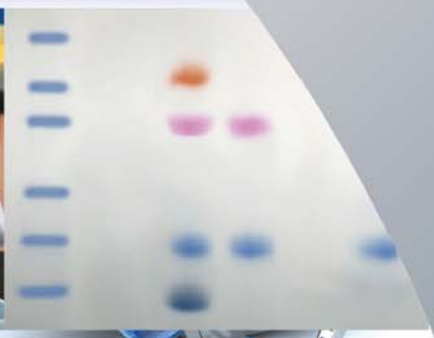
EDVOTEK WORKSHOP

Using Biotechnology to Diagnose HIV/AIDS



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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.

Background Information

Excerpts from Cat. #151

PROTEIN ELECTROPHORESIS

To analyze proteins, researchers often make use of a technique called polyacrylamide gel electrophoresis, or PAGE. This is a simple but powerful method that provides information about the expression and purity of a molecule, along with its molecular weight. PAGE uses acrylamide and bis-acrylamide polymers to create a gel with a network of microscopic pores and channels.

To perform PAGE, a gel is prepared, placed in an electrophoresis chamber and flooded with buffer. Next, the protein samples are loaded into small indentations, or wells, in the top of the gel. Finally, an electrical current is applied to the gel box, pushing the charged proteins through the gel towards the positive electrode (Figure 1). The pore size in polyacrylamide gels is controlled by the gel concentration and the degree of cross-linking, allowing researchers to customize the gel to meet the specific needs of the experiment. As the proteins migrate they are forced through the pores of the gel; smaller proteins have an easier time fitting than larger proteins and will migrate further in the same amount of time.

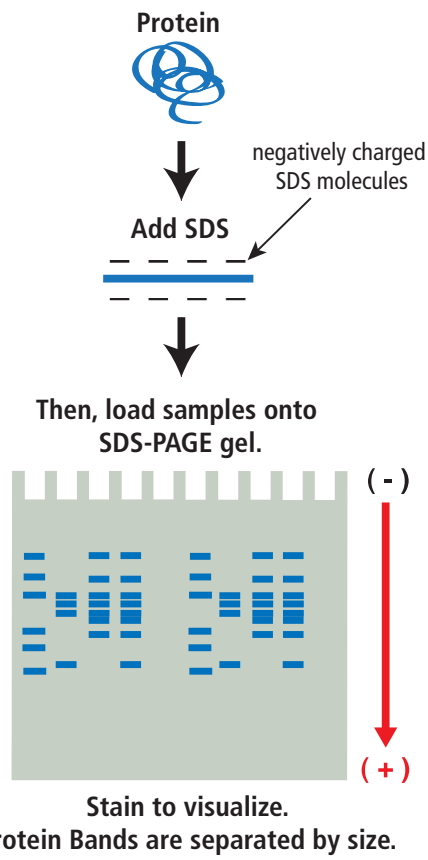


Figure 1: An Overview of SDS-PAGE

Protein denaturation for electrophoresis

Proteins produce a unique challenge for electrophoresis because they have complex shapes and different charges, which affect how they migrate through the gel. Structural differences can cause two proteins with similar molecular weights to migrate at different rates - a complicated, spread-out protein will move slower through the gel than one with a compact shape. Similarly, positively and negatively charged proteins will migrate in different directions through the electric field in a gel. Scientists can solve these problems by using chemicals that denature the proteins, eliminating the complex structure, and neutralize the charge of the native protein.

Sodium Dodecyl Sulfate (SDS) is a common detergent used to disrupt interactions between amino acids. The SDS molecule consists of a hydrocarbon chain bonded to a negatively charged sulfate group. When incubated with proteins and heated, SDS will unfold the protein's three-dimensional structure. To break the stronger disulfide bonds in proteins researchers use reducing agents such as β-mercaptoethanol (β-ME) or Dithiothreitol (DTT). Although the amino acid composition and sequence stay the same, the protein will no longer have biological activity because the specific three-

