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Edvo-Kit # 279

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Investigating Human Health Using the ELISA

Experiment Objective:

In this experiment, students will perform an Enzyme-Linked Immunosorbent Assay (ELISA) to examine the impact of this powerful test on human health. Antibodies will be used to detect miniscule amounts of antigens and determine the status of simulated samples. Three different scenarios can be explored, including pregnancy testing, early detection of heart attacks, and identification of gluten in food products.

See page 3 for storage instructions.

Version 279.190729

EDVOTEK®

Table of Contents

Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets

Experiment Components

COMPONENTS **Store components A-G in the refrigerator.**		Check (✓)
A	10X ELISA Wash Buffer	❑
B	ELISA Dilution Buffer	❑
C	Antigen (lyophilized)	❑
D	Primary Antibody (lyophilized)	❑
E	Secondary Antibody (lyophilized)	❑
F	ABTS (lyophilized)	❑
G	ABTS Reaction Buffer	❑

REAGENTS & SUPPLIES **Store all components below at room temperature.**	Check (✓)
• Microtiter plates	❑
• Transfer pipets	❑
• Snap-top microcentrifuge tubes	❑
• 15 mL conical tubes	❑

Experiment #279 is designed for 10 lab groups.

Three scenarios are included in this experiment - each group will choose one to perform.

Requirements *(not included with this kit)*

- Distilled or deionized water
- Beakers
- Disposable lab gloves
- Safety goggles
- Recommended: Automatic micropipettes (50 µL) and tips

Make sure that glassware is clean, dry, and free of soap residue. For convenience, additional disposable transfer pipets can be purchased for liquid removal and washing steps.

None of the components have been prepared from human sources.
All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

Background Information

ANTIBODIES

Antibodies (also called immunoglobulins, or Igs) are specialized proteins that allow the immune system to distinguish between "self" and "non-self" molecules, known as antigens. These Y-shaped molecules comprise four linked polypeptide chains: two identical "heavy chains" and two identical "light chains" (Figure 1). The antigen binding sites are located at the ends of the short arms of the Y. The amino acid sequence in this region is variable, allowing for each antibody to recognize a unique epitope (a particular location within an antigen).

To create the antibodies used in scientific research, scientists inject animals (i.e. rabbits, goats, and guinea pigs) with the antigen of interest. In response to the injection, the animal's immune cells create multiple antibodies that recognize different epitopes of the antigen. Blood is recovered from the immunized animal, and the antibody-containing serum is separated from the blood cells. The serum, known as a **polyclonal antibody**, contains a heterogeneous mixture of antibodies that recognizes multiple regions of the antigen. If individual immune cells from these animals are isolated and cultured, scientists can create **monoclonal antibodies**. These antibodies recognize a single epitope and thus are very specific.

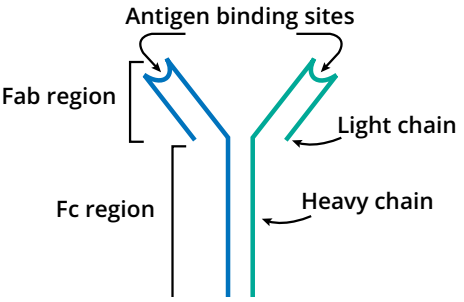


Figure 1:
General Structure of an antibody.

Because of their specificity, researchers can use antibodies to detect the presence of specific biomolecules (i.e. peptides, proteins, antigens and hormones) in a complex sample. One of the most common antibody-based assays is the Enzyme Linked ImmunoSorbent Assay (ELISA). The ELISA uses antibodies to detect a specific compound within a solution. The assay produces a signal that is easy to detect and to quantify, even when starting with complex mixtures like cellular lysates.

THE ELISA

ELISAs were originally developed to measure the quantity of antibodies in a solution, but have since been adapted to detect many different types of antigens. Traditional ELISAs require two antibodies. The first antibody, called the "primary antibody", recognizes the antigen of interest. For example, an ELISA that is designed to detect allergens in food might be designed to use an antibody that recognizes a protein found in peanuts. In an industrial setting this ELISA will be used to screen for foods that are allergen-free before packaging.

