Edvo-Kit # 151

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AIDS Kit III: Simulation of HIV Detection by Protein Electrophoresis

Experiment Objective:

In this experiment, students will use SDS-PAGE to identify HIV proteins in simulated patient samples. The results of this test are used to diagnose an HIV infection.

See page 3 for storage instructions.

Version 151.220222

EDVOTEK®

Table of Contents

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

Experiment Components

	Component	Storage	Check (✓)
A	Standard Protein Markers	-20°C Freezer with desiccant	❑
B	Negative Control Sample	-20°C Freezer with desiccant	❑
C	Positive Control Sample	-20°C Freezer with desiccant	❑
D	Patient #1 Serum Sample	-20°C Freezer with desiccant	❑
E	Patient #2 Serum Sample	-20°C Freezer with desiccant	❑
F	Patient #3 Serum Sample	-20°C Freezer with desiccant	❑

This kit is designed for six (6) groups sharing three polyacrylamide gels.

All remaining components can be stored at room temperature.

- Tris-Glycine-SDS buffer (10x) ❑
- Practice Gel Loading Solution ❑
- FlashBlue™ Protein Stain Powder ❑
- Transfer Pipets ❑
- Microcentrifuge tubes ❑

Experiment Requirements *(NOT included with this experiment)*

- Vertical electrophoresis apparatus (EDVOTEK® Cat. #581 highly recommended)
- D.C. power supply
- Precast 12% SDS polyacrylamide gels (12-well gels recommended)
- Micropipette and tips (Cat #638 Fine Tip Micropipette Tips recommended)
- Microwave or Hot plate
- Distilled or deionized water
- Beakers
- Aluminum foil or foam waterbath float
- White Vinegar
- Ethanol (95% or higher)
- 750 mL or 1 L flask or beaker
- Small plastic tray or large weigh boat
- Plastic wrap
- White light box (recommended)
- Rocking platform (recommended)

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

Proteins are a diverse group of large molecules, or macromolecules, that perform many of the essential functions in our cells. The first observation of proteins occurred by scientists in the 18th century who determined that proteins were critical for maintaining body structure. Since then, it has become clear that proteins also have a role in many cellular processes, including motility, transport, and communication. It is estimated that about 2-4 million proteins per cubic micron are found in mammalian cells.

Proteins are polymers, composed of hundreds to thousands of smaller organic compounds known as amino acids. Amino acids are simple molecules consisting of a central carbon atom bonded to four different groups: an amine group, a carboxyl group, a hydrogen atom, and a unique side chain (Figure 1). The simplest amino acid, glycine, has a single hydrogen atom as a side chain, while other amino acids feature more complex side chains. The chemical properties of side chains determine the polarity of each amino acid and whether the amino acid is acidic, basic, or neutral.

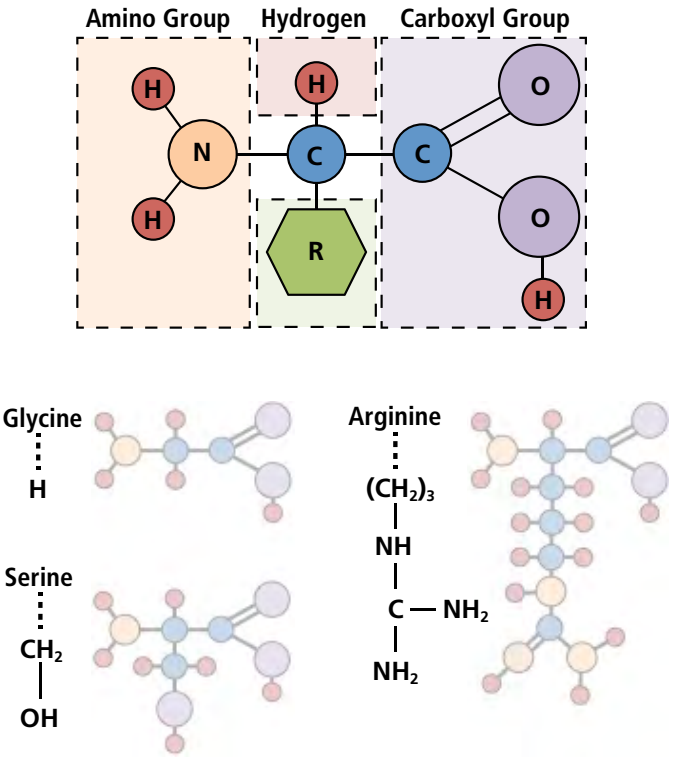


Figure 1: The Structure of Amino Acids.

During protein synthesis a specific sequence of amino acids is connected together to form a continuous chain. Adjacent amino acids in the chain are linked to each other by peptide bonds. These strong covalent bonds link the carboxyl group of one amino acid and the amine group of a second amino acid (Figure 2). A chain of linked amino acids is known as a polypeptide, and one or more polypeptides combine to make a protein. The amino acid sequence gives each protein specific properties. For example, the molecular weight and charge of a protein is based on the number and type of amino acids, while the shape is determined by the order of amino acids. This three-dimensional configuration, including twists, folds, and interactions between multiple polypeptides, is critical to protein function.

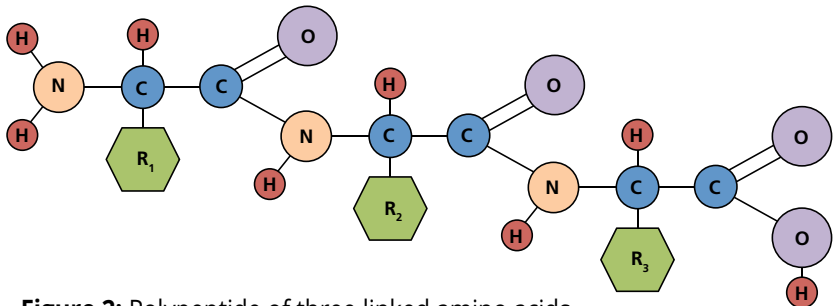


Figure 2: Polypeptide of three linked amino acids.

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 3). 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.

