EDVOTEK® • The Biotechnology Education Company®

Edvo-Kit # 317

Edvo-Kit #317

Western Blot Analysis

Experiment Objective:

The objective of the experiment is for students to understand the theory and applications of Western Blot Analysis.

See page 3 for storage instructions.

Version 317.240409

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

Experiment Components

	Component	Storage	Check ✓
A	Pre-stained Protein Standard Markers (lyophilized)	Refrigerator	❑
B	Negative Control (lyophilized)	Refrigerator	❑
C	BSA high concentration (lyophilized)	Refrigerator	❑
D	BSA low concentration (lyophilized)	Refrigerator	❑
	Immunochemical and Blotting Reagents		
E	Anti-BSA Protein Antibody	Refrigerator	❑
F	Secondary Antibody Conjugate	Refrigerator	❑
G	Hydrogen Peroxide, stabilized	Refrigerator	❑
H	Peroxide Co-substrate	Refrigerator	❑
I	10x Blocking Buffer	Refrigerator	❑
J	10x Wash Buffer	Refrigerator	❑
K	Powdered Milk	Room Temp.	❑
•	10x Tris-Glycine-SDS Buffer (Chamber Buffer)	Room Temp.	❑
•	10x Tris-Glycine-SDS Buffer (Transfer Buffer)	Room Temp.	❑
•	Practice Gel Loading Solution	Room Temp.	❑
•	Nitrocellulose Membrane	Room Temp.	❑
•	Filter Paper (Blotting Paper)	Room Temp.	❑
•	Large Filter Paper (Wick)	Room Temp.	❑

This experiment contains enough reagents for 6 lab groups, with 2 groups sharing a gel.

Enough membrane is provided to accommodate blotting of three 8 x 10 cm gels.

Components A - J should be stored in the refrigerator. All other components can be stored at room temperature.

Experiment Requirements *(NOT included with this experiment)*

- 12% Denaturing Polyacrylamide gels (3) (**Cat. #651** recommended)
- Vertical Gel Electrophoresis Apparatus (**Cat. #581** recommended)
- D.C. Power Supply
- Shaker Platform (optional)
- Adjustable Pipettes with Tips
- Microtest (Microcentrifuge) Tubes
- Beakers
- Transfer Pipets
- Graduated Cylinders
- Plastic Wrap
- Scissors
- Trays or Containers
- Forceps
- Several Packs of Paper Towels
- Latex or Vinyl Lab Gloves
- Safety Goggles
- 95-100% Methanol
- 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. None of the experiment components are derived from human sources.

Background Information

Cells are composed of carbohydrates, lipids, nucleic acids, and proteins. Proteins are an integral part of every cell, and carry out critical cellular functions including membrane stabilization, signaling, transcription/translation, and energy production. Therefore, a wide variety of proteins exist within a given cell. Indeed, within a single human cell it is estimated that there are approximately 10^{10} proteins present. Problems in protein production and mutated proteins are responsible for many genetic disorders. How can we detect problems with a specific protein amongst so many other normal proteins? One method that scientists have developed is the Western Blot.

In Western Blot analysis, the first step is running a denaturing polyacrylamide gel. Similarly to how agarose gel electrophoresis can separate DNA fragments based on size, polyacrylamide gel electrophoresis can separate proteins based on size. Unlike DNA, which always consists of nucleotides arranged in a double-helix structure, the amino acids of proteins can take on large and complex configurations. In order for these proteins to be accurately resolved by their size, they must first be denatured, or unraveled from their 3-dimensional structure.

