Edvo-Kit # 275

Edvo-Kit #275

AIDS Kit II: Simulation of HIV-1 Detection by Western Blot

Experiment Objective:

The objective of this laboratory is to understand the concepts and methodology involved with Western blots. The experiment will test for the presence of simulated viral proteins from three patients.

See page 3 for storage instructions.

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SAMPLE LITERATURE
Please refer to included weblink for correct version

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Table of Contents

	Page
Experiment Components	3
Experiment Requirements	3
Background Information	4
Experiment Procedures	
Experiment Overview and General Instructions	8
Module I: Protein Agarose Gel Electrophoresis	9
Module II: Capillary Western Blot Analysis	12
Module III: Molecular Weight Estimation	15
Study Questions	17
Instructor's Guidelines	
Notes to the Instructor	18
Pre-Lab Preparations	19
Avoiding Common Pitfalls	21
Experiment Results and Analysis	22
Study Questions and Answers	23

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Experiment Components

Components		Storage	Check (✓)
A	Positive Control	Freezer	❑
B	Negative Control	Freezer	❑
C	Patient 1	Freezer	❑
D	Patient 2	Freezer	❑
E	Patient 3	Freezer	❑
F	Standard Molecular Weight Dye Markers	Freezer	❑

This experiment is designed for 6 groups.

All remaining components can be stored at room temperature.

- UltraSpec-Protein Agarose™ ❑
- 10x Tris-Glycine-SDS Buffer (Chamber Buffer) ❑
- 10x Tris-Glycine powdered Buffer (for gel prep only) ❑
- Practice Gel Loading Solution ❑
- 1 mL Pipet ❑

Components for Membrane Transfer and Stain:

- Precut Western Blot Membranes (7 x 7 cm) ❑
- Precut Blotting Filter Papers (7 x 7 cm) ❑
- FlashBlue™ Protein Stain Powder ❑

Requirements

- Horizontal Gel Electrophoresis Apparatus
- D.C. Power Supply
- Automatic Pipettes with Tips
- Shaker Platform
- Incubation Oven (65°C)
- Microwave
- Microcentrifuge Tubes
- 750 mL or 1 L flask or beaker
- Pipets
- Graduated Cylinders
- Trays or Containers that can hold a 7 x 7 cm piece of membrane and 100 mL of liquid
- Disposable Lab Gloves
- Several Packs of Paper Towels
- Plastic Wrap
- Scissors
- Metric Rulers
- White Vinegar
- Ethanol (95% or higher)
- Distilled Water

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.

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Background Information

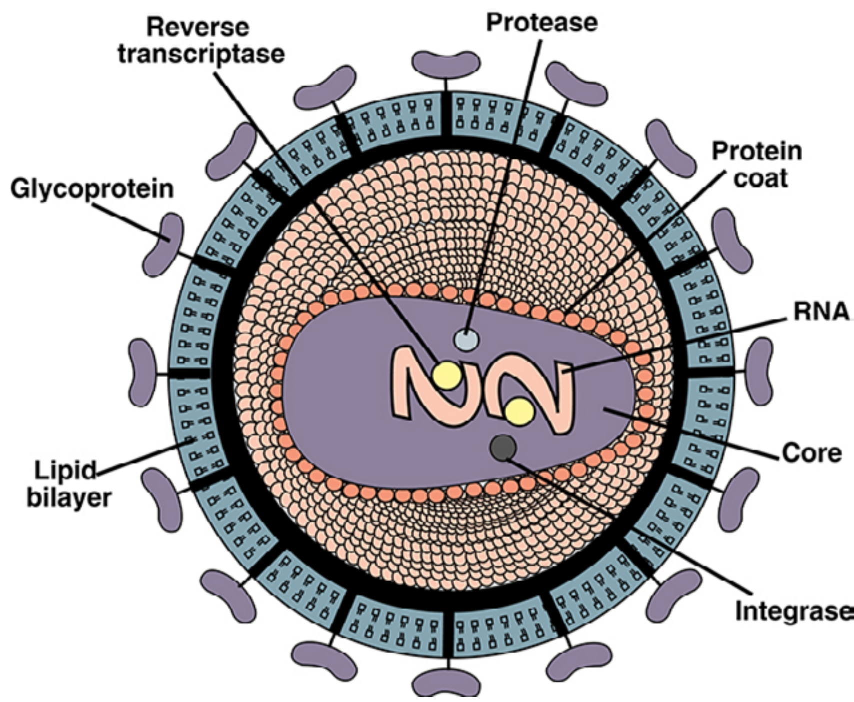
The Biology of HIV/AIDS

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 faulty immunosurveillance. 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.

The HIV Virus

The AIDS etiologic agent is the human immunodeficiency virus type 1 (HIV-1), a retrovirus. HIV-1 contains an RNA genome and the RNA-dependent-DNA-polymerase termed reverse transcriptase. Members of the retrovirus family are involved in the pathogenesis of certain types of leukemias and other sarcomas in humans and animals. The structure and replication mechanism of HIV is very similar to other retroviruses. However, HIV is unique in some of its properties - it specifically targets the immune system, is very immunoevasive, forms significant amounts of progeny virus in vivo during initial stages of infection and can be transmitted during sexual activity.

The HIV viral particle is surrounded by a lipid bilayer derived from the host cell membrane during budding. The viral proteins are identified by the prefix gp (glycoprotein) or p (protein) followed by a number indicating the approximate molecular weight in kilodaltons. The lipid bilayer contains gp120 and gp41. These two proteins are proteolytic products of the precursor gp160. The gp41 anchors gp120 in the bilayer. The protein gp120 is routinely used as a diagnostic marker for HIV in Western Blot Analysis. More recently other viral gp proteins are also included in the test. Beneath the bilayer is a capsid consisting of p17 and p18. Within this shell is the viral core. The walls of the core consist of p24 and p25. Within the core are two identical RNA molecules, 9800 nucleotides in length. Hydrogen bonded to each viral RNA is a cellular tRNA molecule. The viral RNA is coated by tightly bound molecules of p7 and p9. The core also contains approximately 50 molecules of reverse transcriptase.