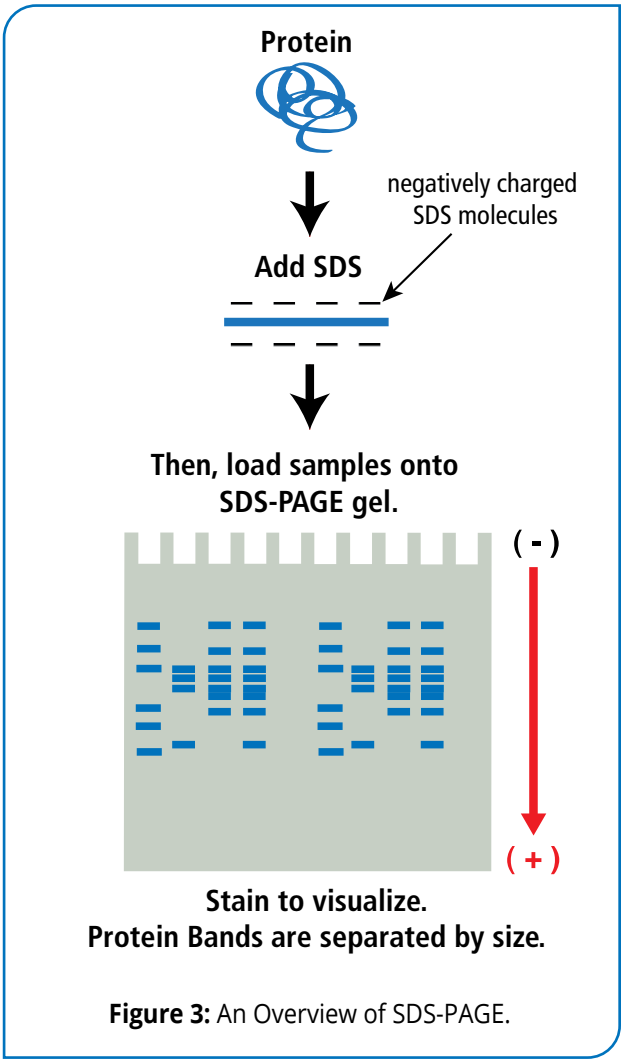


Figure 3: An Overview of SDS-PAGE.

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-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).

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.

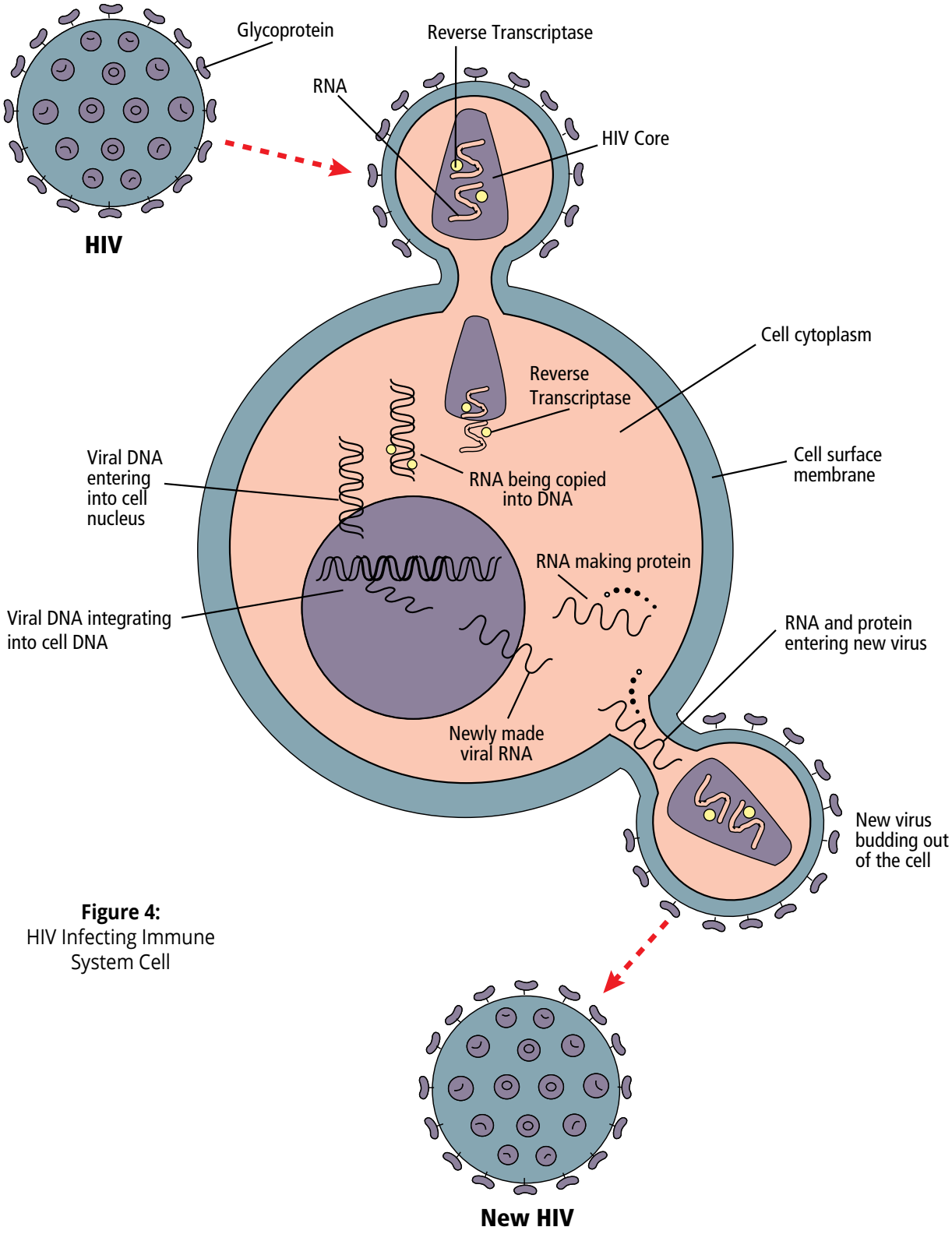


Figure 4:
HIV Infecting Immune System Cell

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 4). 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 5). In total, the HIV genome encodes for 19 proteins necessary for the virus's structure, integration, replication, and disruption of host cell function.

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 6).