The "secondary antibody" in an ELISA recognizes the primary antibody – if mouse immune cells were used to produce the primary antibody we would use a secondary antibody that specifically recognizes mouse antibodies. The secondary antibody is covalently linked to an enzyme called Horseradish Peroxidase (HRP) that lets us detect the presence of the antibody-antigen complex (Figure 2A). HRP has a high catalytic activity – its substrate turnover rates exceed 10^6 per second – allowing us to quickly detect even the smallest amount of antigen.

To perform an ELISA, the samples are added to the wells of transparent microtiter plates made of polystyrene or polyvinyl chloride plastics. During this step the antigens are allowed to bind to the surface through hydrophobic associations (Figure 2B). This step is non-specific – every antigen in the sample can potentially bind to the plastic surface. In a food allergy ELISA, the antigens will be samples of the food in question that are pulverized and dissolved in a solution. After a brief incubation the wells are washed to remove unbound antigens.

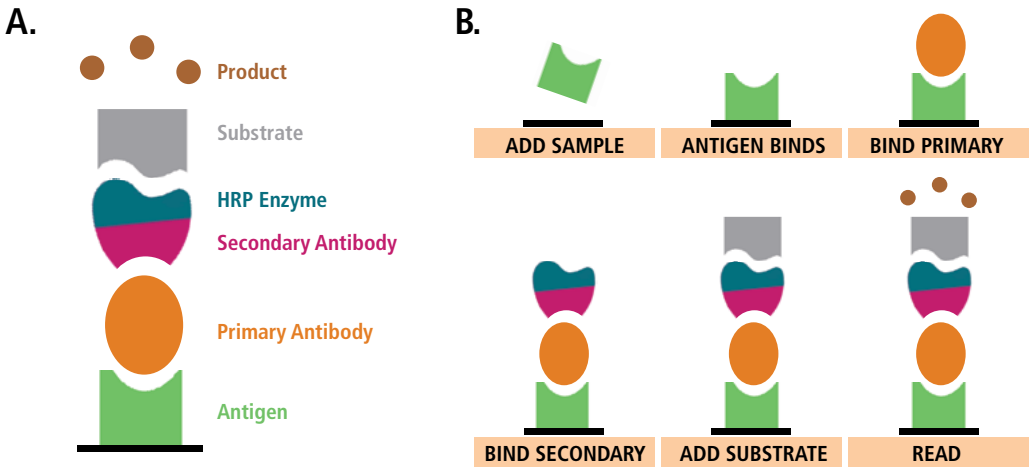


Figure 2: Optimized ELISA workflow.

Next, a primary antibody is added to each well and is allowed to incubate for a short time. If the target antigen is present in the well the antibody will recognize it and bind. Following the incubation period, the wells are washed to remove any primary antibody that did not bind with the antigen.

After the wash, an enzyme-linked secondary antibody is added to the wells where it recognizes and binds to the primary antibody (if present). Importantly, if there was no primary antibody in the initial sample there will be nothing for the secondary antibody to bind. As before, the excess antibody is removed from the wells by washing with buffer. If the secondary antibody has bound to the primary antibody, it will stay in the well.

Finally, a clear, colorless solution of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) and hydrogen peroxide is added to each well. The HRP enzyme on the secondary antibody oxidizes ABTS in wells where the antigen-antibody complex is present, turning the clear substrate solution blue-green. Since each enzyme breaks down many substrate molecules, the ELISA can detect even the smallest amount of antigen. While the color change from clear to blue-green is detectable by eye, measuring the sample's absorbance at 405 nm provides a quantitative result.

SCENARIO I: IMMUNOLOGY OF PREGNANCY TESTS

Home pregnancy tests have become an important tool for the initial detection of pregnancies. In many cases these tests help to ensure a healthy and safe pregnancy for both the mother and the baby. For example, many lifestyle, medical, and dietary changes might be necessary during a pregnancy. Most modern pregnancy tests detect the presence of human chorionic gonadotropin (hCG) in a urine sample. A positive result for hCG indicates that the woman is very likely pregnant. In this scenario you will perform an ELISA to simulate a common pregnancy test on patient samples.

In sexual reproduction, haploid gametes from two individuals unite to form a diploid cell known as the zygote. Within minutes, the chromosomal DNA replicates and the zygote divides into two cells. As the cells continue to replicate their DNA and to divide, they specialize and form tissues that develop into an adult organism. During this time, the growing embryo must be kept in a carefully regulated environment to ensure proper development. In humans and other mammals, the female carries the developing fetus in her body where it is protected and nourished. This time is known as pregnancy.