Background Information

Excerpts from Cat. #151

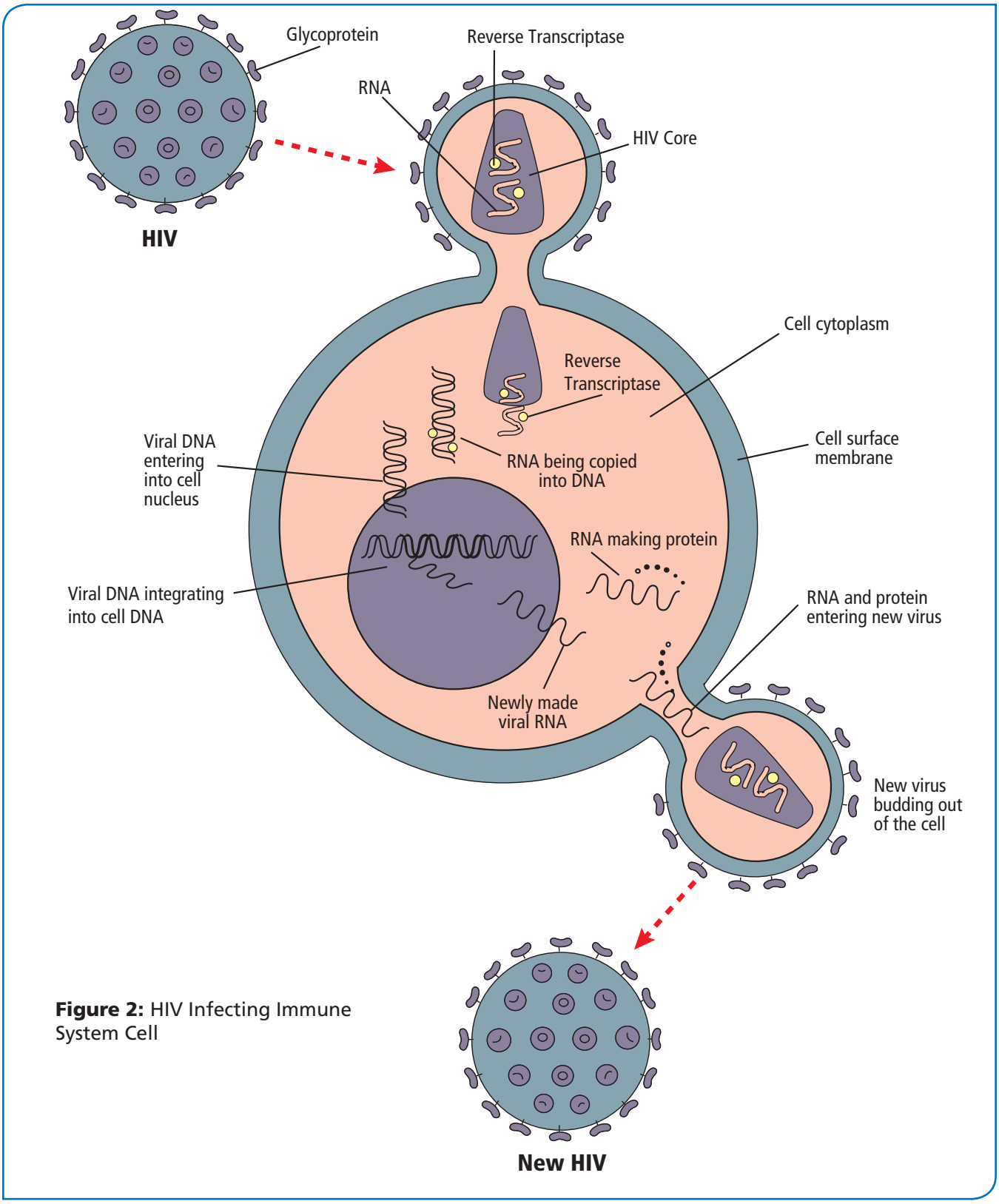


Figure 2: HIV Infecting Immune System Cell

Background Information

Excerpts from Cat. #151

dimensional shape has changed. The prepared protein sample can then be separated on a polyacrylamide gel. This technique is commonly called SDS-PAGE (sodium dodecyl sulfate-PAGE).