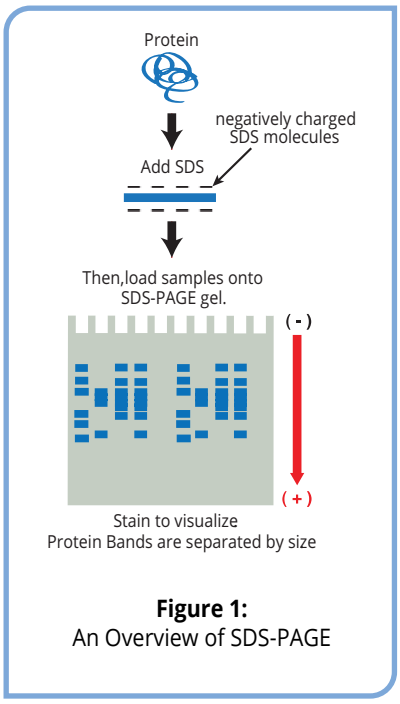


Figure 1:
An Overview of SDS-PAGE

Denatured proteins have lost their specific folding patterns and biological activity, but their amino acid chain remains intact. In most cases, the proteins are denatured through boiling in the presence of sodium dodecylsulfate (SDS) and 2-mercaptoethanol. SDS is a detergent consisting of a hydrocarbon chain bound to a negatively charged sulfate group. SDS binds to amino acids, giving the entire protein a net negative charge. Additionally, SDS binding causes proteins to unfold and helps in the denaturation process. However, even with SDS, some proteins have very strong bonds between amino acids, including covalent crosslinks 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. High concentrations of reducing agents, such as 2-mercaptoethanol, will break disulfide bonds and allow complete denaturation of 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. This is because the polyacrylamide gel serves as a sort of maze for the proteins to travel through. The smaller the proteins, the easier and faster they transport through the gel. The molecular weight of an unknown protein is obtained by the comparison of its position on the gel after electrophoresis to the positions of a standard SDS protein ladder.

The second step of Western Blot Analysis involves the direct transfer of protein from a polyacrylamide gel to a charged nitrocellulose membrane. It is advantageous to transfer proteins to a membrane because membranes are much stronger and more pliable than gels. The charged nitrocellulose membrane binds proteins with a high affinity, and proteins can migrate out of the gel and onto the sheet. After transfer, total protein can be visualized by staining the membrane with protein specific protein specific dyes. However, a much more specific way to detect protein is by using an antibody-coupled method called immunological detection.

For immunological detection, the membrane is placed in blocking buffer which contains detergents and blocking proteins that bind to all unoccupied sites on the membrane. The membrane has a limited protein-binding capacity, so by saturating the sites with a non-specific protein mixture, such as milk proteins, it ensures that no accidental contamination of the membrane occurs. The membrane is then incubated in buffer that contains antibody to one or more of the

blotted proteins. The primary antibody binds specifically to the adsorbed protein antigen. Subsequent washings will remove excess, unbound antibody. A secondary antibody against the primary antibody is then applied. This secondary antibody recognizes the primary antibody and is often linked to an enzyme such as horseradish peroxidase for detection. It is critical that all non-bound secondary antibody is washed off, to be sure that the detection is specific to secondary antibody that is bound to the primary antibody. Finally, the membrane is incubated with a solution containing peroxidase substrates that develops a brown color when it interacts with the horseradish peroxidase on the secondary antibody.

Western blotting has become a staple in almost every laboratory because of its specificity and ability to detect small amounts of protein. It can be used for detection of mutated proteins, the absence of a protein, identification of protein binding partners, and many other research and diagnostic applications.

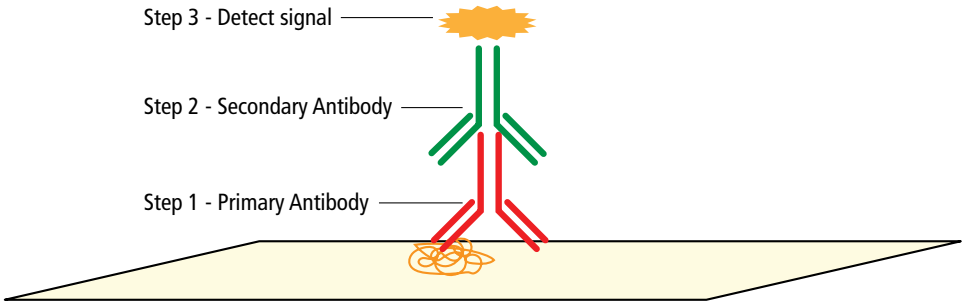


Figure 2: Western Blot Detection

Experiment Overview

EXPERIMENT OBJECTIVE

The objective of this experiment is for students to understand the theory and applications of Western Blot Analysis.