While human males are continually producing gametes (sperm), human females produce a single mature gamete (egg) approximately once every 28 days. Hormonal cues synchronize the release of the egg with the preparation of the uterus, the organ that supports embryonic development. If the egg is fertilized, additional hormones change the female's physiology to

better support the growing fetus. During this time, the fetus establishes germ layers, builds tissues and organs, and grows at a rapid pace. After approximately 40 weeks, the female delivers the offspring to the outside world.

Pregnancy Testing

The term "pregnancy test" is actually is a misnomer. Most pregnancy tests do not actually determine pregnancy, but rather the level of human chorionic gonadotropin (hCG) in the blood or urine. This hormone is a glycoprotein, which is made up of two subunits, the alpha subunit, which has a molecular weight of 18,000, and the beta chain, which has a molecular weight of 32,000. hCG is produced throughout pregnancy by the placenta and is essential for a successful pregnancy. It should be noted that several glycoprotein hormones such as hCG, luteinizing hormone and follicle-stimulating hormone share a common alpha subunit. The pregnancy test detects only the unique beta-subunit of hCG. Since hCG is only produced in the developing embryo (except in rare cases of secretion of hCG by hydatidiform moles or choriocarcinoma), and since it is detectable within a few days of implantation, it is a very specific and early test for pregnancy.

In this scenario, students will use the ELISA to test simulated urine samples for hCG. First, urine samples from patients are added to the wells and any antigens are allowed to bind through hydrophobic interactions with the plastic. After washing, any hCG present in the samples can be bound by an anti-hCG primary antibody. Next, a secondary antibody that has been cross-linked to horseradish peroxidase is used to detect the primary antibody. Finally, a substrate will be used to detect if the secondary antibody is present in the wells. A color change is only possible if hCG was contained in the original urine sample, allowing for the primary and secondary antibodies to bind. At the conclusion of this experiment, students will examine their results and determine the pregnancy status of two hypothetical patients.

SCENARIO II: HEART ATTACK DETECTION BY ELISA

In patients suspected of having a heart attack, clinical blood tests are performed to examine levels of cardiac Troponin proteins. Following damage to cardiac muscles, Troponin proteins are released into the blood, where even a slight increase in protein is detectable. In this scenario an ELISA will be run to examine "patients" for evidence of a heart attack.

Detecting a Heart Attack in a Patient

Heart attacks, or myocardial infarctions, are one of the leading causes of death in the developed world. In the United States approximately 1 million people suffer from a heart attack each year. The most common symptoms of a heart attack include sudden chest pain, shortness of breath, sweating, and fatigue, although many patients experience only minor sensations. Rapid detection and treatment of heart attack patients is critical to minimize damage to the heart and prevent further complications or death.

During a heart attack, the cardiac muscle suffers injury due to a lack of oxygen to the heart tissues. Most heart attacks are due to a blockage of fat and cholesterol within a coronary artery, leading to reduced blood flow over time. Eventually the amount of blood traveling through the arteries is reduced to the point where it can no longer support the heart cells and a patient will experience a heart attack. During a heart attack, oxygen-deprived heart cells rapidly die and, unlike the cells in many other tissues, can never grow back. This results in a permanently scarred area on the heart, which can lead to additional complications in the future.

Injured heart cells will undergo the process of necrosis, or cell death, rupturing and releasing their cellular components into the surrounding area. This can injure neighboring cells, increasing the area of damaged tissues. In addition, the dying heart cells will release proteins that can enter the bloodstream and travel systemically throughout the body. This is useful to doctors who can perform blood tests to monitor the levels of cardiac proteins, such as troponin and creatine kinase, for evidence that a heart attack has taken place.

Troponin is a complex of three regulatory proteins (troponin C, troponin I, and troponin T) that is integral to muscle contraction in skeletal muscle and cardiac muscle, but not smooth muscle. Certain subtypes of troponin (specifically troponin I and T) are very sensitive and specific indicators of damage to the heart muscle. A person suffering from a heart attack will

have an area of damaged heart muscle, leading to elevated cardiac troponin levels in the blood. This increase in troponin starts within 2-3 hours, peaks in approximately 24 hours, and persists for 1-2 weeks. In a clinical setting, blood levels of both cardiac troponin T and I can be rapidly measured by immunoassay methods.