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There are several other viral proteins whose precise functions are not fully understood. The virus can be grown in tissue culture for diagnostic and research purposes. Several of the viral proteins have been cloned and generated in relatively large quantities.

An individual can receive an inoculum of HIV through an abrasion in a mucosal surface (e.g., genital and rectal walls), a blood transfusion, or by intravenous injection with a contaminated needle. Virus or virally infected cells are found in body fluids such as semen and blood. The most important target for the virus is hematopoietic cells such as bone marrow derived monocytes, myelocytes and lymphocytes. Infection of immune system effector cells such as T cells and macrophages ultimately produce the most profound clinical consequences. Gp120 binds to the CD4 receptors on the surface of T helper (TH) cells. These receptors are membrane bound glycoproteins involved in T cell activation. Under normal conditions CD4 acts as a receptor for major histocompatibility class II (MHC II) membrane bound molecules that are present on the surface of macrophages and several other types of cells. TH cells are required for the body's overall immunological responses. The viral lipid bilayer fuses with that of the cells' membranes and the viral protein capsid becomes internalized via receptor mediated endocytosis. Subsequently, the rest of the CD4 receptors are down-regulated and gp120 appears on the T cell surface. Through a complex mechanism, the reverse transcriptase synthesizes a double stranded DNA copy of the genomic RNA template. The tRNA molecule acts as the primer of the first strand synthesis. The RNAse H activity of the reverse transcriptase degrades the RNA strand of the RNA-DNA duplex and the enzyme synthesizes a complimentary DNA strand. The DNA reverse transcripts (double-stranded DNA) migrate into the cell nucleus where they become covalently integrated into the cellular genomic DNA. The integration is partly catalyzed by viral proteins. The copy DNA integrates via specific, self-complimentary sequences at both ends called long terminal repeats (LTRs). These sequences also have important functions in viral transcription. The integrated copy DNA is called proviral DNA or the provirus. The provirus enters a period of latency that can last for several years. The proviral DNA is replicated along with the cellular DNA and can be inherited through many cell generations. The HIV proviral DNA contains the major genes common to all non-transducing retroviruses. These genes are gag, pol and env. HIV-1 also contains five or six smaller genes.

Unlike other cellular DNA polymerases, HIV DNA polymerase (reverse transcriptase) has a high error rate. These frequent mutations continually change the viral protein epitopes. This is believed to be the main mechanism of HIV immunoevasion. The gag gene is translated into a polypeptide that is cleaved by a viral protease into four proteins that form the inner shells. The protease is encoded in the pol gene. The pol gene encodes the reverse transcriptase and the integrase which is responsible for the genomic incorporation of copy DNA. The env gene encodes the surface glycoproteins the viral particles acquire as they bud from the cells. Viral replication causes the destruction of the TH cells.

Enzyme linked immunoadsorbent assay (ELISA) is an important immunochemical method used for the detection of low levels of antigens. ELISA is used for clinical screening for HIV in the blood supply. ELISA testing for HIV detects the patient's circulating IgG directed toward the viral antigens. A positive reaction in the ELISA requires more definitive testing for verification by the use of a Western Blot. One reason for this problem is that antibodies sometimes exhibit cross reactivity.

Properties of Proteins

Denaturing SDS gel electrophoresis separates proteins based on their size. SDS (sodium dodecylsulfate) is a detergent which consists of a hydrocarbon chain bonded to a highly negatively charged sulfate group. SDS binds strongly to most proteins and causes them to unfold to a rod-like chain and makes them net negative in charge. In the absence of a denaturing agent such as 2-mercaptoethanol, no covalent bonds are broken in this process. Therefore, the amino acid composition and sequence remains the same. Since its specific three-dimensional shape is abolished, the protein no longer possesses biological activity.

Proteins that have lost their specific folding patterns and biological activity but have intact polypeptide chains are called denatured. Proteins which contain several polypeptide chains that are non-covalent bonds will be dissociated by SDS into

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separate, denatured polypeptide chains. Proteins can contain covalent cross-links known as disulfide bonds. These bonds are formed between two cysteine amino acid residues that can be located in the same or different polypeptide chains. Treatment of proteins at 100°C for 3 minutes in the presence of high concentrations of reducing agents, such as 2-mercaptoethanol, will break disulfide bonds. This allows the SDS to completely dissociate and denature the protein.

During electrophoresis, the SDS denatured proteins migrate through the gel towards the positive electrode at a rate that is inversely proportional to their molecular weight. In other words, the smaller the protein, the faster it migrates. The molecular weight of the unknown is obtained by the comparison of its position after electrophoresis to the positions of standard SDS denatured proteins electrophoresed in parallel.

Western Blot Analysis

Western Blot Analysis involves the direct transfer of protein bands from an agarose or polyacrylamide gel to a charged nylon membrane for analysis. Following an electrophoresis experiment, the gel is removed from the tray and the nylon membrane is placed directly on the gel. (Nylon membranes are much stronger and more pliable than gels and can undergo many manipulations without tearing.) Protein bands are transferred to the surface of the nylon membrane and are adsorbed on the membrane by hydrophobic bonds. This transfer is achieved electrophoretically in specially designed chambers, by capillary flow or by the application of vacuum.

The total protein transferred can then be visualized by staining the membrane with protein dyes. Visualizing a specific protein within a mixture of proteins is usually detected by immunochemical methods. It cannot be detected by protein staining because the amount may be too low and the banding of the protein mixture may block it from view.