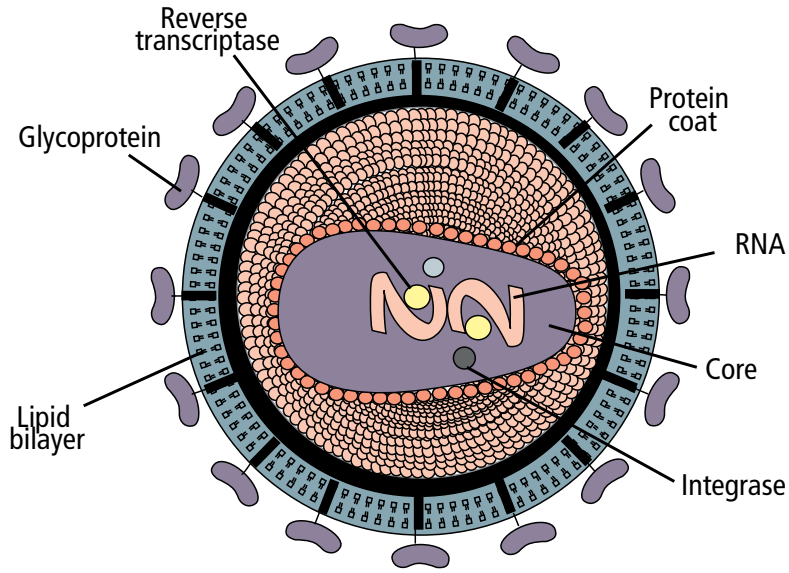


Figure 5: The structure of an HIV virus

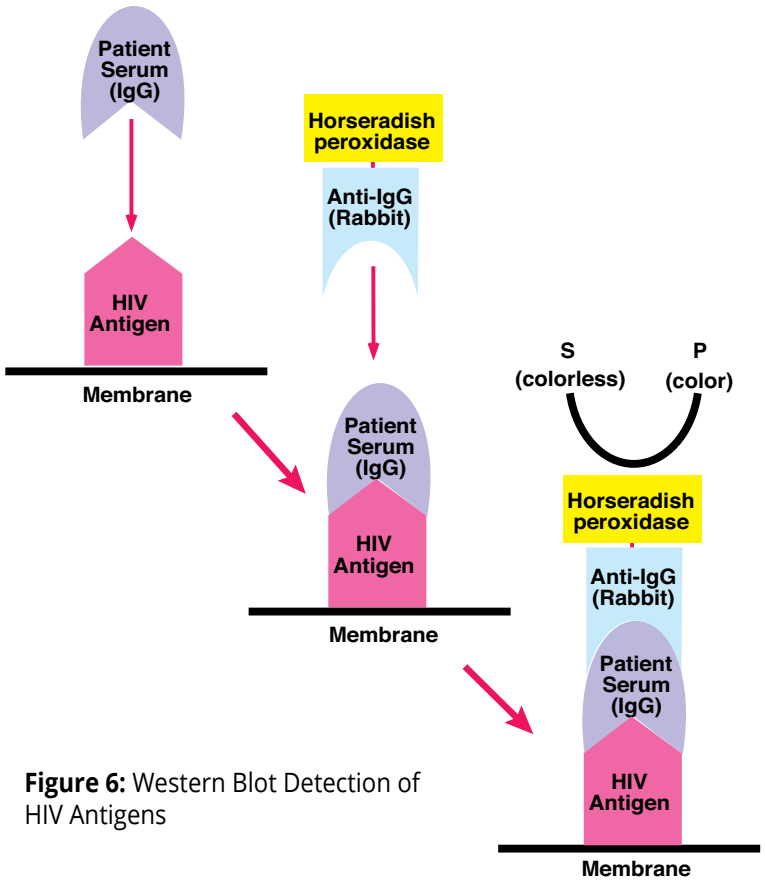


Figure 6: Western Blot Detection of HIV Antigens

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.

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

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. Students will also stain their gels with a fast and classroom friendly protein stain for greater resolution. They will then analyze their results and provide an HIV diagnosis or further testing recommendation for each patient.

Experiment Overview

EXPERIMENT OBJECTIVE:

In this experiment, students will use SDS-PAGE to identify HIV proteins in simulated patient samples. The results of this test are used to diagnose an HIV infection.

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.
- Exercise caution when working in the laboratory – you will be using equipment that can be dangerous if used incorrectly.
- Wear protective gloves when working with hot reagents like boiling water and melted agarose.
- DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
- Always wash hands thoroughly with soap and water after working in the laboratory.

Wear gloves and safety goggles

Acrylamide is a known neurotoxin and carcinogen and should be handled with extreme caution. Liquid acrylamide, used in the manufacture of SDS-PAGE gels, should only be handled in a chemical fume hood while wearing gloves and goggles. Polymerized acrylamide, including precast acrylamide gels, is safe but should still be handled with caution at all times.

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a 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.

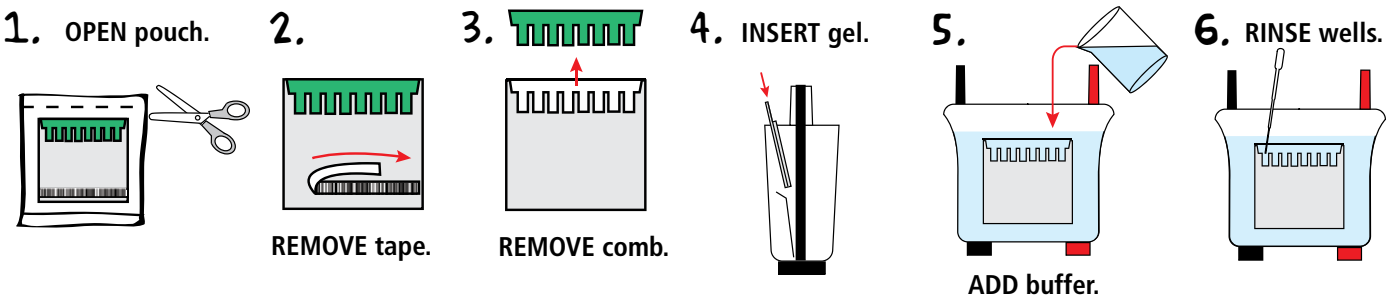
During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Module I-A: Preparing Precast Polyacrylamide Gels For Electrophoresis

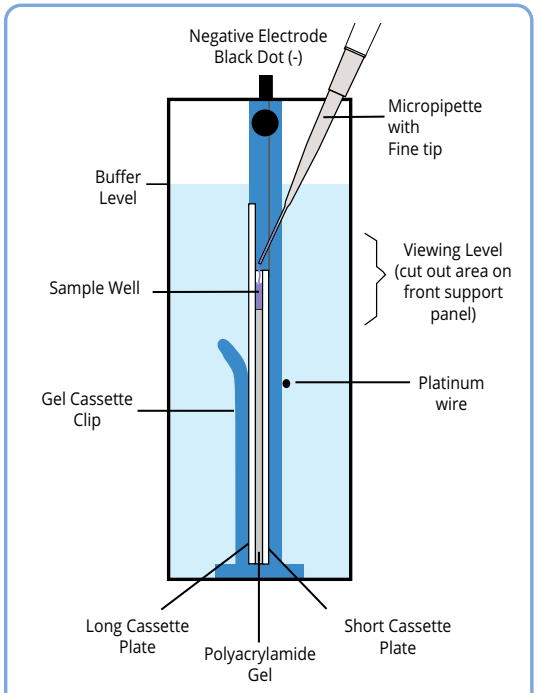


PREPARING THE POLYACRYLAMIDE GEL AND CHAMBER

NOTE: Although precast polyacrylamide gels and protein chambers will vary slightly in design, the procedure for their use will be similar.

