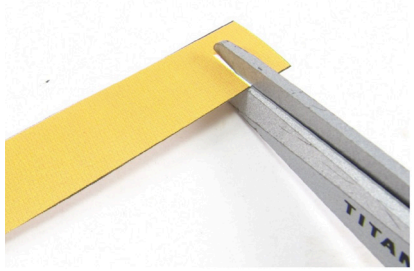
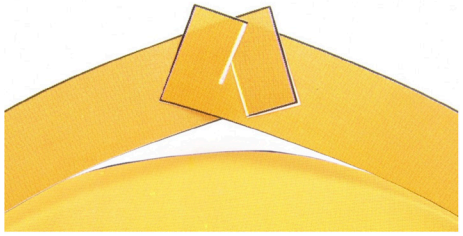
Building an HIV Structure Model

Origami Organelles are downloadable paper models that you print and make as many times as you like! When you purchase a model, you are licensed for unlimited use on a single site or campus. This activity takes only 30 minutes!

1. Print out the pages for your paper model.
2. Cut out the shapes. Fold and tape where indicated.
3. Join the shapes together. The 3D model visualizes the main features of the HIV structure!





Related Product

Cat# EVT-138

Origami Organelles: HIV Structure Model

Teach your class about the structure of HIV!

- Students make their model of HIV out of the colorful parts.
- The 3D model illustrates the envelope, capsid, RNA genome, reverse transcriptase, protease, integrase, matrix and glycoproteins.
- Takes just 30 minutes!

Details for this product and MORE can be found on our website!

WORKSHOP Using Biotechnology to Diagnose HIV/AIDS

EDVOTEK®

Designed for the Classroom

SINCE 1987

Introduction

The Human Immunodeficiency Virus (HIV) causes acquired immune deficiency syndrome (AIDS), a serious disease that suppresses a patient's immune system, leaving them susceptible to infections. In this simulation, we'll perform two common tests (western blot, ELISA) used by doctors to diagnose an HIV infection.

The Human Immunodeficiency Virus (HIV)

Acquired immune deficiency syndrome (AIDS) is a disease characterized by the progressive deterioration of an individual's immune system. The immunological impairment allows infectious agents such as viruses, bacteria, fungi and parasites to invade the body and propagate unchecked. In addition, the incidence of certain cancers dramatically increases in these patients because of their compromised immune system. AIDS is a serious threat to human health and is a global problem. Intensive research is being done to advance methods of detection, clinical treatment and prevention.

ABOUT HIV-1

The AIDS etiologic agent (HIV-1) is the human immunodeficiency virus type 1, which is a retrovirus. HIV-1 contains an RNA genome and the RNA dependent DNA polymerase termed reverse transcriptase. The structure and replication mechanism of HIV is very similar to other retroviruses. Members of the retrovirus family are involved in the pathogenesis of certain types of leukemia and other sarcomas in humans and animals.

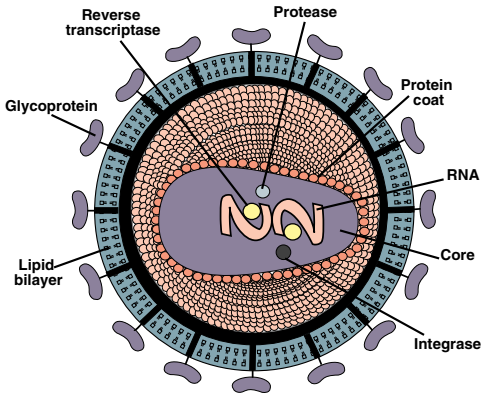


Figure 1: The HIV Virus

HIV is unique in some of its properties since it specifically targets the immune system, is very immune-evasive, forms significant amounts of progeny virus *in vivo* during the later stages of the disease, and can be transmitted through bodily fluids.

The HIV viral particle is surrounded by a lipid bilayer, derived from the host cell membrane during budding, and a protein capsid (Figure 1). Within the core are two identical RNA molecules 9000 nucleotides in length. Hydrogen bonded to each viral RNA is a cellular tRNA molecule. The core also contains approximately 50 molecules of reverse transcriptase. The viral proteins are identified by the prefix "gp" (glycoprotein) or "p" (protein) followed by a number indicating the approximate molecular weight in kilodaltons (Table 1). In total, the HIV genome encodes for 19 proteins necessary for the virus's structure, integration, replication, and disruption of the host cell.

Table 1: Examples of HIV Proteins

HIV Protein Name	Category	Protein Description
gp41	Envelope Antigens	Transmembrane protein
gp120	Envelope Antigens	CD4 binding protein
p17	Core Antigens	Matrix protein
p24	Core Antigens	Capsid protein
p31	Enzymes	Integrase
p51	Enzymes	Reverse transcriptase

WORKSHOP **USING BIOTECHNOLOGY TO DIAGNOSE HIV/AIDS**

Simulation of HIV Detection by ELISA

7 Incubate for 10 minutes. **37°C**

8 Remove all liquid using the transfer pipet designated for each row.

9 Wash each well with PBS buffer by adding the PBS buffer until each well is almost full. The capacity of each well is approximately 0.2 ml. Do not allow the liquids to spill over into adjacent wells. Remove all the PBS from each of the wells with the transfer pipet designated for each row.