LABORATORY SAFETY

Wear gloves and safety goggles

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

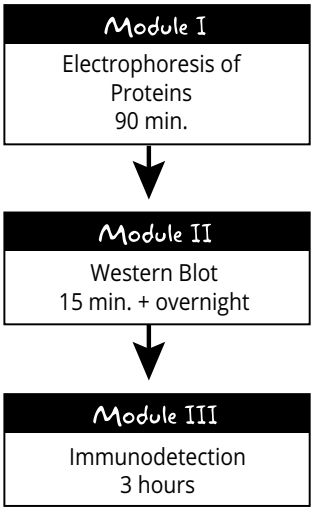
LABORATORY NOTEBOOKS

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

Before starting the Experiment:
- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

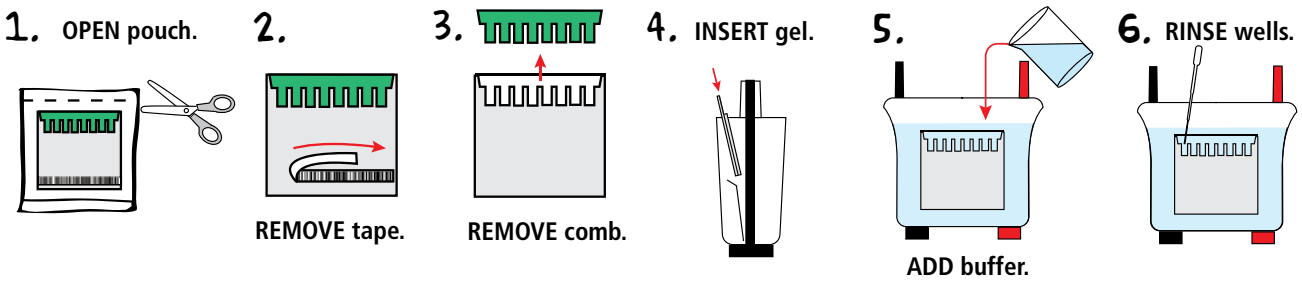
During the Experiment:
- Record (draw) your observations, or photograph the results.

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

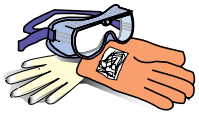


Module I: Electrophoresis of Proteins

PREPARING THE POLYACRYLAMIDE GEL AND CHAMBER



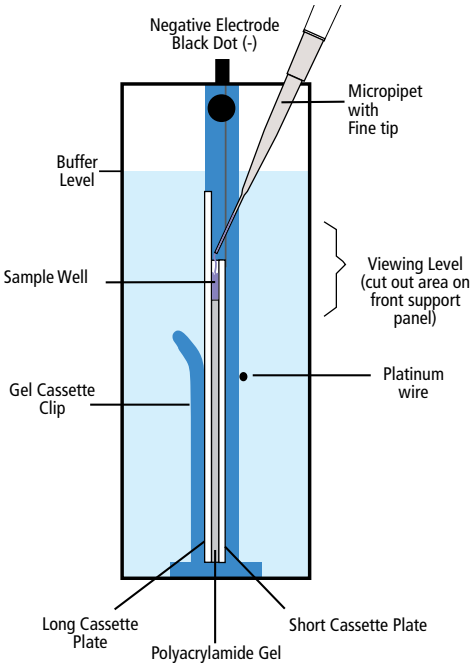
NOTE: Although precast polyacrylamide gels and protein chambers will vary slightly in design, the procedure for their use will be similar.