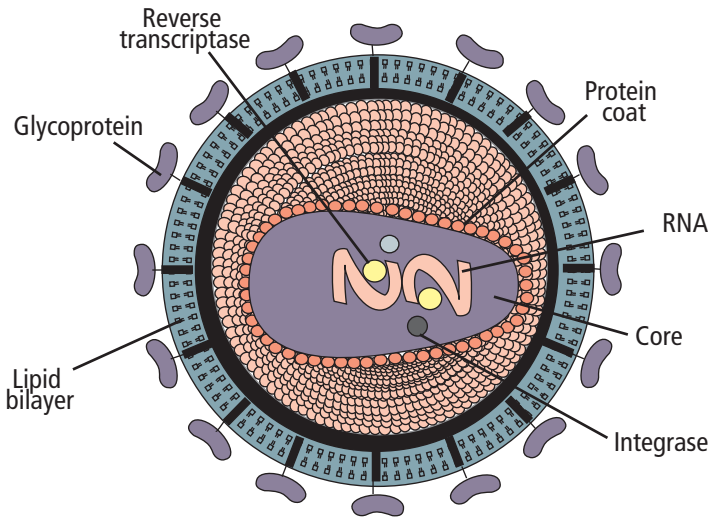


Figure 3: The structure of an HIV virus

Detecting HIV Proteins in Patient Samples

Acquired immune deficiency syndrome (AIDS) is a life threatening viral disease that is caused by the Human Immunodeficiency Virus (HIV). Infection by HIV suppresses a patient's immune system by infecting and destroying antibody-producing helper T-cells. Due to a lack of immunosurveillance, patients are extremely susceptible to infections from viruses, bacteria, fungi, and parasites. An individual can contract HIV through unprotected sexual contact, a blood transfusion, or by intravenous injection with a contaminated needle. AIDS is a global threat to human health, and early detection and care of patients is essential to controlling the spread and suffering from the disease.

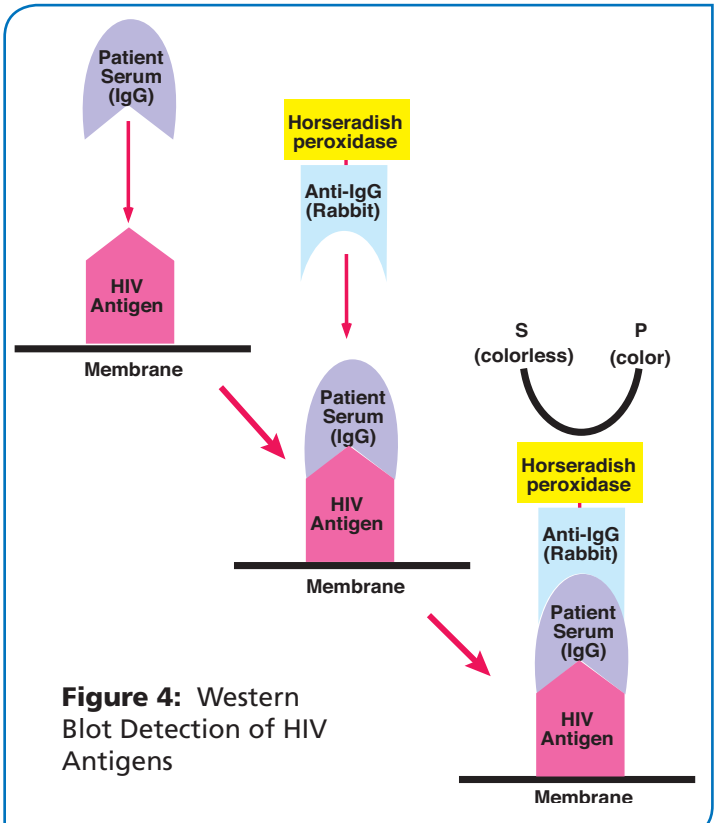


Figure 4: Western Blot Detection of HIV Antigens

HIV-1 is a retrovirus, which means it has an RNA genome and an RNA-dependent-DNA-polymerase termed reverse transcriptase. The reverse transcriptase protein allows the virus to create a DNA template that will integrate into the patient's genome. Once inserted into the host cell's genome the viral DNA can lie dormant for long periods of time, known as the latent stage of infection. During active viral production the DNA will be transcribed into mRNA, which can initiate production of viral proteins. The proteins and RNAs assemble at the surface of the cell, forming a new virus particle (Figure 2). Finally, the completed virus buds off from the cell membrane and is ready to infect healthy T-cells.

Structurally, the HIV virus features a viral envelope, composed of glycoproteins and a lipid membrane, and a protein capsid surrounding two copies of the RNA genome (Figure 3). In total, the HIV genome encodes for 19 proteins necessary for the virus's structure, integration, replication, and disruption of host cell function.