For immunological detection of specific protein, the unstained membrane is placed in a blocking buffer that contains detergent and protein that bind to all unoccupied sites on the nylon membrane. The membrane is then incubated in buffer that contains antibody to one (or more) of the blotted proteins. The antibody binds to the adsorbed protein (antigen) and subsequent washings removes unbound antibody. A secondary antibody that is covalently linked to an enzyme such as alkaline phosphatase or horseradish peroxidase is used for detection. The conditions for cross-linking the enzyme to the secondary antibody does not appreciably affect the antigen binding specificity, the affinity of the antibody, or the catalytic activity of the enzyme.

The membrane is then incubated in a solution of the secondary antibody where it will bind selectively to the bound antigen-primary antibody complex. Following this treatment, the membrane is washed to remove the unbound secondary antibody-enzyme complex and is then incubated in a solution containing a phosphatase or peroxidase substrate. The products of the enzymatic reaction yield chromogenic products that are easily visible on the nylon membrane.

In this experiment, students will use a modified Western Blot Analysis to detect an HIV protein.

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Experiment Overview

EXPERIMENT OBJECTIVE:

The objective of this laboratory is to understand the concepts and methodology involved with Western blots. The experiment will test for the presence of simulated viral proteins from three patients.

LABORATORY SAFETY

- 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.
- Always wash hands thoroughly with soap and water after working in the laboratory.

Wear gloves and safety goggles

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.

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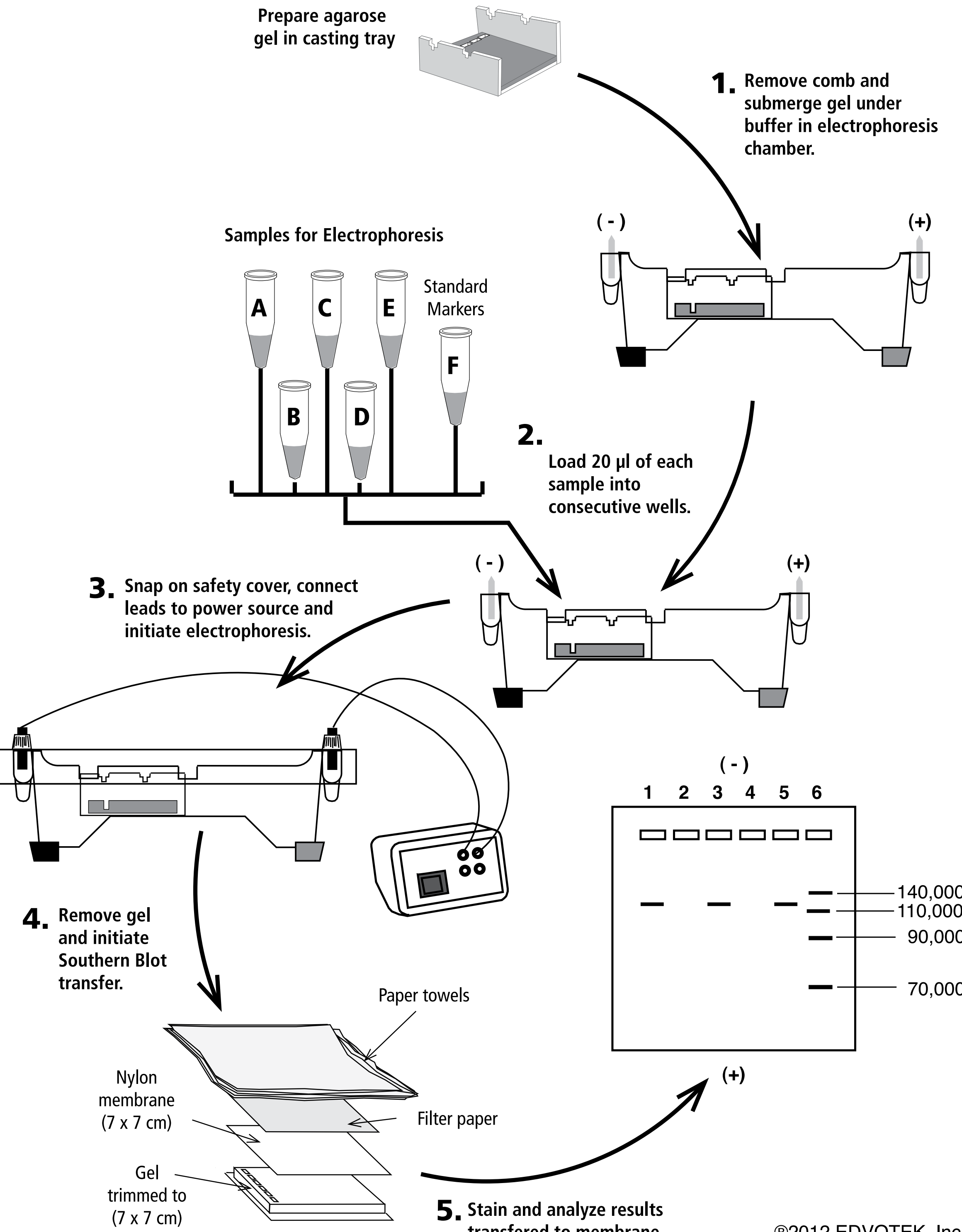
Experiment Overview, continued



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Module I: Protein Agarose Gel Electrophoresis

NOTE: When preparing the 2.5% protein agarose gel, make sure to use the 1x Tris-glycine buffer prepared by your instructor (DO NOT use the 1x Tris-glycine SDS electrophoresis running buffer).

PREPARATION OF 2.5% PROTEIN AGAROSE GEL

1. To make 2.5% agarose in 1X Tris-glycine Buffer, **DETERMINE** volume of agarose required for your gel tray. Refer to Table A to determine volume required.