Wear gloves and safety goggles

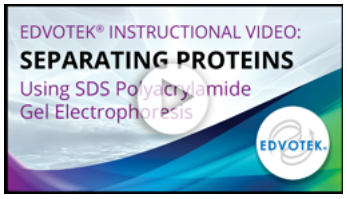
1. **OPEN** the pouch containing the gel cassette. Remove the cassette and place on bench with the shorter front plate facing up.
2. Gels may feature a sticker or tape at the bottom of the front plate. **REMOVE** the tape (if present) to expose the bottom of the gel.
3. Carefully **REMOVE** the comb 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 or sample loading.



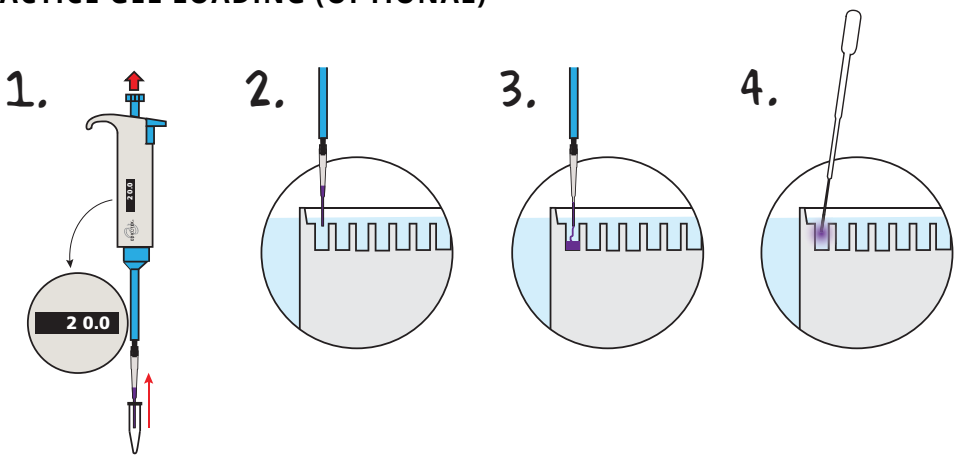
A polyacrylamide gel cassette in the EDVOTEK® Vertical Electrophoresis Apparatus, Model #MV10.

RELATED VIDEO:



Module I: Electrophoresis of Proteins, continued

PRACTICE GEL LOADING (OPTIONAL)



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.**

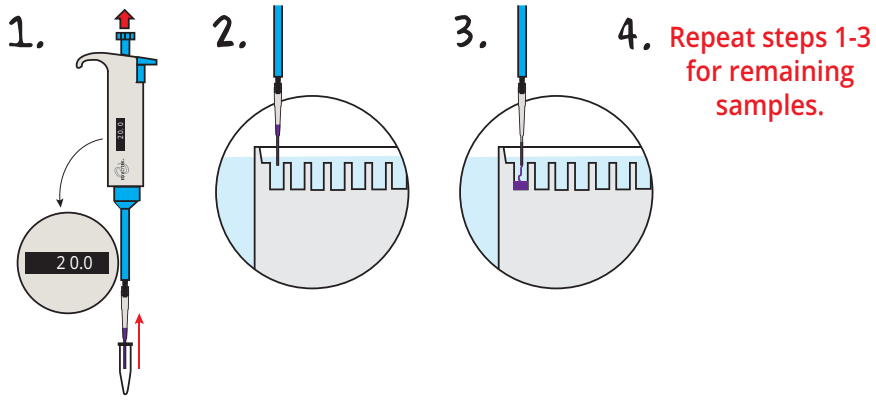

Wear gloves and safety goggles

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 pipette 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 I: Electrophoresis of Proteins, continued

LOADING THE PROTEIN SAMPLES



Two student groups can share one gel. The first group should load samples into wells 1 - 4. The other group sharing the gel should load samples into wells 7 - 10 (see Table 1).