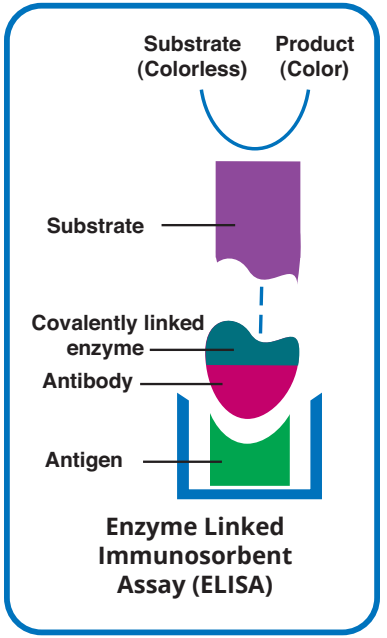
SUBSTRATE:

10 Add 0.1 mL or 5 drops of the substrate solution to all of the wells.

11 Incubate for 10 minutes. **37°C**

ANALYSIS:

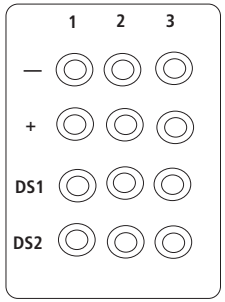
12 Remove the plate for analysis. If color is not fully developed after 10 minutes, incubate at 37°C for a longer period of time.



Simulation of HIV Detection by ELISA

This ELISA detects an HIV infection by looking for HIV antibodies in the patient's blood. To perform the ELISA, the patient samples are added to a microtiter plate coated with HIV antigens. If HIV antibodies are present in the blood, they will bind to the antigens on the plate. The HIV antibodies are detected with an enzyme-linked secondary antibody that can change a colorless substrate brown. When the substrate is added to the wells, a color change is observed in any samples positive for HIV antibodies.

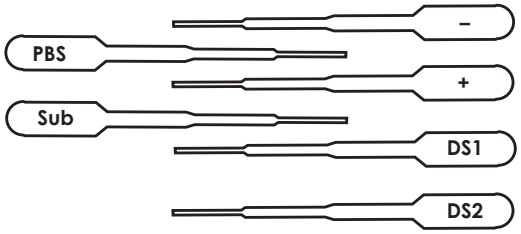
LABEL MATERIALS:



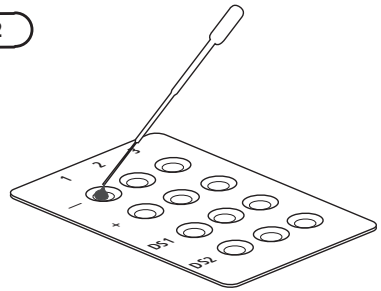
1. Label wells: "-", "+", "DS1", and "DS2" directly on the microtiter plate, or place the plate on a labeled sheet of paper.

   Put your initials or group number on the plate.

2. Label pipets: "-", "+", "DS1", "DS2", "PBS", and "Sub" . These are designated for adding samples and removing washes - save these pipets!



ANTIBODY CONTROLS AND SAMPLES:



3. Add 3 drops of Negative Control to all three wells in the "–" Row.

4. Add 3 drops of Positive Control to all three wells in the "+" Row.

5. Add 3 drops of Donor Serum 1 to all three wells in the "DS1" Row.

6. Add 3 drops of Donor Serum 2 in all three wells in the "DS2" Row.

DETECTING HIV INFECTION IN PATIENT SAMPLES

An individual can be infected with HIV through unprotected sex, a blood transfusion, or by intravenous injection with a contaminated needle. Virus or virally infected cells are found in bodily fluids such as semen and blood. During the early stages of infection in an immunocompetent person the HIV virus elicits immune responses. However, the viral reverse transcriptase has a high error rate, leading to an extremely high rate of mutations. This allows some of the viral variants to survive and produce progeny having a similar capacity to escape immunosurveillance.

Due to the highly infectious nature of HIV it is essential that patients are identified as quickly as possible following infection. This allows for patients to understand their potential risks, minimize transmission of the virus, and immediately begin treatment if necessary. Almost all modern HIV detection tests collect serum or saliva samples from the patient to identify the presence of antibodies against HIV proteins. These antibodies are produced within a few weeks of HIV infection and are incredibly specific, making them ideal for HIV screening.

ANTIBODIES

Antibodies (also called immunoglobulins, or Igs) are specialized proteins that allow the immune system to distinguish between "self" and "non-self" proteins or polysaccharides. These Y-shaped molecules comprise four linked polypeptide chains: two identical "heavy chains" and two identical "light chains" (Figure 2). 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).