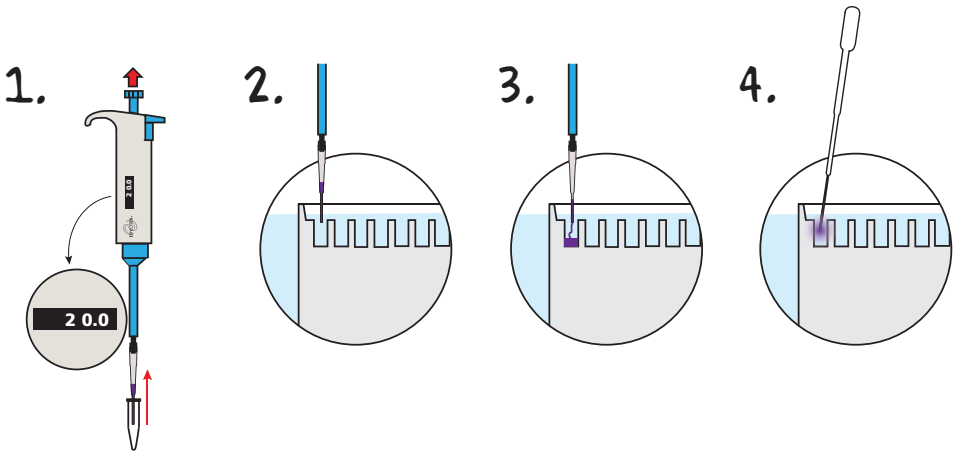
1. **OPEN** the pouch containing the gel cassette. Remove the cassette and place on bench with the shorter front plate facing up.
2. Gels may feature a sticker or tape at the bottom of the front plate. **REMOVE** the tape (if present) to expose the bottom of the gel.
3. Carefully **REMOVE** the comb by gently pulling upwards. Pull the comb straight up to prevent damage to the wells of the gel.
4. **INSERT** the gel into the electrophoresis chamber. Orient the gel according to the manufacturer's instructions. ***NOTE: For EDVOTEK® vertical electrophoresis chambers, the short plate should face the middle of the apparatus.***
5. **ADD** diluted electrophoresis buffer to the chamber. The buffer should cover the top of the shorter plate.
6. **RINSE** each well by squirting electrophoresis buffer into the wells using a transfer pipet. Using the transfer pipet, carefully straighten any wells which may have been distorted during comb removal or rinsing.

The gel is now ready for practice gel loading.



A polyacrylamide gel cassette in the EDVOTEK® Vertical Electrophoresis Apparatus, Model #MV10.

Module I-B: Practice Gel Loading



Wear gloves and safety goggles

NOTE: EDVOTEK® Cat. #638, Fine Tip Micropipette Tips are recommended for loading samples into polyacrylamide gels. A regular microtip may damage the cassette and result in the loss of protein samples.

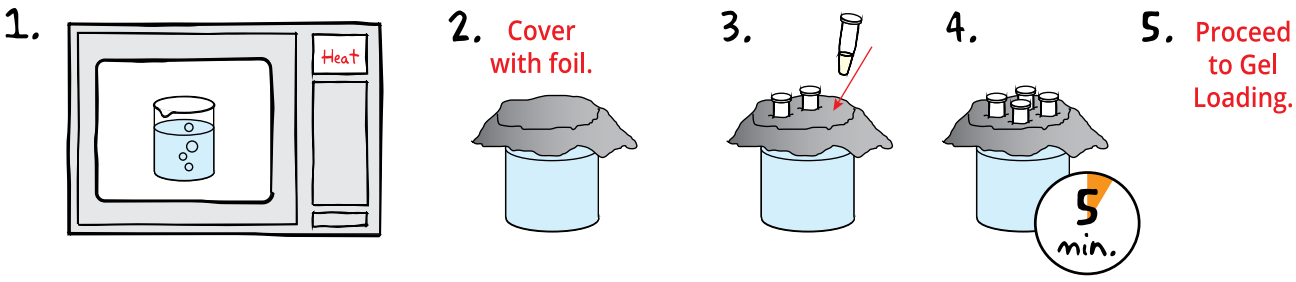
1. **PLACE** a fresh tip on the micropipette. **REMOVE** 20 µL of practice gel loading solution.

2. **PLACE** the lower portion of the pipette tip below the surface of the electrode buffer, directly over a sample well. The tip should be at an angle pointed towards the well. The tip should be partially against the back plate of the gel cassette, but the tip opening should be over the sample well. **Do not try to jam the pipette tip in between the plates of the gel cassette.**

3. **EJECT** all the sample by steadily pressing down on the plunger of the automatic pipette. Do not release the plunger before all the sample is ejected. Premature release of the plunger will cause buffer to mix with sample in the micropipette tip. Release the pipette plunger after the sample has been delivered and the pipette tip is out of the buffer.