1. Using a fresh fine tip micropipette tip, **MEASURE** 20 µL of the first sample as indicated in Table 1. Sample A is the protein standard markers and should go in the first lane of your group's half of the gel.

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 for remaining samples in Table 1, changing the tip between each new sample.

NOTE: Be sure to change pipette tips between loading each sample!

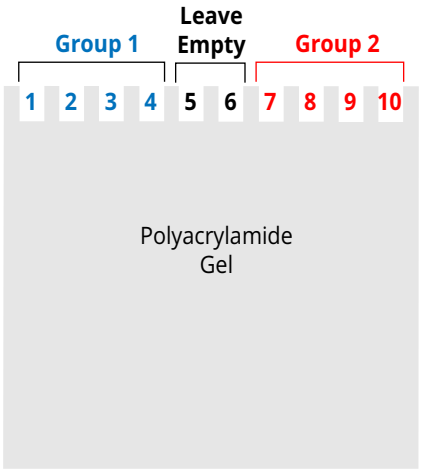
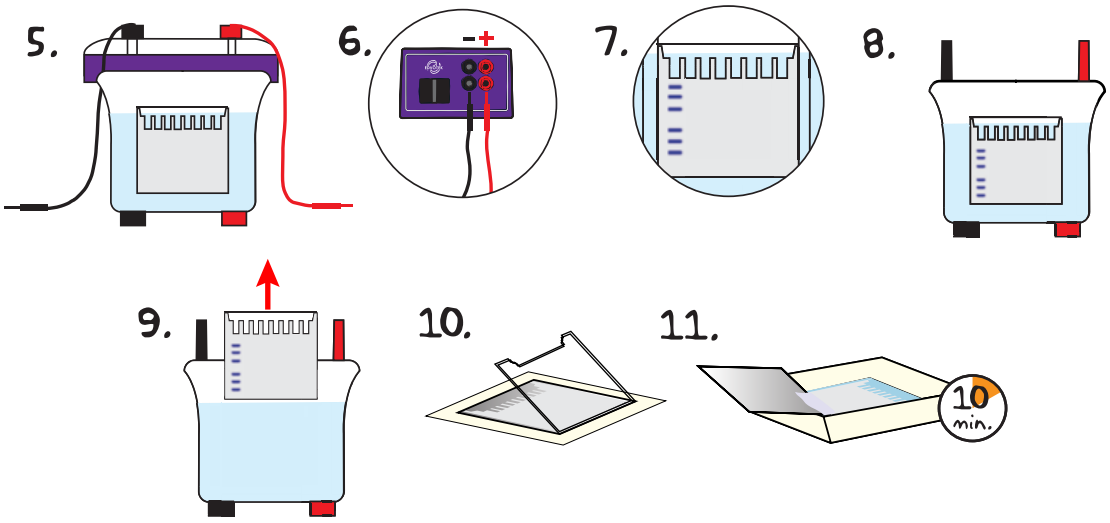


TABLE 1: GEL LOADING

	LANE	SAMPLE
GROUP 1	1	Sample A (Standard Marker)
	2	Sample B
	3	Sample C
	4	Sample D
XX	5	LEAVE EMPTY
XX	6	LEAVE EMPTY
GROUP 2	7	Sample A (Standard Marker)
	8	Sample B
	9	Sample C
	10	Sample D

Module I: Electrophoresis of Proteins, continued

RUNNING THE GEL



5. Once all samples have been loaded, carefully **PLACE** the cover onto the electrode terminals.

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. *Note: When the current is flowing, you should see bubbles forming on the electrodes.*

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.

8. After the electrophoresis is finished, **TURN OFF** the power supply, disconnect the leads, and carefully **REMOVE** the cover.

9. **REMOVE** the gel cassette from the electrophoresis apparatus and **BLOT** off excess buffer with a paper towel.