2. **ADD** the required amount of protein agarose powder to the required volume of Tris-glycine buffer. **SWIRL** to disperse clumps.

3. With a marking pen, **INDICATE** the level of the solution volume on the outside of the flask.

4. **DISSOLVE** the agarose powder by boiling the solution.
   a. **MICROWAVE** the solution on high for 1 minute.
   b. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask.
   c. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved. (The solution should be clear like water.)
   d. **REMOVE** your solution from the microwave.
   e. **OBSERVE** if any evaporation has occurred. If it has, **ADD** distilled water until the solution is back to its original volume as marked in step 2.
   f. **MIX** by swirling.

CAUTION:
Melting at high temperatures or without stirring will result in scorching the agarose.

5. **COOL** the agarose to 55°C with swirling to promote even dissipation of heat. If detectable evaporation has occurred, **ADD** hot distilled water to bring the volume of the solution up to the original volume as marked on the flask.

Table A: Individual 2.5% Protein Agarose Gels

Size of Gel Casting tray	Amt of Agarose	+ Volume 1X Tris-glycine Buffer	= TOTAL Volume
7 x 7 cm	0.6 g	29.4 mL	30 mL
14 x 7 cm	1.2 g	58.8 mL	60 mL

IMPORTANT:
7x7 cm gels are preferred. 14x7 cm gels will need to be shared by two groups in Module I and then carefully cut into 7x7 cm squares before Module II so that both groups can independently perform a western blot.

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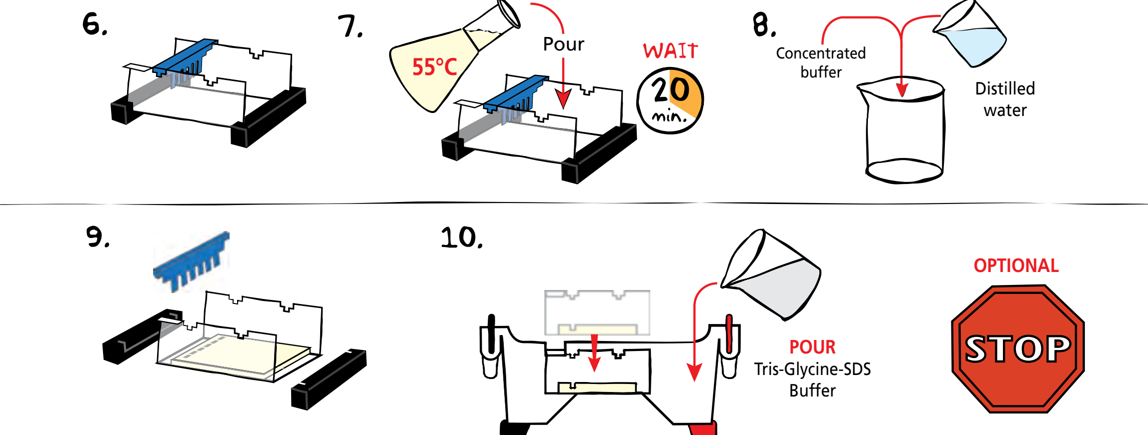
Module I: Protein Agarose Gel Electrophoresis, continued



6. While the agarose is cooling, **SEAL** the ends of a gel casting tray with the rubber end caps. **PLACE** the well template (comb) in the first set of notches. ***NOTE: 7 x 7 cm gels are preferred.***

7. Slowly, **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes.

8. While the agarose solidifies, **PREPARE** the electrophoresis (chamber) buffer according to Table B.

9. **REMOVE** both end caps and then remove the comb by pulling straight up. Work slowly and carefully to prevent damage to the wells.

10. **PLACE** the gel (still on the tray) into the electrophoresis chamber. **COVER** the gel with prepared Electrophoresis Buffer using the recommended volume in Table B. The gel should be completely submerged.

Table B: 1x Tris-Glycine-SDS (Chamber) Buffer

EDVOTEK Model #	Total Volume Required	Dilution: Conc. Buffer +	Dilution: Distilled Water
M12	400 mL	40 mL	360 mL
M36	1000 mL	100 mL	900 mL

OPTIONAL STOPPING POINT:
Gels can be stored overnight submerged in the electrophoresis chamber. Gels can also be stored for several days in the refrigerator. Keep refrigerated gels hydrated by storing each gel in a watertight plastic bag with a small amount of electrophoresis buffer.

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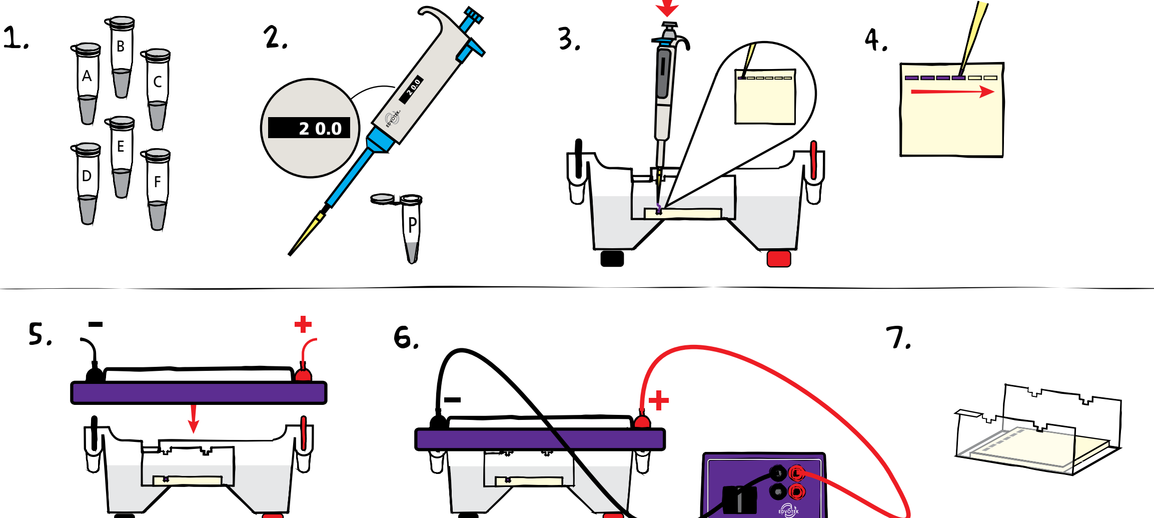
Module I: Protein Agarose Gel Electrophoresis, continued