4. **REMOVE** the practice gel loading solution from the sample wells. **FILL** a transfer pipet with buffer and **SQUIRT** a stream into the sample wells. This will displace the practice gel loading solution, which will be diluted into the buffer and will not interfere with the experiment. ***NOTE: Practice gel loading solution must be removed from the sample wells prior to sample loading.***

Module II: Performing SDS-PAGE with Protein Samples



PROTEIN DENATURATION:

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

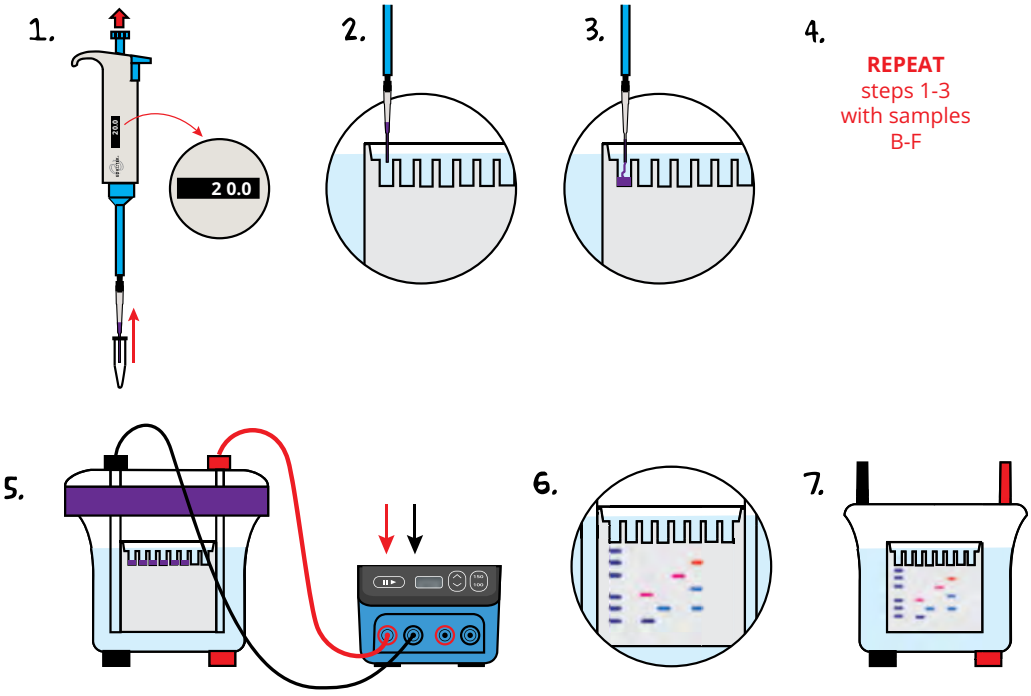
1. Using a hot plate or microwave, **HEAT** a beaker of water until it boils.
2. **COVER** with aluminum foil and carefully remove from heat.
3. Tightly **CAP** sample tubes. **PUSH** tubes through foil to suspend in the boiling water.
4. **INCUBATE** the samples for 5 minutes.
5. Immediately **PROCEED** to loading the gel. (For loading, the samples can be aliquoted into individual microcentrifuge tubes or placed at a classroom pipetting station for students to share.)

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 gel loading.

Samples must be boiled in screw top microcentrifuge tubes!

Module II: Performing SDS-PAGE on Protein Samples, continued



LOADING THE PROTEIN SAMPLES:

1. Using a fresh pipette tip, **MEASURE** 20 µL of the Standard Protein Marker (A).
2. **PLACE** the pipette 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 and **CONNECT** the electrical leads to the power supply.
6. **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.
7. **TURN OFF** the power supply and carefully **REMOVE** the lid. The gel can now be removed from the chamber and stained.

Wear gloves and safety goggles

Table 2: Gel Loading

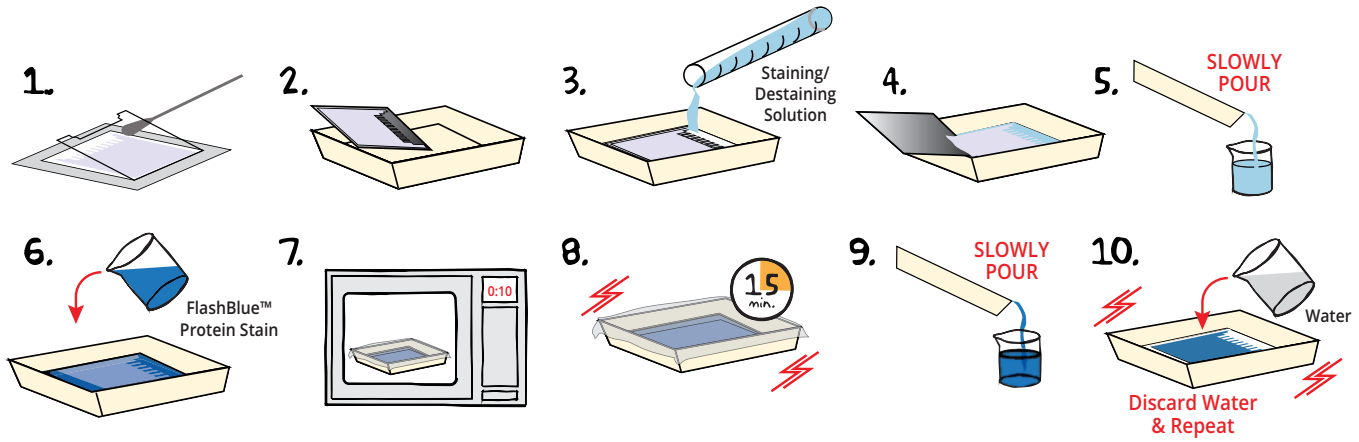
Lane		
1	Tube A	Standard Protein Marker
2	Tube B	Negative Control
3	Tube C	Positive Control
4	Tube D	Patient 1
5	Tube E	Patient 2
6	Tube F	Patient 3

Table A Time and Voltage Guidelines

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

Module III: Gel Staining with FlashBlue™ Protein Stain

Although the protein samples are provided in a pre-stained format it is possible to increase the intensity of the bands by using FlashBlue™ Protein Stain. Staining is rapid and sensitive. Student groups that shared a polyacrylamide gel during electrophoresis should also stain this gel together.



1. After electrophoresis, **LAY** the cassette down and **REMOVE** the front plate by placing a thin spatula or screwdriver at the side edge and gently lift it away from the larger back plate. In most cases, the gel will stay on the back plate. If it partially pulls away with the front plate, let it fall onto the back plate. ***Handle very carefully as the thin gels are extremely fragile.***

2. **TRANSFER** the gel on the back plate to a clean tray.

3. **ADD** a sufficient volume (approximately 50-75 mL) of the staining/destaining solution into the tray to **COVER** the gel and back plate.

4. Carefully **REMOVE** the back plate from the tray, leaving just the gel in the tray containing the staining/destaining solution. Bands may be easier to see once the cassette is removed. **OBSERVE** the gel and take a photo/sketch the banding pattern in your notebook before continuing. ***NOTE: If the gel sticks to the plate, gently nudge the gel off the plate using two GLOVED fingers.***

WEAR GLOVES AND SAFETY GOGGLES

Gloves must be worn during this procedure. Avoid touching the gel without gloves.