Background Information

Excerpts from Cat. #151

HIV infections in patients can be detected by multiple methods. The most common initial test involves a rapid immunoassay that detects the presence of HIV antibodies in serum or saliva. A positive result on the rapid test is confirmed by a second test, including screening for viral nucleic acids and proteins, or performing a western blot to identify serum HIV antibodies. HIV western blots are both sensitive and inexpensive, making them one of the preferred methods to confirm an HIV diagnosis. Unlike the rapid immunoassay, the western blot is normally performed by a trained technician and can require a few days or weeks to obtain results.

During the HIV western blot, HIV proteins are separated on an SDS-PAGE gel and then transferred to a membrane for analysis. Next, the membrane is incubated with patient serum, allowing any antibodies in the blood to bind to viral proteins. The patient serum is washed away, and the membrane is incubated with a second antibody and detection reagent (Figure 4).

Serum from infected patients will contain antibodies to multiple HIV proteins, including envelope, capsid, and functional proteins (Table 1). These antibodies can appear within three months of infection, allowing for early detection in the HIV western blot. A negative result for the western blot test will show zero bands, while positive results can vary depending on the provider of the test. Typically, a positive diagnosis requires the detection of at least one envelope and one capsid protein, although most positive patients will show additional bands. In addition, it is possible for the results to reveal one or more bands without reaching the criteria required for a positive test; in these cases the patient is said to be "indeterminate" and will require additional testing to confirm HIV infection. The requirement for multiple bands helps to prevent the misdiagnosis of patients infected with other viruses which are occasionally detected by the western blot.

This experiment replicates the clinical screen to detect HIV antibodies in a patient blood sample. Simulated patient samples have been pre-stained with dyes, making them visible during the electrophoresis. These proteins are mixed with a sample buffer containing SDS, DTT, glycerol, and a tracking dye. The tracking dye will migrate ahead of the smallest proteins in these samples where it serves as a marker to show how far the gel has run. Since the proteins are pre-labeled it is not necessary to perform a western blot analysis; instead, HIV protein bands will be visible in each sample as the gels are run. This allows for analysis of each patient sample immediately after removing the gel from the electrophoresis chamber. Students will then provide an HIV diagnosis or further testing recommendation for each patient.

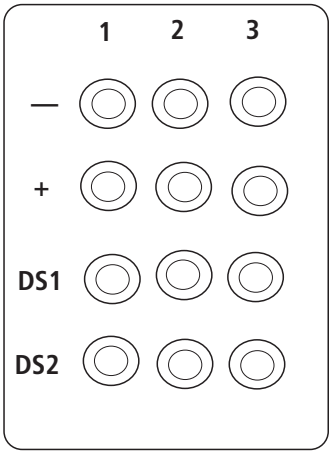
Protein Size	HIV Protein Name	Category	Protein Description
72,000 Da	p65	Enzyme	Reverse Transcriptase
38,000 Da	p41	Transmembrane	Envelope protein
20,000 Da	p24	Structural	Capsid protein
14,000 Da	p18	Structural	Matrix protein

Table 1: HIV proteins analyzed by Western Blotting

Simulation of HIV Detection by ELISA

Excerpts from Edvokit 271

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.

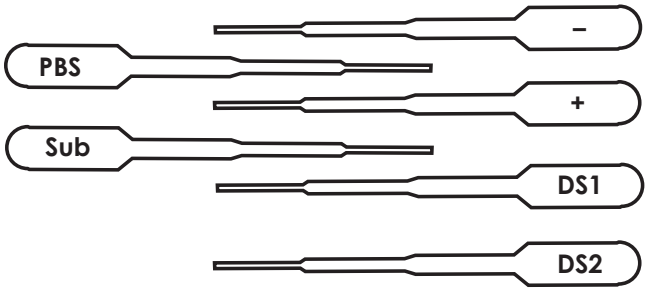


LABEL MATERIALS:

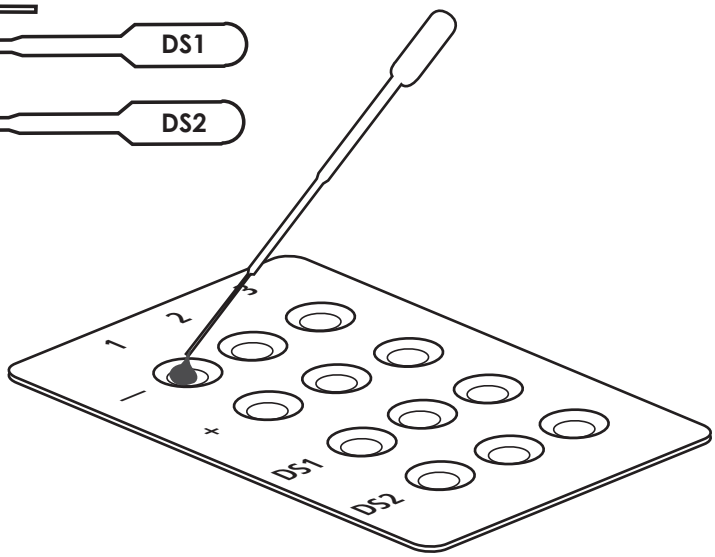
1. Label wells: "-", "+", "DS1", and "DS2" directly on the microtiter plate, or place the plate on a labeled sheet of paper.

   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 1st Row.

4. Add 3 drops of Positive Control to all three wells in the 2nd Row.

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

6. Add 3 drops of Donor Serum 2 in all three wells in the 4th Row.

Simulation of HIV Detection by ELISA

Excerpts from Edvokit 271

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:

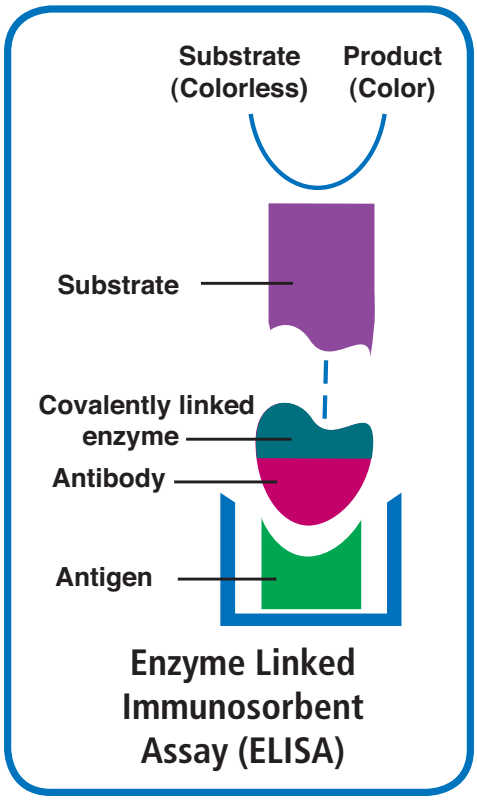
10 Add 0.1 ml or 5 drops of the substrate solution to all of the wells.

10 Incubate for 10 minutes. 37°C

ANALYSIS:

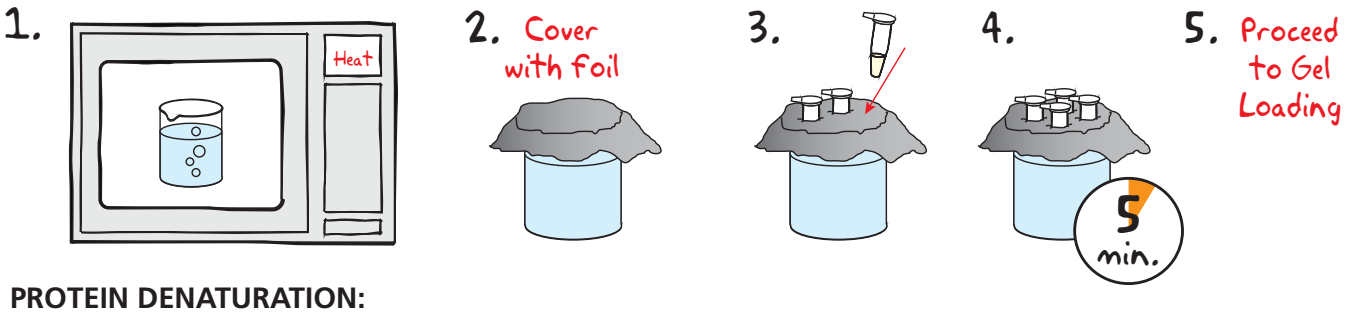
12 Remove the plate for analysis.