It is important to note that cardiac troponins are a marker of all heart muscle damage, not just myocardial infarction, which is the most severe form of heart disorder. An elevated troponin level can identify patients at high risk for adverse cardiac events, and elevated levels have been associated with an increase in cardiac mortality rate.

This experiment replicates a clinical screen to detect the presence of cardiac Troponin I in simulated patient blood samples. Students will incubate the simulated patient samples in a microtiter plate to allow for any antigens to bind. They will then wash each well to remove unadsorbed proteins, and then incubate with an anti-Troponin I antibody. An HRP-linked secondary antibody is then added to detect primary antibodies, if present. Finally, substrate is added to each well and monitored to determine the status of the assay. Patients who have recently suffered from a heart attack will show elevated levels of Troponin I, revealing damage to cardiac cells. At the conclusion of the experiment students will provide a diagnosis for each patient.

SCENARIO III: DETECTION OF GLUTEN IN FOOD PRODUCTS

Celiac disease is an immune disorder that causes problems in the small intestine which can lead to serious health complications. Patients with celiac must maintain a strict gluten-free diet, making informed dietary choices an essential part of their daily life. Many products are now tested and certified as "gluten-free", providing a greater range of choices and freedom for consumers. In this scenario, you will run an ELISA to test simulated food samples for the presence of gluten and then make recommendations for a patient with celiac disease.

Celiac disease is a serious digestive disorder that afflicts approximately 1 in every 141 Americans. Patients with celiac must carefully regulate their diet to avoid gluten, a protein found in wheat, rye, and barley. Gluten can comprise up to 80% of the total proteins in the grain, and is typically processed by enzymes in the digestive system. Unfortunately, in patients with celiac, dietary gluten triggers an immune response that can lead to pain, bloating, and severe damage to the small intestine if untreated.

Celiac disease is often incredibly difficult to diagnose. Many of the symptoms are shared with other digestive disorders, such as lactose intolerance, irritable bowel syndrome, or Crohn disease. Doctors will generally order a blood test as an initial screen, followed by an intestinal biopsy to confirm. The blood test will look for the presence of antibodies against endomysium or tissue transglutaminase, two markers of damage to the intestinal epithelial cells. A positive result on the blood test will almost always be followed up by a biopsy to confirm that the patient has celiac disease.

Treatment for celiac disease involves following a strict gluten-free diet. This means that the patient must completely remove all gluten from the foods and drinks that they consume, including anything with wheat, barley, rye, and spelt. Unfortunately, this is often challenging since many products can be unexpected sources of gluten. For example, gluten is commonly found in processed foods, is used in sauces as a thickening agent, and can even be found in lipsticks or other cosmetics (Table I). Since even miniscule quantities of gluten can cause serious reactions it is essential that products are tested and confirmed to be gluten-free.

In addition to individuals with celiac disease there are other people with non-celiac gluten sensitivity (NCGS). These patients are negative for celiac disease but still show varying levels of sensitivity to wheat products. Although little is known about the causes of NCGS, many of these individuals report better health by avoiding gluten.

TABLE 1:
Foods that might contain gluten

- Ice cream
- Corn bread
- Soy sauce
- Granola and granola bars
- Processed meats and meat substitutes
- Candy bars
- Malt vinegar
- Salad dressing
- Baking powder and spice blends

Due to the number of people requiring gluten-free diets many food products are now tested to ensure that they are gluten-free. To entice customers, many companies have started to test and label their products. Unfortunately, there is no current standard for what constitutes "gluten-free". The U.S. Food and Drug Administration requires foods to contain less than 20 parts per million (ppm) gluten, while many celiac support organizations recommend even lower levels. In all cases, determining what is best for the patient should be decided after a discussion with a doctor.

In this scenario students will be performing an ELISA to simulate the certification of various products as gluten-free. The simulated food samples will be added to the wells of a microtiter plate and allowed to bind during a brief incubation. Next, an anti-gluten primary antibody will be added to each well. If gluten was present in the food sample the antibody will remain bound to the wells after washing. Next, an HRP-linked secondary antibody will be added to detect primary antibodies, if present. Finally, substrate is added to each well and monitored for a color-change reaction. Samples that are positive for gluten will change from a colorless to green. At the conclusion of the assay the students will determine which food samples should be certified as "gluten-free".