Polyacrylamide gels are very thin and fragile. Use care in handling to avoid tearing the gel.

5. **DISCARD** the staining/destaining solution. ***Pour slowly to keep the gel in the container.***

6. **ADD** 30 mL of prepared FlashBlue™ Protein Stain.

7. (OPTIONAL) **COVER** the container with plastic wrap and **MICROWAVE** for 10 seconds to gently heat the solution.

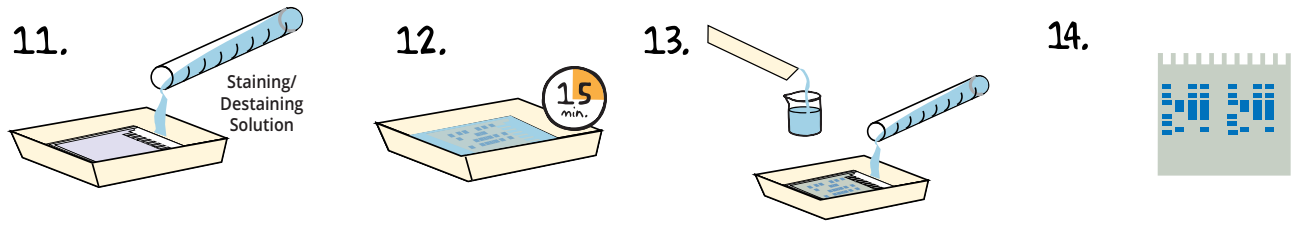
8. **INCUBATE** for 15 minutes at room temperature, **SHAKING** occasionally.

9. **DISCARD** the FlashBlue™ Protein Stain solution. ***Pour slowly to keep the gel in the container.***

10. **WASH** the gel by partially filling container with water and gently rocking back and forth several times. **DISCARD** the used water and **REPEAT** with fresh water.

continued

Module III: Gel Staining with FlashBlue™ Protein Stain, continued



11. **ADD** 30 mL of staining/destaining solution to the gel.

12. **INCUBATE** for 15 minutes at room temperature. **EXAMINE** the gel.

13. (OPTIONAL) **DISCARD** the used staining/destaining solution and **ADD** an additional 30 mL of staining/destaining solution. **INCUBATE** for 15-60 minutes at room temperature until the appearance and contrast of the protein bands against the background improves.

14. After staining, protein bands will appear medium to dark blue against a light background. A white light box can be used to better visualize the protein bands. **OBSERVE** the number of bands in the positive and negative controls as well as the three patients. **ESTIMATE** the size of each band using the standard markers*. Finally, **DETERMINE** the diagnosis for each of the three patients, if a result is inconclusive you can recommend retesting.

*For the most accurate size estimates measure migration distances and plot these on a semilog graph (see Appendix A).

STORING THE GEL

- Gel may be left in deionized water for several hours with no loss in sensitivity and band intensity. This step should be performed once a desired background and stained protein bands are obtained. Pour off the destaining solution from Step 12 (or 13) and add a sufficient amount of deionized water to cover the gel.

- For permanent storage, the gel can be dried between two sheets of cellophane (saran wrap) stretched in an embroidery hoop. Air dry the gel for several days until the gel is paper thin. Cut the “extra” saran wrap surrounding the dried gel. Place the dried gel overnight between two heavy books to avoid curling. Tape it into a laboratory book.

Study Questions

1. Describe protein electrophoresis. Why was it necessary to boil the proteins in the presence of SDS before loading them onto the gel?

2. How does the HIV western blot test work?

3. What are the advantages of a western blot test for detecting diseases like HIV? Can you think of any possible disadvantages?

4. Why do doctors require multiple positive bands on a western before determining that the patient is infected with HIV?

Instructor's Guide

ORGANIZING AND IMPLEMENTING THE EXPERIMENT

Prior to starting this experiment, carefully check the list of components and requirements on page 3 to ensure you have all the necessary components and equipment.

This experiment requires three 12% polyacrylamide Gels to be shared by the 6 student groups. Each group requires 6 sample wells. If necessary it is possible to omit sample B, the negative control, to allow two groups to share a 10 well gel.

Preparation For:	What to do:	When:	Time Required:
Module I: Preparing Precast Polyacrylamide Gels for Electrophoresis	Prepare diluted electrophoresis buffer	Up to one day before performing the experiment.	15 min.
	Rehydrate and aliquot protein samples	Up to one day before performing the experiment and stored at -20˚ C.	15 min.
Module II: Performing SDS-PAGE on Protein Samples	Prepare waterbaths for denaturing proteins	Up to one day before performing the experiment.	15 min.
	Denature proteins (optional)	No more than 10 min. before performing the experiment.	10 min.
Module III: Gel Staining with FlashBlue™ Protein Stain	Prepare staining solutions	Anytime before performing the experiment.	10 min.

Pre-Lab Preparations

PREPARING PROTEIN SAMPLES

1. Add 160 µL of distilled or deionized water to each tube (A-F) and allow the samples to hydrate for several minutes. Vortex or flick tube vigorously to mix. Resuspended proteins may be kept at room temperature for immediate use or frozen until needed.
2. The protein samples must be heated in their original 1.5 ml screw-top microcentrifuge tubes before use. This step can be completed by laboratory instructors immediately before the lab period or it can be performed by the students during the lab period. For instructions on denaturing the protein samples please refer to Module II, page 12.
3. Label six (6) snap-top microcentrifuge tubes for each protein sample (A-F).
4. After boiling, aliquot 25 µL of each sample into the appropriate tubes. The proteins should be aliquoted and then loaded by students as quickly as possible once heated.

PREPARING ELECTROPHORESIS BUFFER

Prepare the electrophoresis buffer by adding and mixing 1 part Tris-Glycine-SDS 10x buffer concentrate to 9 parts distilled water.

The approximate volume of 1x electrophoresis buffer required for EDVOTEK Protein Vertical Electrophoresis units are listed in Table B. The buffer should just cover the back plate of the gel cassette.

Table B — Tris-Glycine-SDS Electrophoresis (Chamber) Buffer

EDVOTEK Model #	Total Volume Required	Concentrated Buffer (10x)	+ Distilled Water
MV10	580 mL	58 mL	522 mL
MV20	950 mL	95 mL	855 mL

ELECTROPHORESIS TIME AND VOLTAGE

Your time requirements will dictate the voltage and the length of time it will take for your samples to separate by electrophoresis. Approximate recommended times are listed in Table A.