10. **REMOVE** the front plate by:
    a. Lay cassette down on the table and locate the gap between the front and back plates on the corners of the cassette.
    b. Use a spatula, or a similarly thin object, to torque the plates apart at the upper left corner.
    c. Repeat in all 4 corners of the plate and, if necessary, in the center of the left and right sides of the cassette.
    d. Continue to repeat steps (b) and (c) until the 2 plates loosen apart.
    e. Carefully remove the top plate.

11. **PLACE** the gel in transfer buffer and carefully **REMOVE** the gel from the back plate. **SAVE** the gel plates for the Western Blot procedure. **SOAK** the gel for 10 minutes. The gel is now ready for the Western Blot Procedure.

Module II: Western Blot Procedure

1. **PRE-SOAK** wick, blotting paper, and nitrocellulose membranes in transfer buffer for 5-10 min. When handling nitrocellulose membranes, be sure to handle them by their edges. Carefully **SLIDE** the membrane out of the blue protective covers and **TRANSFER** using forceps to the transfer buffer.

2. **PLACE** large gel plate on top of a container approximately 16 x 9 x 4 cm (L x W x D). **ADD** transfer buffer to the tray and **PLACE** presoaked wick onto gel plate such that ends are submerged in 2 cm of transfer buffer (see Figure 3).

Wear gloves when handling nitrocellulose membrane to avoid transferring oil from your skin which will interfere with the protein transfer.

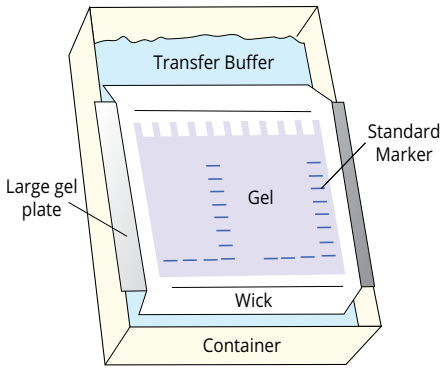


Figure 3: Top view of the blot transfer assembly.

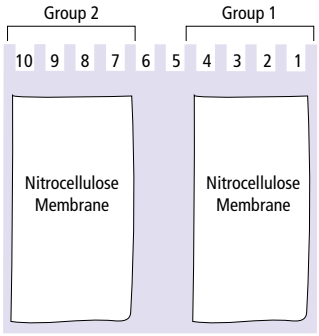


Figure 4: Placing the nitrocellulose membranes.

3. **FLIP** the gel so that the standard marker is now on the right, and **PLACE** the gel flat on top of the wick. **SMOOTH** over top of gel to remove air bubbles. An easy way to smooth the gel over is to use a large pipette tip as a roller.

4. **PLACE** nitrocellulose membranes on top of the groups's respective gel lanes (Figure 4).

5. **PLACE** the two pieces of blotting paper (from step 1) on top of the membrane. **SMOOTH** over to remove all air bubbles underneath. An easy way to smooth the gel over is to use a large pipette tip as a roller.

6. **PLACE** a 6 cm stack of paper towels on top of the blotting paper. Finally, **PLACE** a glass plate (or the small gel plate) and a 1 kg weight on top of the stack to complete the assembly, as shown in Figure 5.

STOPPING POINT

Allow transfer to take place overnight (12-15 hours).