PERFORMING ELECTROPHORESIS

Table 1: Gel Loading		
Lane 1	Tube A	Positive control
Lane 2	Tube B	Negative control
Lane 3	Tube C	Patient 1
Lane 4	Tube D	Patient 2
Lane 5	Tube E	Patient 3
Lane 6	Tube F	Standard Molecular Weight Dye Markers

1. **COLLECT** the ***rehydrated and heated*** samples (A-E) and the standard protein marker (F) from your instructor. ***NOTE: Load samples A-E while they are warm.***
2. Using a fresh pipet tip, **MEASURE** 20 µL of the first sample.
3. **PLACE** the pipet tip under the buffer and directly above the sample well. Slowly **DISPENSE** the sample by depressing the plunger.
4. **REPEAT** steps 2-4 with the remaining samples.
5. **PLACE** safety cover on the chamber. **CHECK** that the gel is properly oriented. Remember that the samples will migrate toward the positive (red) electrode.
6. **CONNECT** leads to the power supply and **PERFORM** electrophoresis (See Table C for time and voltage guidelines.) **PROCEED** with electrophoresis until the blue tracking dye has traveled at least 4 - 4.5 cm from the wells.

   NOTE: To optimize lab time students should begin to set up their western blot (Module II, steps 1-5) during electrophoresis.

7. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and immediately **PROCEED** to Wester Blot Analysis.

Table C: Time and Voltage

	Recommended Time	
Volts	Minimum	Maximum
150	20	45
125	25	55
100	30	60

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Module II: Capillary Western Blot Analysis

SETTING UP THE WESTERN BLOT

Wear Gloves - Do Not Touch The Nylon Membrane With Bare Hands.

Note: Only touch your Western Blot membrane with pre-washed lab gloves. Hands leave oil residues that prevent proteins from properly binding to the membrane. Many gloves contain powders which also interfere with binding or create background. To remove powder put gloves on and then wash them under tap water.

1. **PLACE** a piece of plastic wrap on your bench top. Be sure it is smooth and flat. If necessary, tape the four edges to keep wrap tight and secure. (The filter paper, gel, membrane, and paper towels will be placed onto it to make the blotting sandwich.)
2. Wearing gloves, carefully **REMOVE** the cover sheets (blue) from the Western Blot membrane (white). Using forceps, **TRANSFER** the membrane to a plastic tray.
3. **PRE-WET** the membrane (7 x 7 cm) by immersing it in approximately 20 mL of 95-100% ethanol for 10 seconds. **POUR** and **SAVE** the ethanol.
4. Immediately **IMMERSE** the membrane in distilled water for about 5 minutes to remove the ethanol.
5. **POUR** off the water and **IMMERSE** the membrane in diluted Transfer Buffer. Let the membrane sit until needed for the gel, at least 10 minutes.
6. Remove the gel from the tray and **IMMERSE** the gel into a separate tray that contains the Transfer Buffer. **SOAK** for 10 to 15 minutes.
7. **SATURATE** 1 piece of 7 x 7 cm filter paper with Transfer Buffer. **PLACE** the filter paper on the plastic wrap on your bench.
8. Carefully **REMOVE** the gel from the Transfer Buffer and place ***upside down*** on the filter paper. **ROLL** a pipet over the surface to remove air bubbles that may be trapped under the gel.
9. **PIPET** 1 to 2 mL of Transfer Buffer over the top of the gel and place the Western Blot membrane over the gel. ***NOTE: If the membrane appears to have a smooth surface on one side and a rough surface on the other side, make sure the rough surface is in direct contact with the gel.*** **ROLL** a pipet over the surface to remove air bubbles.
10. Use a pencil to lightly **TRACE** the location of each of the bands in lane 6. Beside each mark, **INDICATE** the color of the respective band. (B1 = Blue 1, B2 = Blue 2, P = Purple, and R = Red). ***NOTE: The protein marker used in this experiment is a dye that will not transfer to the membrane so it must be traced onto the membrane before blotting.***
11. **SATURATE** 1 piece of filter paper with Transfer Buffer and cover the membrane with the wet filter paper. **ROLL** a pipet over the surface to remove air bubbles.
12. **ADD** the second piece of dry filter paper to the top of the stack. **REMOVE** air bubbles.
13. Evenly **PLACE** a stack of 7 x 7 cm paper towels 4 to 6 cm in thickness on top of the stack.
14. **PLACE** a plastic tray or plate on top of the stack. Place a light weight beaker (400 mL size) on top.
15. **ALLOW** the protein transfer to proceed overnight or for a minimum of 4 hours.

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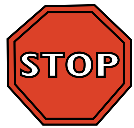
Module II: Capillary Western Blot Analysis, continued

PROCESSING THE BLOT MEMBRANE

1. **REMOVE** the tray, paper towels and filter paper from the top of the membrane. **LEAVE** the membrane in place on top of the gel.
2. Using a pencil, lightly **TRACE** the outline of each well and number according to loading sequence. ***(Remember the gel is upside down.)***
3. Using forceps, **REMOVE** the membrane from the gel and **PLACE** it on a clean piece of filter paper. ***The side that was in contact with the gel should be facing up.***
4. With a pencil, on one of the lower corners **WRITE** "F" (for front). On the other corner, **WRITE** your group number or initials.
5. **PLACE** the membrane in a 65°C incubation oven for 10 minutes to **FIX** the samples.