If color is not fully developed after 10 minutes (step 12), incubate at 37°C for a longer period of time.



Performing SDS-PAGE with Protein Samples

Excerpts from Edvokit 151



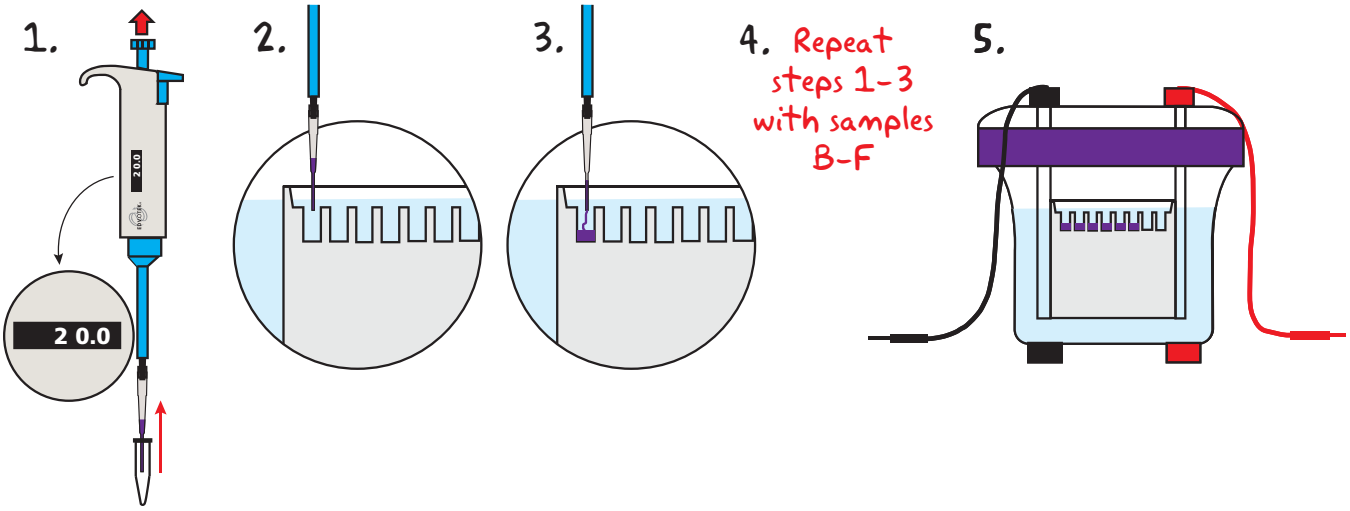
PROTEIN DENATURATION:

NOTE: PROCEED to gel loading if your lab instructor has already heated the protein samples.

1. Using a hot plate or microwave, **HEAT** a beaker of water until it boils.
2. **COVER** with aluminum foil and carefully remove from heat.
3. Tightly **CAP** sample tubes. **PUSH** tubes through foil to suspend in the boiling water.
4. **INCUBATE** the samples for 5 minutes.
5. Immediately **PROCEED** to loading the gel while the samples are still warm.

Wear gloves and safety goggles

FREEZING PROTEINS:
Unused portions of the protein samples can be frozen for later use. When needed, repeat steps 1-4 and proceed to Loading the Protein Samples.

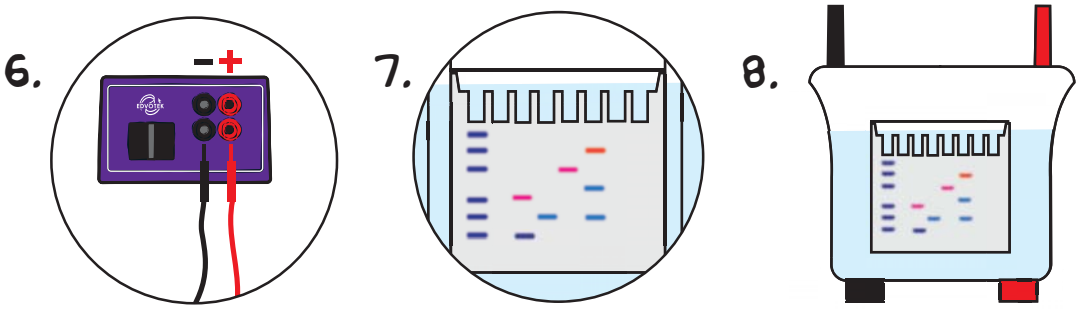


LOADING THE PROTEIN SAMPLES:

1. Using a fresh pipet tip, **MEASURE** 20 µl of the Standard Protein Marker (A).
2. **PLACE** the pipet tip under the buffer and directly above the sample well, resting gently against the back plate of the gel cassette.
3. Slowly **DISPENSE** the sample by depressing the plunger.
4. **REPEAT** steps 1-3 with protein samples B-F, changing the tip between each new sample.
5. Once all samples have been loaded, carefully **PLACE** the cover onto the electrode terminals.