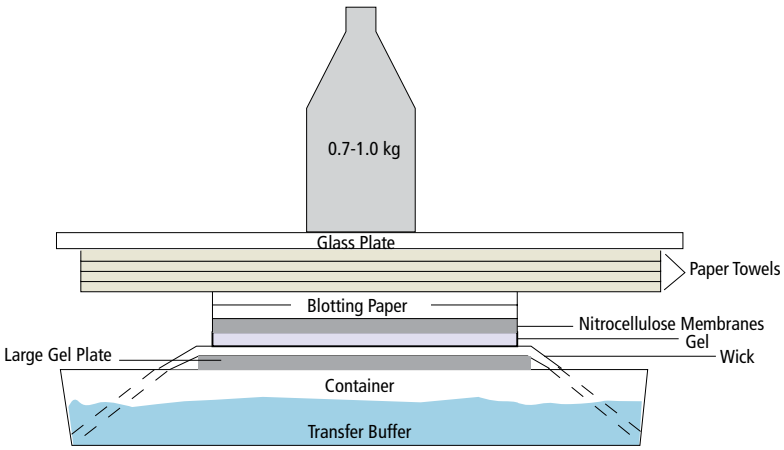
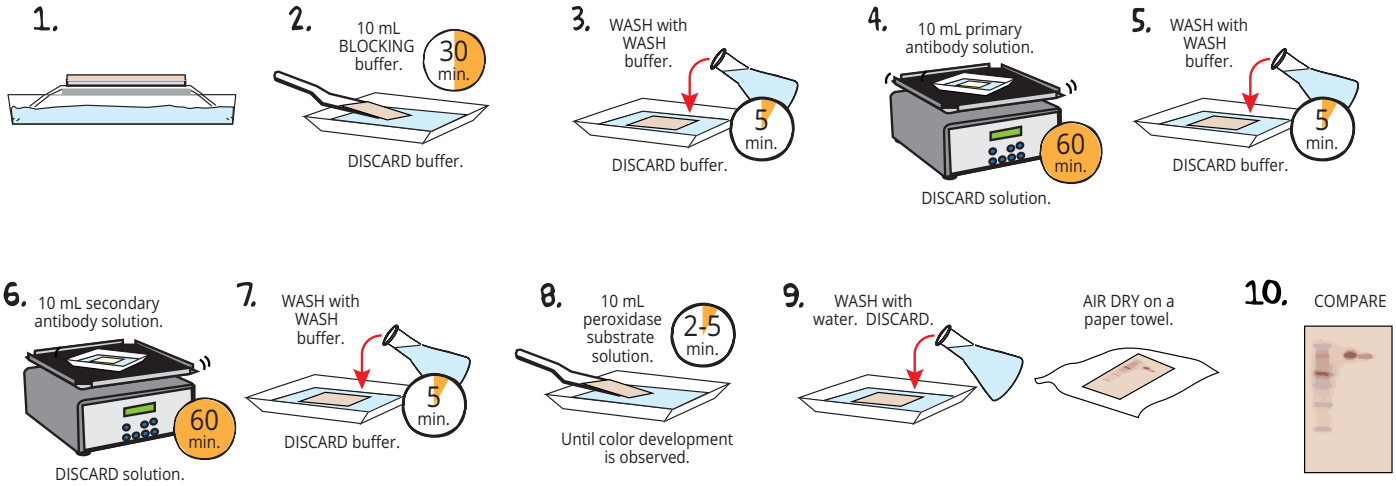


Figure 5: Side view of the blot transfer assembly.

Module III: Immunodetection



1. **DISMANTLE** the stack above the membrane. Carefully **REMOVE** the membrane from the gel with forceps or a spatula. **VERIFY** that the transfer occurred by the presence of prestained protein standard markers on the membrane.

2. **TRANSFER** the group's membrane to a small tray or small sealable plastic bag containing 10 mL blocking buffer (membrane should be submerged) and occasionally agitate for 30 minutes. **DISCARD** the blocking buffer.

3. **WASH** the membrane with 10 mL wash buffer for 5 minutes. **DISCARD** the buffer.

4. **ADD** 10 mL of primary antibody solution which has been prepared by your instructor. **INCUBATE** for 60 min. at room temperature on a rotating or shaking platform. **DISCARD** the primary antibody solution.

5. **WASH** the membrane with 10 mL wash buffer for 5 minutes. **DISCARD** the buffer.