NOTE:
Put on gloves and wash them under tap water before handling membrane to ensure best results.



OPTIONAL STOPPING POINT

After the membrane has been fixed, the membrane can be held for several days before staining.

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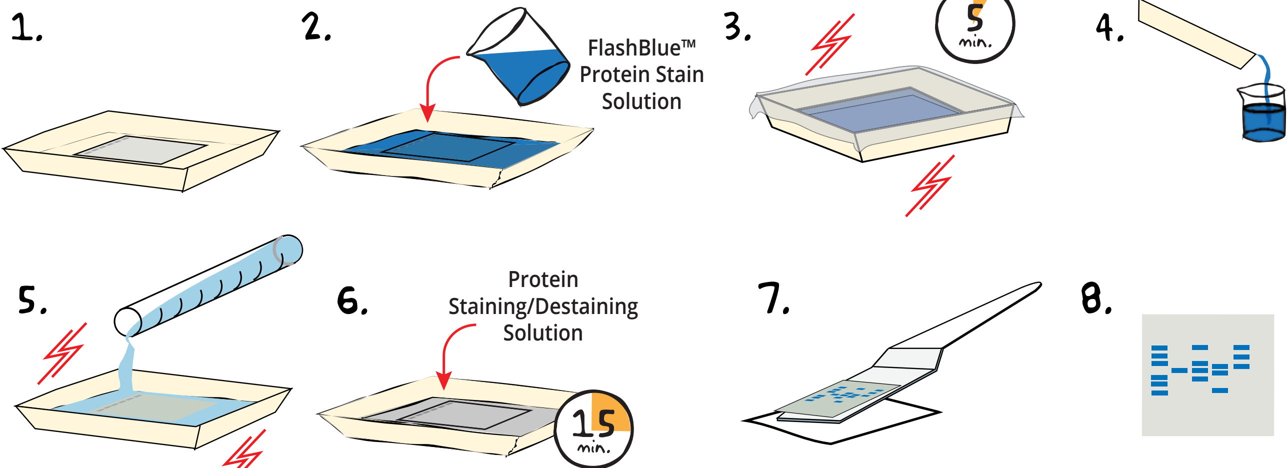
Module II: Capillary Western Blot Analysis, continued



STAINING THE BLOT MEMBRANE

1. **PLACE** the membrane into a shallow container, with the labeled "front" side facing up.
2. **ADD** 30 mL of FlashBlue™ Protein Stain. The membrane should be completely covered with liquid.
3. **INCUBATE** the membrane for 5 minutes at room temperature, **SHAKING** occasionally.
4. **DISCARD** the FlashBlue™ Protein Stain solution.
5. **WASH** the membrane by partially filling container with distilled water and gently rocking the membrane back and forth several times. **DISCARD** the used water and **REPEAT** one or two times with fresh distilled water.
6. **ADD** 30 mL of Protein Staining/Destaining solution to the membrane and **INCUBATE** for 15 minutes at room temperature. **EXAMINE** the membrane. If more contrast is desired between the background and bands, **DISCARD** the used Protein Staining/Destaining solution and **ADD** an additional 30 mL of Protein Staining/Destaining solution. **INCUBATE** for an additional 15 minutes at room temperature so that the appearance and contrast of the protein bands against the background improves.
7. **REMOVE** membrane from the solution and **LAY** on a piece of filter paper.
8. **COMPARE** the three patient samples to the positive and negative controls.

REMEMBER:
The Western HIV/AIDS diagnostic test establishes the presence of the viral coat protein gp120 by antibody interaction and also confirms the size of the protein band to be 120,000 daltons ±10%.

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Module III: Molecular Weight Estimation of Simulated Viral gp120 Protein

1. **MEASURE** the distance of each band traced on the membrane for the standard dye markers and the positive viral samples. Each measurement should be from the bottom of the well to the bottom of each band.

   The sizes for the standard dye markers are (in daltons):

   Blue 1 140,000
   Blue 2 110,000
   Purple 90,000
   Red 70,000

2. Using semilog graph paper, **PLOT** the distance travelled by each band on the x-axis and their molecular weight on the y-axis.

3. **DETERMINE** the molecular weight of the viral protein by the extrapolating from the standard curve.

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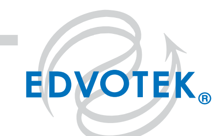