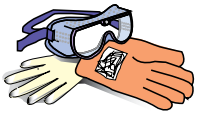
Experiment Overview

EXPERIMENT OBJECTIVE:

In this experiment, students will perform an Enzyme-Linked Immunosorbent Assay (ELISA) to examine the impact of this powerful test on human health. Antibodies will be used to detect miniscule amounts of antigens and determine the status of simulated samples. Three different scenarios can be explored, including pregnancy testing, early detection of heart attacks, and identification of gluten in food products.

LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS OR BULBS.
3. Always wash hands thoroughly with soap and water after handling contaminated materials.



Wear gloves and safety goggles

LABORATORY NOTEBOOKS:

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

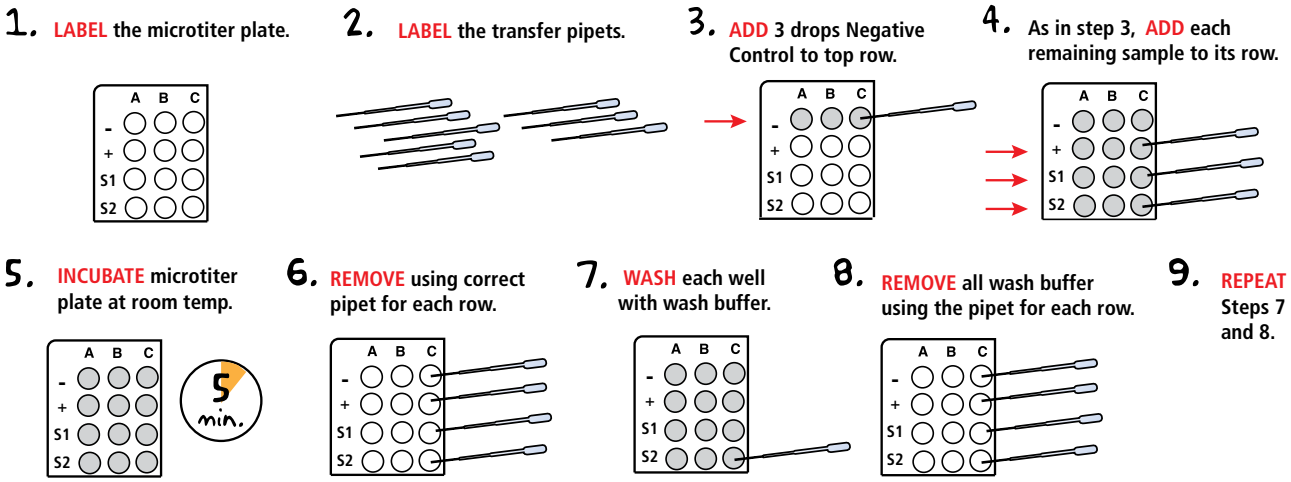
During the Experiment:

- Record (draw) your observations, or photograph the results.

After the Experiment:

- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.