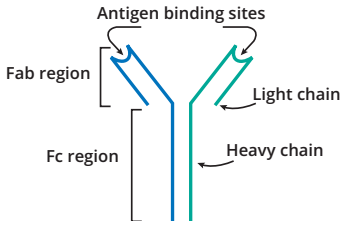


Figure 2:
General Structure of an antibody.

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 technique, called western blotting, identifies a particular protein in a mixed sample. Immunohistochemistry uses antibodies to label specific antigens within a cell or tissue. To quantitatively measure the amount of an antigen within a sample, scientists use the Enzyme Linked ImmunoSorbent Assay (ELISA). 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 detects the HIV virus might be designed to use an antibody that recognizes one of the virion's coat proteins. In a clinical HIV ELISA, the assay will determine if HIV antibodies are present in the patient samples.

The "secondary antibody" recognizes the primary antibody – since our primary antibody is produced by human immune cells we would use a secondary antibody that specifically recognizes human 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 3A). 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 and the antigens are allowed to adsorb to the surface through hydrophobic associations (Figure 3B). In an HIV ELISA, the antigens will be viral proteins prepared ahead of time. ELISAs are often performed in transparent microtiter plates made of polystyrene or polyvinyl chloride plastics. Scientists add antigens to the wells and allow them to non-specifically stick to the plastic through hydrophobic and electrostatic interactions. After washing away any excess fluid, the wells are "blocked" with a protein-containing buffer, which prevents non-specific interactions between the antibody and the plastic wells.

Next, a patient sample, or a control sample, is added to the wells and the mixture is allowed to incubate for a short time. If anti-HIV antibodies are present in the patient sample, they will recognize and bind to the HIV antigens. 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 patient 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.

This experiment replicates a clinical screen to detect HIV antibodies in a simulated patient blood sample. Students will incubate prepared antigens in a microtiter plate, wash to remove unadsorbed protein, and then incubate with control and patient samples. If a patient is positive for HIV, their blood will contain anti-HIV antibodies that can bind to the proteins in the well. An HRP-linked secondary 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. At the conclusion of the experiment students will provide an HIV diagnosis for each patient.

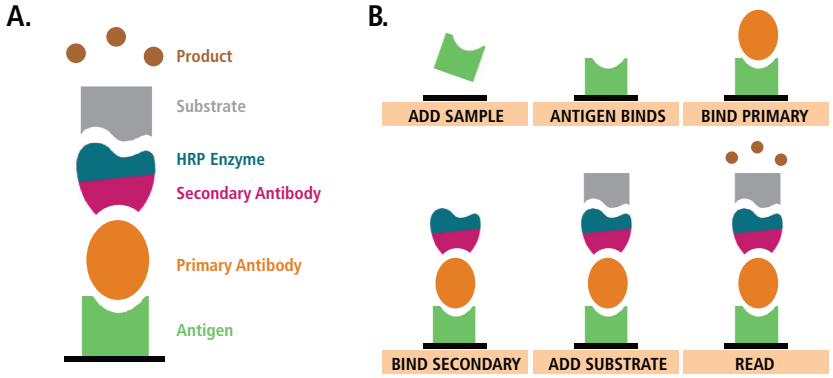


Figure 3: Optimized ELISA workflow

Experiment Overview

LABORATORY SAFETY

Be sure to READ and UNDERSTAND the instructions completely BEFORE starting the experiment. If you are unsure of something, ASK YOUR INSTRUCTOR!

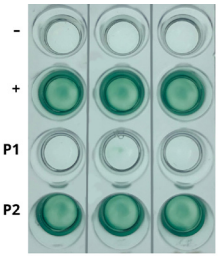
- Wear gloves and goggles while working in the laboratory.
- DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
- Always wash hands thoroughly with soap and water after working in the laboratory.

Related Products

Cat# 271

AIDS Kit I: Simulation of HIV Detection by ELISA

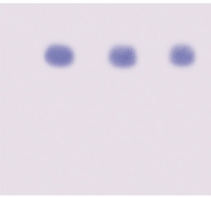
An HIV test detects HIV infection indirectly using an ELISA test against HIV antibodies in the blood. The test works by taking antibodies from the patient's blood and adding them to a microtiter plate coated with HIV antigen. If HIV antibodies are present in the blood, they will bind to the antigens on the plate. This binding is detected with an enzyme-linked secondary antibody that causes a color change upon addition of substrate. In this experiment, your students will perform an ELISA test by coating microtiter plate wells with simulated HIV antigen and then test simulated donor serum for anti-HIV antibodies.



Cat# 275

AIDS Kit II: Simulation of HIV Detection by Western Blot

The second assay used to confirm a positive HIV ELISA result is the Western Blot. Students separate protein samples from hypothetical patients on agarose gels, transfer the samples to a membrane and detect the simulated HIV proteins. This kit is an introductory level experiment. For a comprehensive advanced course, we recommend Cat. #317.



- Understand the theory and applications of Western blots
- Learn the molecular biology of HIV and the pathogenesis of AIDS
- Perform electrophoresis and a modified western blot to detect simulated viral proteins
- Easy set up using standard (horizontal) electrophoresis equipment