Run the gel until the bromophenol blue tracking dye is near the bottom edge of the gel.

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.

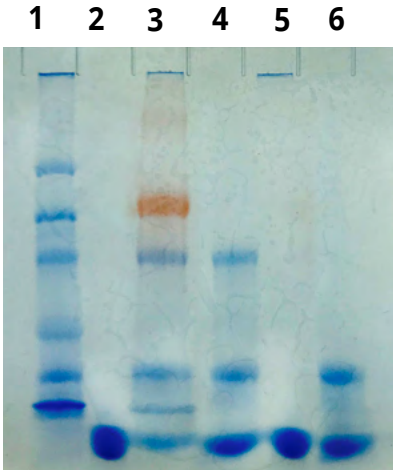
PREPARATION FOR STAINING GELS

1. Prepare a stock solution of white vinegar and ethanol* by combining 400 mL white vinegar with 200 mL ethanol. Gently mix. Label as "Staining/Destaining Solution".
2. Add 125 mL of the Staining/Destaining Solution to a 250 mL flask or beaker. Add the entire contents of the FlashBlue™ Protein Stain powder and briefly stir or shake to mix. Residual powder can be rinsed from the tube using an additional 1 mL of Staining/Destaining Solution.
3. Store both solutions at room temperature until needed.
4. TWO student groups will share: 30 mL FlashBlue™ Protein Stain, 140 mL Staining/Destaining Solution, water, a staining tray, and plastic wrap.

**White vinegar, sometimes called distilled or spirit vinegar, is an easy to find cooking and cleaning vinegar with an acetic acid concentration between 5-8% and a pH ~2.6. Ethanol is a common lab supply which is available at various concentrations. Our FlashBlue™ Protein Stain has been designed to work with a wide range of white vinegars. However, we do recommend using 95% Ethanol or higher.*

Experiment Results and Analysis

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	Negative Control	0 bands	HIV negative
3	Positive Control	4 bands (72kD, 38kD, 20kD, 14kD)	HIV positive
4	Patient 1	2 bands (38kD, 20kD)	HIV positive
5	Patient 2	0 bands	HIV negative
6	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

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

Appendices

A Size Determination of Unknown Proteins

B EDVOTEK® Troubleshooting Guide

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

Appendix A

Size Determination of Unknown Proteins

	Migration Distance (cm)	Molecular Weight (daltons)
Marker Protein 1 (Phosphorylase)		94,000
Marker Protein 2 (Bovine Serum Albumin)		67,000
Marker Protein 3 (Ovalbumin)		38,000
Marker Protein 4 (Carbonic anhydrase)		30,000
Marker Protein 5 (Soybean Trypsin Inhibitor)		20,000
Marker Protein 6 (Lysozyme)		14,000
Unknown Protein Sample 1		
Unknown Protein Sample 2		
Unknown Protein Sample 3		

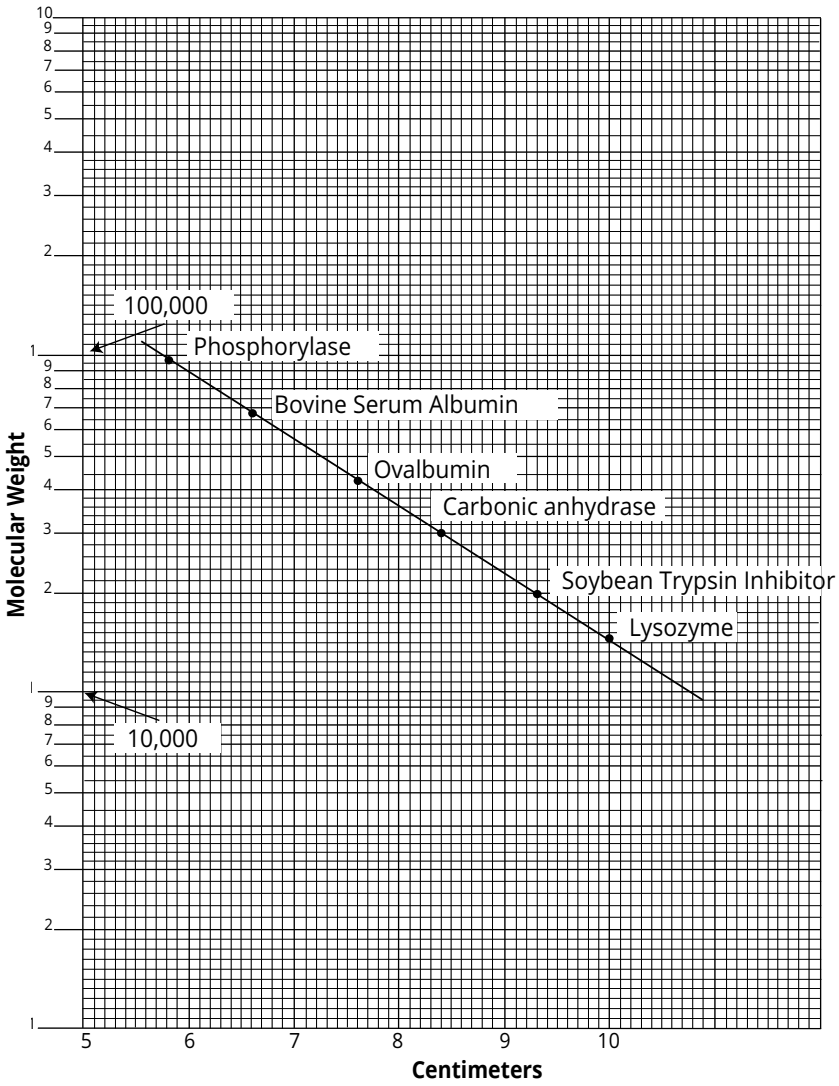
1. **MEASURE** the migration distance, in centimeters (to the nearest millimeter) of every major band in the gel. All measurements should be from the bottom of the sample well to the bottom of the protein band.