Experiment Procedures



PERFORMING THE ELISA

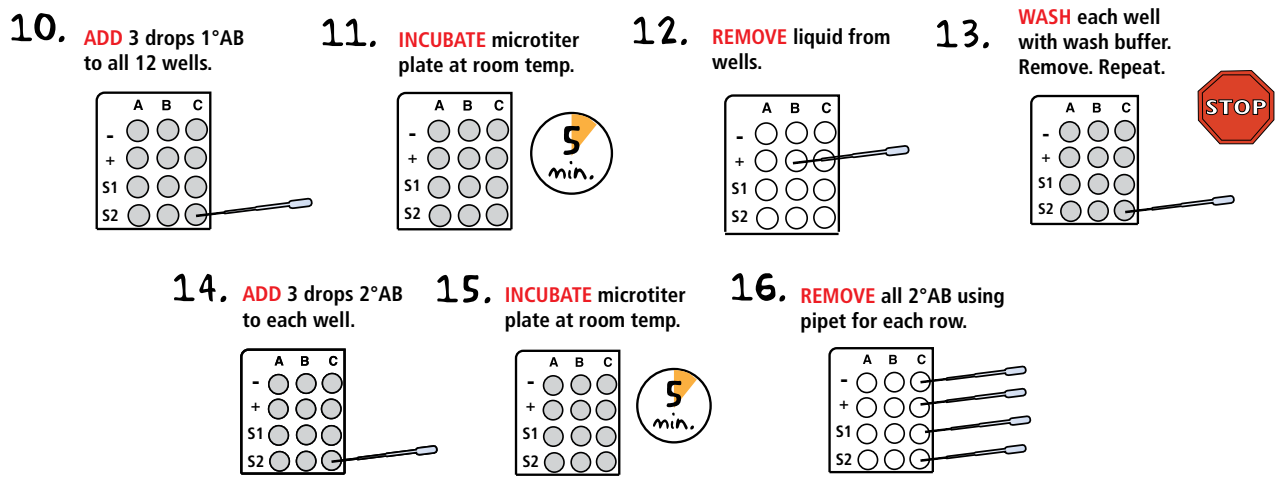
Wear gloves and safety goggles

1. **LABEL** the wells of the microtiter plate as shown.
2. **LABEL** the transfer pipets as outlined in the box below. These 8 pipets will be used to add and remove liquid from the wells.

(Wash)	1x PBST Wash Buffer	(S1)	Sample 1
(-)	Negative Control	(S2)	Sample 2
(+)	Positive Control	(1°AB)	Primary Antibody
(ABTS)	ABTS Substrate	(2°AB)	Secondary Antibody

3. Using the "-" transfer pipet or a micropipette, **ADD** 3 drops or 50 µL of the negative control to all three wells in the top row.
4. As in step 3, **ADD** the "+", "S1", and "S2" samples to all three wells in the appropriate rows, taking care to use the correct pipets or changing tips between each sample.
5. **INCUBATE** the plate at room temperature for 5 minutes.
6. Using the correct transfer pipets for each row, **REMOVE** all of the solutions from each well.
7. Using the "Wash" transfer pipet **WASH** each well by adding wash buffer until the wells are almost full (~200 µL). Do not allow the buffer to spill over into adjacent wells.
8. **REMOVE** all of the wash buffer using the transfer pipet designated for each row.
9. **REPEAT** steps 7 and 8 to wash the wells once more.

Experiment Procedures, continued

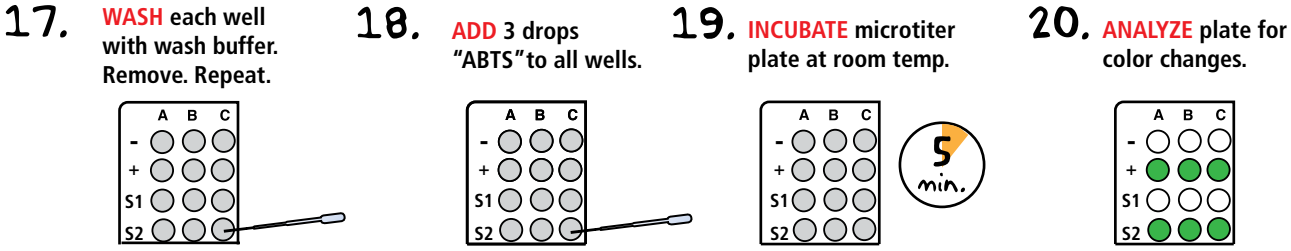


10. Using the "1°AB" transfer pipet or a micropipette, **ADD** 3 drops or 50 µL of Primary Antibody (1°AB) to all 12 wells.
11. **INCUBATE** the plate at room temperature for 5 minutes.
12. Using the "1°AB" pipet **REMOVE** all of the liquid from the wells.
13. **WASH** each well twice with fresh wash buffer. Between washes **REMOVE** all of the wash buffer using the transfer pipet designated for each row.

OPTIONAL STOPPING POINT: For overnight storage, **ADD** 200 µL of wash buffer to each well. Carefully cover the samples and place the plate in the refrigerator. The experiment should be resumed during the next lab period. Remove the wash buffer and continue with Step 14.

14. Using the "2°AB" labeled transfer pipet or a micropipette, **ADD** 3 drops or 50 µL of the secondary antibody to each well.
15. **INCUBATE** the plate at room temperature for 5 minutes.
16. Using the labeled transfer pipet for each row, **REMOVE** all of the secondary antibody from each well.

Experiment Procedures, continued



17. **WASH** each well twice with fresh wash buffer. Between washes **REMOVE** all of the wash buffer using the transfer piper designated for each row.

18. Using the "ABTS" labeled transfer pipet or a micropipette, **ADD** 3 drops or 50 µL of ABTS substrate to all wells.

19. **INCUBATE** the plate at room temperature for 5 minutes.