Performing SDS-PAGE, continued

Excerpts from Edvokit 151



6. **CONNECT** the electrical leads to the power supply.
7. **SET** the voltage of the power supply and **PERFORM** electrophoresis (See Table A for time and voltage guidelines). Allow the proteins to separate on the gel for the recommended length of time, or until the tracking dye reaches the bottom of the gel.
8. **TURN OFF** the power supply and carefully **REMOVE** the lid. The gel can now be removed from the chamber and analyzed.

Note: For easier analysis the gel can be removed from the cassette. Using a metal spatula or screwdriver carefully pry apart the two plastic plates and gently remove the gel for analysis. The gels are very fragile and must be handled with care.

OPTIONAL STAINING: Although the protein samples are provided in a pre-stained format it is possible to increase the intensity of the bands by using Protein InstaStain® cards. Staining is rapid and sensitive, and gels are ready for visualization in as short as 1-3 hours.

Table 2: Gel Loading

Lane 1	Sample 1	Standard Protein Marker
2	Sample 2	Positive Control
3	Sample 3	Patient 1
4	Sample 4	Patient 2
5	Sample 5	Patient 3

Table A — Time and Voltage Guidelines

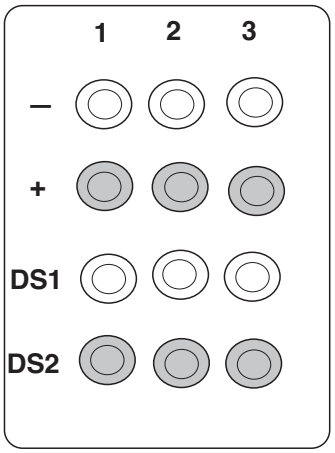
	Recommended Time	
Volts	Minimum	Optimal
100	80 min.	95 min.
125	60 min.	75 min.
150	50 min.	60 min.

Related Video – Protein Electrophoresis

Tech Video

www.youtube.com/EdvotekInc

Results and Data Analysis



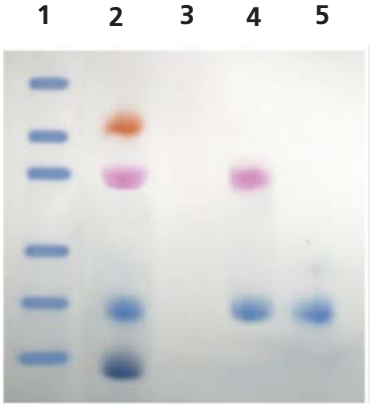
SIMULATION OF HIV DETECTION BY ELISA

Donor 2 should show positive for HIV. The color should look similar to the positive control.

Row A -- Negative Control (–)
Row B -- Positive Control (+)
Row C -- Donor Serum 1 (DS1)
Row D -- Donor Serum 2 (DS2)

PERFORMING SDS-PAGE WITH PROTEIN SAMPLES

The expected banding patterns are shown below relative to the standard protein marker. The actual results may differ slightly due to variations in gel quality or composition, or due to fluctuations in how the samples run in the gel.



Standard Protein Marker Molecular Weights
94,000 Da
67,000 Da
38,000 Da
30,000 Da
20,000 Da
14,000 Da

Lane	Sample Name	Number/Size of bands	Diagnosis
1	Standard Protein Marker	----------	----------
2	Positive Control	4 bands (72kD, 38kD, 20kD, 14kD)	HIV positive
3	Patient 1	0 bands	HIV negative
4	Patient 2	2 bands (38kD, 20kD)	HIV positive
5	Patient 3	1 band (20kD)	Inconclusive – should be retested by doctor

Protein Size	HIV Protein Name	Category	Protein Description
72,000 Da	p65	Enzyme	Reverse Transcriptase
38,000 Da	p41	Transmembrane	Envelope protein
20,000 Da	p24	Structural	Capsid protein
14,000 Da	p18	Structural	Matrix protein

Related Products

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, they will bind to the antigens on the plate. 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.