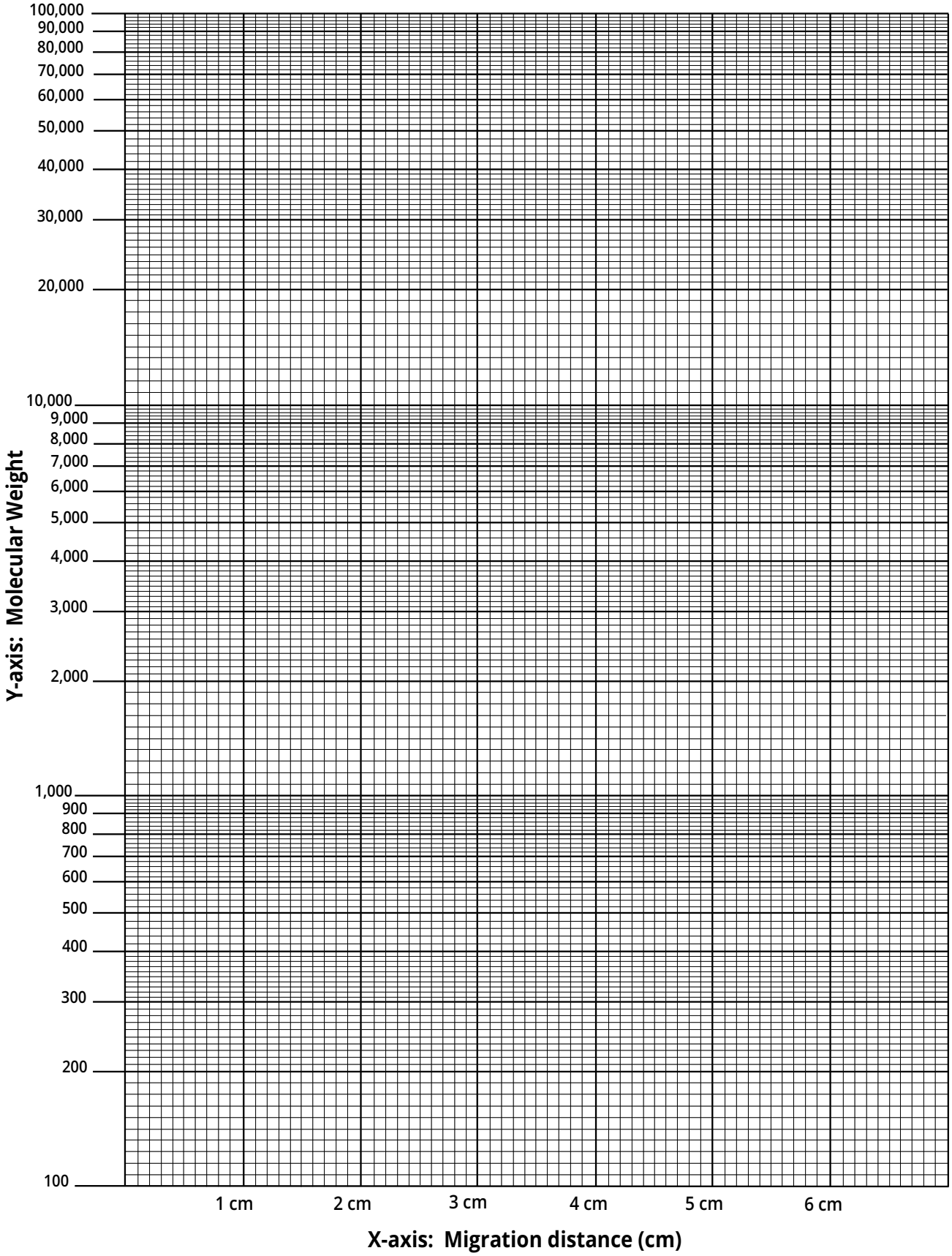
2. Using semilog graph paper, **PLOT** the migration distance or relative mobility (Rf) of each standard protein on the non-logarithmic x-axis versus its molecular weight on the logarithmic y-axis. **CHOOSE** your scales so that the data points are well spread out.

3. **DRAW** the best average straight line through all the points. This line should roughly have an equal number of points scattered on each side of the line. As an example, refer to the figure at left. This method is a linear approximation.

4. Using your standard graph, **DETERMINE** the molecular weight of the three unknown proteins. This can be done by finding the Rf (or migration distance) of the unknown band on the x-axis and drawing a straight vertical until the standard line is intersected.

5. A straight line is then made from the intersection across to the y-axis where the approximate molecular weight can be determined.





Appendix B
EDVOTEK® Troubleshooting Guide

PROBLEM:	CAUSE:	ANSWER:
Gel is not running properly.	Running buffer was not properly prepared.	Check buffer protocol, make fresh buffer.
	Wrong buffer used.	Check gel recipe, buffer must be compatible with the gel.
	Buffer volume is too low.	Buffer must fully cover the sample wells throughout the entire experiment.
	Gel is inserted in the wrong orientation.	Check with manufacturer for proper setup of the electrophoresis chamber.
	Malfunctioning electrophoresis chamber or power supply.	Consult with manufacturer of electrophoresis chamber or power supply.
	Tape at bottom of precast gel not removed.	Carefully remove tape before running the gel.
	Buffer volume is too low.	Buffer must fully cover the sample wells throughout the entire experiment.
	Electrodes not connected or polarity reversed.	Check electrode connections at the gel box and power supply.
Poor band resolution or separation.	Diffusion of samples before power was turned on.	Minimize time between loading samples and the start of electrophoresis.
	The gel is old or expired.	Make fresh gels or order new pre-cast gels.
	Wrong concentration of acrylamide gel.	The kit is designed for 12% acrylamide gels, other concentrations will affect results.
Smiling or frowing of bands.	Proteins have been overloaded.	EDVOTEK ® has optimized this kit to avoid overloading. Be sure to load the amount recommended by the protocol.
	Wrong buffer was used.	Check gel recipe, the buffer must be compatible with the gel.
	Incorrect voltage supplied to the gel.	Check the protocol for the recommended voltage (page 13).
No bands on gel/ smallest bands missing from gel.	Proteins ran off gel.	Use the appropriate length of time for the chosen voltage. Be sure to monitor the tracking dye while the gel is running. For best results, the tracking dye should run 8-9 cm and should not be allowed to run off the gel.
Proteins have accumulated in the wells of the gel.	Proteins have aggregated.	Ensure proteins have fully denatured; boil proteins for 5 min. and load while still warm.
Bands are smeary and distorted.	The gel has overheated.	Reduce voltage, check buffer concentration and dilute if necessary.
Bands are faint.	Proteins have diffused or faded.	Repeat staining with increased staining times and/or increased destaining times.
	Too little protein was loaded.	EDVOTEK ® has optimized this kit to avoid underloading. Be sure to load the amount recommended by the protocol.