20. Immediately **ANALYZE** the plate for color changes in the substrate. If the color is not fully developed it can be left for a longer period of time.

Study Questions

1. Describe the mechanism of ELISA. Why is ELISA so sensitive?
2. Why is it important to have a positive and negative control?
3. Why is it necessary to block unoccupied binding sites in the microtiter wells?
4. What is being detected in a standard home pregnancy test?
5. What is being measured in by the heart attack ELISA and why is this useful to physicians?
6. Why is it important to know if gluten is present in a food product? Why would it be necessary to know the detection limits of the ELISA being used to test a product?

Instructor's Guide

OVERVIEW OF INSTRUCTOR'S PRELAB PREPARATIONS:

This experiment contains samples that can be used to perform any of the three scenarios outlined in the background sections: pregnancy testing by ELISA, heart attack detection, and identification of gluten in food products. Each group will have enough reagents to perform one of the tests – the instructor can assign scenarios to individual groups, have the entire class perform the same scenario, or let each group choose independently.

The reagents used require some preparation before performing the experiment. The table below gives a guideline to the time required for each component and when it can be prepared and aliquoted. Detailed instructions are contained on pages 15 and 16.

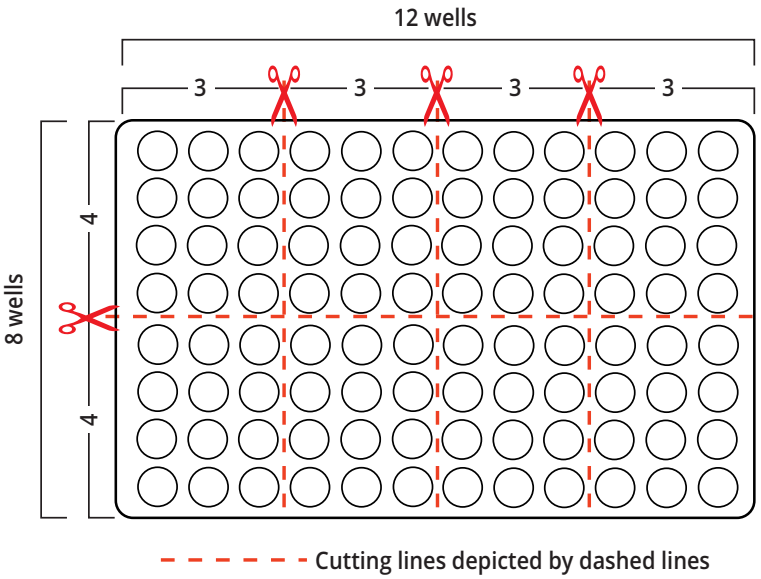
Component:	What to do:	When:
10X ELISA Wash Buffer (A)	Dilute to 1X solution and aliquot	Anytime before the experiment. Cover and store in the refrigerator.
ELISA Dilution Buffer (B)	Aliquot for negative control and patient samples	Anytime before the experiment. Store tubes in the refrigerator.
Whey Antigen (C)	Rehydrate and aliquot	Up to one week before performing the experiment.
Anti-Whey Primary Antibody (D)	Rehydrate and aliquot	Up to one week before performing the experiment.
Secondary Antibody (E)	Rehydrate and aliquot	Up to one day before performing the experiment.
ABTS Substrate (F)	Rehydrate and aliquot	Up to one week before performing the experiment.

Red = Prepare immediately before module. Yellow = Prepare shortly before module. Green = Flexible / prepare up to a week before the module.

Pre-Lab Preparations

Preparation of the Microtiter Plates

Carefully cut and divide the 8 x 12 well microtiter plate to create EIGHT 4 x 3 well pieces (as shown in the figure to the right). Each piece will contain 3 wells on one axis and 4 wells on the other axis. Each group will receive one piece.



Preparation of the Wash Buffer

1. Add all of the 10x ELISA Wash Buffer (A) to 180 mL of distilled water and mix well. Label as “Wash Buffer”.
2. Dispense 18 mL into small beakers for each lab group.

Preparation of the Controls and Patient Samples

Each group should receive one positive control, one negative control, and two unknown samples. The instructions below dictate that sample 1 is negative and sample 2 is positive, but instructors can adjust the samples depending on their desired lesson. For example, you can create additional samples to be tested and assign them randomly to student groups. The students can then pool data to examine a greater number of samples as a class.