Kit includes: instructions, simulated HIV antigens, positive and negative controls, simulated donor serum, secondary antibody, substrate, microtiter plates, transfer pipets, microtubes.

All you need: 37°C incubation oven

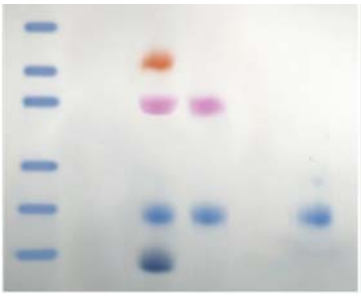
Storage: Some Components Require Refrigerator Storage.

For 10 Groups

Complete in 60 min.

Cat. #271

AIDS Kit III: Simulation of HIV Detection by Protein Electrophoresis



This experiment replicates the clinical screen to detect HIV antibodies in a patient blood sample. Simulated patient samples have been pre-stained with dyes, making them visible during the electrophoresis. These proteins are mixed with a sample buffer containing SDS, DTT, glycerol, and a tracking dye. The tracking dye will migrate ahead of the smallest proteins in these samples where it serves as a marker to show how far the gel has run. Since the proteins are pre-labeled it is not necessary to perform a western blot analysis; instead, HIV protein bands will be visible in each sample as the gels are run. This allows for analysis of each patient sample immediately after removing the gel from the electrophoresis chamber. Students will then provide an HIV diagnosis or further testing recommendation for each patient.

Kit includes: instructions, standard protein markers, positive and negative control samples, patient samples, Tris-Glycine-SDS buffer, practice gel loading solution, Protein InstaStain®, transfer pipets, screw-top microcentrifuge tubes.

All you need: vertical electrophoresis apparatus, D.C. power supply, precast 12% SDS polyacrylamide gels, micropipet and tips, hot plate, distilled or deionized water, beakers, aluminum foil or foam waterbath float.

Storage: Some Components Require Freezer Storage.

For six (6) groups sharing three polyacrylamide gels.

Complete in 80 min.

Cat. #151

Related Products

MV10 Vertical Protein Electrophoresis Apparatus

Our newly re-designed MV10 is by far the most user-friendly vertical protein electrophoresis unit with simple gel clip system. It runs one vertical polyacrylamide gel. All parts are color coded to ensure proper orientation.

For 1 Group

Cat. #581

MV10 Features:

- Gel support cassette clip - holds gel securely, allows for easy placement and remove of gel
- New, sleek design
- Domed lid with sturdy overmold grip feature
- Modified shape improves gel running speed by 10-15%
- Stabilizing feet - raises unit off bench to promote cooling and improves stabilization
- Placement of color-coded anode/cathode sides completely eliminate the chance of improper or reverse lid placement
- Electrode lead 90° right angle shape improves stability by lowering the overall profile and thus minimizes tipping
- Patent pending

TetraSource™ 300 Power Supply (30-300 V)

Power any combination of EDVOTEK electrophoresis units with this mighty 750 mA power supply! Features an easy-to-use, fully programmable interface for setting voltage, current or timer control with each parameter displayed in real-time. Programs may be paused or resumed at any point. Run experiments in the least time possible with this powerful and versatile unit!

30-300 V for 1 to 4 units

Cat. #5010

EDVOTEK® Variable Micropipet

Eeasy to use, sturdy, highly accurate and use standard micropipet tips. The volume is easily selected by twisting the top. The lightweight design and tip ejector makes operation fast & easy. A tool and instructions are included for self-calibration.

5 - 50 µl Micropipet

Cat. # 590

White Light Box

EDVOTEK's all new White Light Box features a spacious 25 x 25 cm viewing area illuminated by long life LEDs and is housed in a slim aluminum body. It's designed to safely enhance the visualization of DNA stained with FlashBlue™, proteins stained with Coomassie Blue and autoradiograms.

Features:

- 25 x 25 cm Viewing Area
- 90 Lumens LED Chips
- Power Rating: 8 Watts
- Dual Voltage: 110/220 V
- Frequency: 50/60 Hz
- Two-Year Warranty
- Made in the USA

Cat. #552