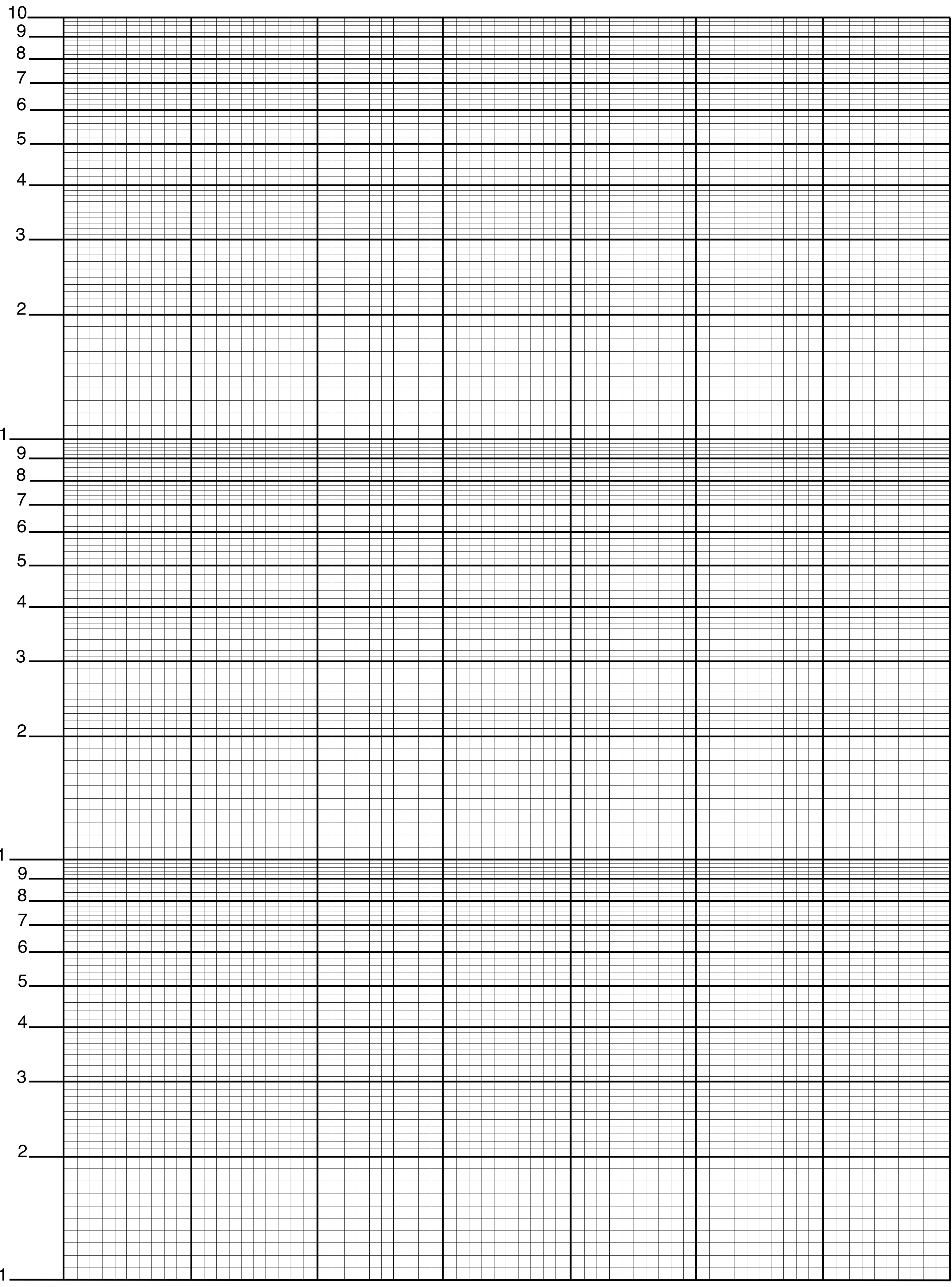

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Study Questions

1. Why are the electrophoretically fractionated proteins transferred to a membrane for detection?

2. Would higher or lower percentage gels favor transfer to a membrane? Would larger or smaller proteins transfer better?

3. What is the purpose of the negative and positive controls?

4. What is the difference between a Western, Northern and Southern Blot?

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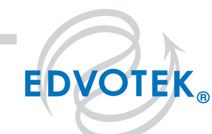

Instructor's Guide

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 is supplied with reagents for six groups. Each group can be provided with protein agarose gel or can create their own.

Preparation for:	What to do:	When?	Time Required:
MODULE I	Distribute student supplies OR	Anytime before Module I	10 minutes
	Batch prepare gels and electrophoresis buffer	Up to one week before Module I.	30 minutes
MODULE II	Rehydrate protein samples	Up to one week before Module II.	10 minutes
MODULE III	Prepare stain solutions	Up to one week before Module III.	10 minutes

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Pre-Lab Preparations

PREPARATION OF MEMBRANES

- Remember to wear rinsed and dried lab gloves when handling Western blot membranes. Oils from hands or powders from gloves will interfere with the procedure.
- Protect Western blot membranes by keeping them between the two blue cover sheets until Student Module II step 2.
- Six precut 7 x 7 cm Western blot membranes and six precut 7 x 7 cm blot squares are provided for this experiment. If you are working with smaller gels you will need to alter the size of your membranes, blot squares, and towels to the exact size of your gel to ensure an optimal transfer.

PREPARATION OF BUFFERS
(On the day of the lab)

Preparing the Tris-Glycine Buffer (for gel preparation only)

1. Add the powder contents of the Tris-Glycine Buffer to a flask or beaker (500 mL size or larger).
2. Add 300 mL distilled or deionized water to the powdered buffer. Swirl and stir to dissolve the powder (a stir plate, if available, may be useful). This is 1x buffer, ready for use in preparing the gel(s).
3. (Optional) To save time you can prepare the gels for your class as part of the prelab by following the kit instructions on pages 9 and 10. ***NOTE: 7 x 7 cm gels are preferred.***

Transfer Buffer (required first day)

1. To 350 mL of distilled water, add 50 mL of 10X Tris-Glycine-SDS liquid concentrate.
2. Add 100 mL of 95 - 100% ethanol to the buffer. Mix. Keep tightly covered at room temperature until ready to use.

Electrophoresis Buffer, Tris-glycine-SDS Buffer

1. Add 1 part EDVOTEK® 10X Tris-Glycine-SDS buffer to every 9 parts distilled or deionized water.
2. Make enough 1X buffer for all electrophoresis units used. The approx. volume of 1X electrophoresis buffer required for EDVOTEK Horizontal Electrophoresis Chambers are listed in Table B in Module I.

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Pre-Lab Preparations

PREPARATION OF LYOPHILIZED PROTEIN SAMPLES FOR ELECTROPHORESIS
(On the day of the lab - Required first day)

1. Add 130 µL of distilled or deionized water to each tube (A-E). Incubate the samples at room temperature for 5 minutes. Vortex or mix vigorously.
2. Bring a beaker of water, covered with aluminum foil, to a boil. Remove from heat.
3. Make sure the sample tubes A through E are tightly capped and well labeled. The bottom of the tubes should be pushed through the foil and immersed in the boiling water for 5 minutes. The tubes should be kept suspended by the foil.