1. Transfer 7 mL of ELISA Dilution Buffer (B) to a 15 mL conical tube. Label the tube “Antigen”.
2. Carefully remove the stopper from the vial of lyophilized Antigen (C) and transfer approximately 0.5 mL of the ELISA Dilution Buffer from the tube in step 1. Close the stopper and gently shake the vial to mix.
3. Transfer the entire contents of reconstituted Antigen back to the 15 mL tube from step 1. Mix well.
4. Label 10 microcentrifuge tubes "-CTRL" and 10 tubes as "S1", or whichever sample you want to be negative. Dispense 200 µL ELISA Dilution Buffer (B) into each tube.
5. Label 10 microcentrifuge tubes "+ CTRL" and 10 tubes as "S2". Dispense 200 µL of the antigen solution from step 3 into each tube.

Preparation of the Primary Antibody

1. Transfer 7 mL of ELISA Dilution Buffer (B) to a 15 mL conical tube. Label the tube “1°AB”.
2. Carefully remove the stopper from the vial of lyophilized Primary Antibody (D) and transfer approximately 0.5 mL of the ELISA Dilution Buffer from the tube in step 1. Close the stopper and gently shake the vial to mix.
3. Transfer the entire contents of reconstituted Primary Antibody back to the 15 mL tube from step 1. Mix well.
4. Label 10 microcentrifuge tubes "1°AB" and dispense 650 µL into each tube.

Pre-Lab Preparations

Preparation of Secondary Antibody

(NOTE: Prepare on same day as needed for the experiment.)

1. Transfer 7 mL of ELISA Dilution Buffer (B) to a 15 mL conical tube. Label the tube "2°AB".
2. Carefully remove the stopper from the vial of lyophilized Secondary Antibody (E) and transfer approximately 0.5 mL of the ELISA Dilution Buffer from the tube in step 1. Close the stopper and gently shake the vial to mix.
3. Transfer the entire contents of reconstituted Secondary Antibody back to the 15 mL tube from step 1. Mix well.
4. Label 10 microcentrifuge tubes "2°AB". Dispense 650 µL per tube.

Each Lab Student Group Should Receive:

1	Microtiter plate (3 x 4 well)
1	Microcentrifuge tube containing 650 µL Primary Antibody
1	Microcentrifuge tube containing 650 µL Secondary Antibody
1	Microcentrifuge tube containing 650 µL ABTS
1	Microcentrifuge tube containing 200 µL - Control
1	Microcentrifuge tube containing 200 µL + Control
1	Microcentrifuge tube containing 200 µL S1
1	Microcentrifuge tube containing 200 µL S2
8	Transfer pipets
1	Beaker containing 18 mL Wash Buffer
1	Empty beaker for waste

Preparation of ABTS Substrate

1. Transfer 10 ml ABTS Reaction Buffer (G) into a 15 mL conical tube. Label the tube "ABTS".
2. Carefully remove the stopper from the vial of lyophilized ABTS (F) and transfer approximately 0.5 mL of the ABTS from the tube in step 1. Close the stopper and gently shake the vial to mix.
3. Label 10 microcentrifuge tubes "ABTS". Dispense 650 µL per tube.

Avoiding Common Pitfalls

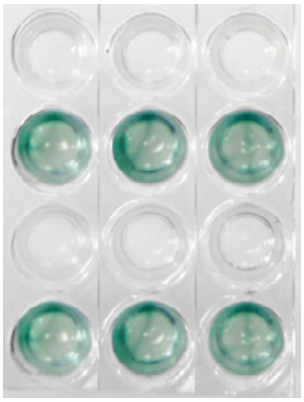
1. Students should be advised to be very careful when transferring solutions into and out of the microtiter plate wells.
2. Use only clean or appropriately labeled pipets.
3. Do not attempt to empty the microtiter wells by shaking it out. This will often result in contaminating adjacent wells.
4. Wash the wells gently and slowly, without force.

Experiment Results and Analysis

The ABTS substrate will change color to dark green in wells containing a positive result on the ELISA.

Students should first confirm that the results from the control samples are correct. The wells in the first row (-CTRL) should have no color change, while wells in the second row (+CTRL) should be dark green.

The samples should be compared to the control samples to identify positive or negative results. In the example results, S1 is negative while S2 is positive.



Please refer to the kit insert for the Answers to Study Questions