources related to ELISAs.
- Complete the student worksheet including the vocabulary list and questions.

Vocabulary list:

antibody, antigen, assay, ELISA, direct ELISA, indirect ELISA, sandwich ELISA, competitive ELISA, enzyme, immunoassay, microplate reader, primary antibody, secondary antibody, spectrophotometry, standard curve, substrate

Worksheet questions:

- List the four different types of ELISAs and illustrate the order of layers for each one.
- What is the difference between a qualitative and a quantitative ELISA?
- Explain the importance of washing the wells between each step when conducting an ELISA.
- How is the standard curve used to analyze the ELISA test results?

### Explain

Time: 20-30 minutes

Have students:

- Work in small groups to create presentations on one type of ELISA. Groups can choose which they want to feature or the teacher can assign the type to ensure presentations on each type.

### Elaborate

Time: 20-30 minutes

- Work in small groups to research the mechanics of how the ELISA plate reader works. Students can be given a general directive to research the mechanics of how an ELISA plate reader works or, depending on the class and other topics that have been covered, they may be directed to focus on how ELISA plate readers employ the physics of light and color to generate information.

### Evaluate

Time: 25-45 minutes

Work in small groups to create a learning tool (infographic, poster, skit, etc.) to describe what was learned during the small group research activity.

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### Extension (Optional)

Time: Variable

Have students:

- Engage in the process of performing an ELISA protocol using a classroom activity kit, such as the ELISA Immuno Explorer Kit from Bio-Rad which offers three protocol options:
  - Protocol I: ELISA for Tracking Disease Outbreaks
  - Protocol II: Antigen Detection ELISA
  - Protocol III: ELISA Antibody Test

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**RUBRIC: STUDENT WORKSHEET – Lesson Vocabulary**

Antibody	Y-shaped proteins produced by B cells that are specific to a particular pathogen and can neutralize it. Five different classes of antibodies occur which have distinct functions.
Antigen	Part of a pathogen that generates an immune system response because it is recognized by cells of the immune system.
Assay	A procedure used in a biology lab to generate data related to an experimental hypothesis.
ELISA	Stands for Enzyme-Linked Immunosorbent Assay, this is a type of assay used to detect quantities of a protein, antibody, or other biological component in samples.
Direct ELISA	An ELISA assay that uses a labeled antibody to bind directly to the protein or antigen of interest.
Indirect ELISA	An ELISA assay that detects a protein or antigen of interest using a two-step method. The first step binds an antibody to the protein or antigen of interest, and the second step binds a second antibody to the first antibody. The second antibody has a fluorescent label that allows it to be detected in the ELISA assay.
Sandwich ELISA	An ELISA assay that quantifies the amount of antigen in a sample by using two antibodies. The first antibody is used to bind to the protein or antigen in a sample, and the second antibody is labeled, so that it can be detected in the ELISA assay. The labeled antibody is added at the end to bind protein or antigen that was bound by the first antibody.
Competitive ELISA	An ELISA assay that measures the amount of protein or antigen in a sample by adding and measuring a competitor antibody. In this manner, the more color that develops, the lower the amount of protein or antigen present in the sample.
Enzyme	A substance that increases the speed of a chemical reaction.
Immunoassay	A procedure to measure or detect specific molecules in a sample, such as proteins.
Microplate reader	A tool used in the laboratory to measure the concentration of color in samples in a microplate. A type of spectrophotometer.
Primary antibody	As used in an ELISA, these are antibodies that bind directly to the antigen or protein of interest.
Secondary antibody	As used in an ELISA, these antibodies bind to a primary antibody and are typically labeled with a fluorescent marker, so that the amount of protein or antigen of interest in a sample can be measured.
Spectrophotometry	A laboratory technique used to measure how much light a sample absorbs. Allows scientists to determine the concentration of labeled target molecule in the sample.
Standard curve	A graph that allows scientists to determine the concentration of a sample by comparing it to samples of known concentration.
Substrate	A molecule that reacts with an enzyme.

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**RUBRIC: STUDENT WORKSHEET – Lesson Questions**

1. List the four different types of ELISAs and illustrate the order of layers for each one.

*Teacher note:* You may choose to have students either verbalize or draw and label the order of layers. Illustrations showing the order can be found on the Intergra Biosciences page: <https://www.integra-biosciences.com/united-states/en/blog/article/types-of-elisa-tests>

- Direct ELISA
- Indirect ELISA
- Indirect Sandwich ELISA
- Competitive direct ELISA

2. What is the difference between a qualitative and a quantitative ELISA?

Answers may vary. Student responses should include that qualitative ELISAs provide a positive or negative result, whereas quantitative ELISAs offer an indication of how much of the target protein or antigen is present in the sample.

3. Explain the importance of washing the wells between each step when conducting an ELISA.

Answers may vary. Students should be able to articulate that washing the wells when conducting an ELISA is important to maintain test sensitivity by removing non-bound reagents and reduce background noise.

4. How is the standard curve used to analyze the ELISA test results?

Answers may vary. Students should articulate that a standard curve is used in quantitative ELISAs to determine the protein concentration in a sample by comparing the light absorbance of the ELISA sample to a protein sample of known concentration.