NOTES:
Store any unused portion of reconstituted sample at -20°C and repeat steps 2 - 4 when using samples at a later time.

Note: DO NOT boil the Standard Molecular Weight Dye Markers (Component F).

4. Remove the samples and tap or briefly microcentrifuge to get condensate at the top of the tubes back into the sample.
5. Aliquot 20 µL of each sample plus the Standard Markers (Component F) for each lab group. Have students load samples onto the gel while the heated samples (A-E) are still warm to avoid aggregation.

STAINING THE MEMBRANE

Prepare Staining Solutions

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 100 mL of the Staining/Destaining Solution to the bottle of FlashBlue™ Protein Stain. Shake briefly to mix. Pour into a new beaker or bottle and add an additional 80 mL of the Staining/Destaining Solution. Swirl or shake to mix.
3. Store both solutions at room temperature until needed.
4. Aliquot 60 mL of the Staining/Destaining Solution and 30 mL of the FlashBlue™ Protein Stain Solution to each student group.

**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.*

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Avoiding Common Pitfalls

Potential pitfalls and/or problems can be avoided by following the suggestions and reminders listed below.

- To ensure that protein bands are well resolved, make sure the gel formulation is correct and that gels are quickly poured as high percentage gels can cool quickly and then set unevenly. If this happens you can remelt the gel.
- Correctly dilute the concentrated buffer for preparation of both the gel and electrophoresis (chamber) buffer. Remember that without buffer in the gel, there will be no protein mobility. Use only distilled or deionized water to prepare buffers. ***Do not use tap water.***
- For optimal results, use fresh electrophoresis buffer and Staining Solution prepared according to instructions.
- This experiment kit contains practice gel loading solution. If you are unfamiliar with gel electrophoresis, it is suggested that you practice loading the sample wells before performing the actual experiment.
- To avoid loss of protein bands into the buffer, make sure the gel is properly oriented so the samples are not electrophoresed in the wrong direction off the gel.
- The protein marker used in this experiment is a dye that will not transfer to the membrane. It must be traced onto the membrane before blotting.

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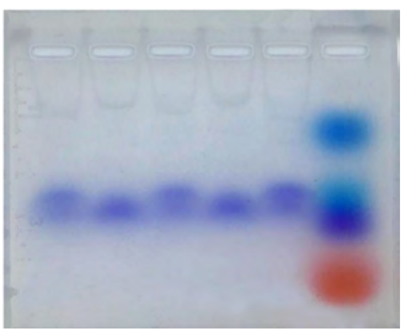
Experiment Results and Analysis

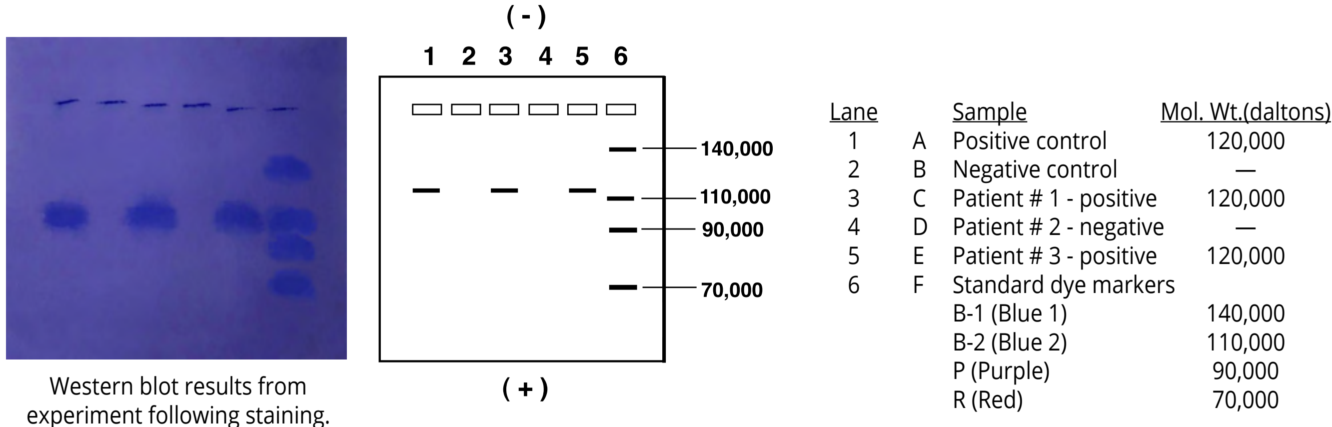


MODULE I

Gel results following electrophoresis.

MODULE II

Samples containing the simulated HIV protein and the positive control should show a protein band. The positive control, Patients 1 and 3 show bands. The negative control and Patient 2 should not have any visible bands.



Western blot results from experiment following staining.

Lane		Sample	Mol. Wt.(daltons)
1	A	Positive control	120,000
2	B	Negative control	—
3	C	Patient # 1 - positive	120,000
4	D	Patient # 2 - negative	—
5	E	Patient # 3 - positive	120,000
6	F	Standard dye markers	
		B-1 (Blue 1)	140,000
		B-2 (Blue 2)	110,000
		P (Purple)	90,000
		R (Red)	70,000

The idealized schematic shows the relative positions of the protein bands, but is not drawn to scale. The molecular weight of the viral glycoprotein for the positive control and the positive patients can be extrapolated from the standard curve. Students should plot the distance in millimeters traveled by each of the standard proteins on the x-axis and the respective molecular weights on the y-axis using semi-log graph paper.

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