6. **ADD** 10 mL of secondary antibody solution which has been prepared by your instructor. **INCUBATE** for 60 min. at room temperature on a rotating or shaking platform. **DISCARD** the secondary antibody solution.

7. **WASH** the membrane with 10 mL wash buffer for 5 minutes. **DISCARD** the buffer.

8. **ADD** 10 mL peroxidase substrate solution which has been prepared by your instructor. **INCUBATE** for 2-5 minutes or until color development is observed. **DISCARD** the substrate solution.

9. **WASH** membrane with water. **DISCARD** the water and **AIR DRY** on a paper towel.

10. **COMPARE** the size of the samples containing the various concentrations relative to the protein standard markers.

NOTE:
The lid from a micro-pipette rack (200 µL size) works well for a tray to incubate the membrane.

NOTE:
Substrate is prepared by your instructor just prior to use.

Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. Why are the electrophoretically fractionated proteins transferred to a membrane for immunological detection?
2. Why is the membrane blocked before incubation with milk?
3. What is the advantage of performing a Western Blot over visualizing proteins using a total protein stain?
4. What is the purpose of the negative and positive controls?

Instructor's Guide

NOTES TO THE INSTRUCTOR

This experiment requires three 12% Denaturing Polyacrylamide Gels to be shared by six groups (2 groups per gel). Each group requires 4 sample wells.

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances.

If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK web site. In addition, Technical Service is available from 8:00 am to 5:30 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

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

Preparation For:	What to do:	When:	Time Required:
Module I: Electrophoresis of Proteins	Reconstitution of Lyophilized Proteins.	Anytime before performing the experiment.	10 min.
	Prepare Chamber Buffer (Tris-Glycine-SDS buffer).	Anytime before performing the experiment.	10 min.
Module II: Western Blot Procedure	Prepare Transfer Buffer (Tris-Glycine-SDS Powdered buffer).	Anytime before performing the experiment.	10 min.
	Prepare Membranes and Blotting Paper.	Anytime before performing the experiment.	10 min.
Module III: Immunodetection	Prepare buffers for immunodetection.	Anytime before performing the experiment.	15 min.
	Reconstitute and dilute antibodies.	Up to 2 hours before the lab.	10 min.
	Prepare Peroxidase Substrate.	During the PBS washes following the secondary antibody.	10 min.

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

Pre-Lab Preparations - Module I: Electrophoresis of Proteins

Specific Requirements for this Experiment

This experiment requires three 12% Denaturing Polyacrylamide Gels to be shared by six student groups (2 groups per gel). Each group will require 4 sample wells. (Cat. #651 is recommended.)

Reconstitute Lyophilized Proteins

The protein standard markers (Component A) and lyophilized proteins (Components B, C, and D) must be reconstituted.

1. **ADD** 135 µL distilled water to each of the tubes, A-D. **VORTEX** each tube for 30 seconds each, or until completely dissolved. Resuspended proteins may be kept at room temperature for immediate use or frozen until needed.

2. **ALIQUOT** 20 µL of each sample per group.

   This experiment 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. Refer to instructions entitled "Practice Gel Loading" on page 8.

Prepare Tris-glycine-SDS Buffer (Chamber Buffer for Electrophoresis Only)

1. **ADD** 1 part 10x Tris Glycine SDS Buffer to every 9 parts of distilled water.

2. **MAKE** enough 1X chamber buffer for the 3 electrophoresis units (2 liters for three Cat. #581 EDVOTEK® units).

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 samples' 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.

REQUIREMENTS FOR ELECTROPHORESIS
(Reagents for Two Groups Sharing a Gel)

- One 12% Denaturing Polyacrylamide gel
- Components A - D (20 µL of each sample per group)
- Practice gel loading solution (optional)
- Chamber buffer

Pre-Lab Preparations - Module II: Western Blot Procedure

(Prepare Any Time Before the Lab - Required First Day)

Tris-Glycine SDS Buffer (Transfer Buffer Only)

NOTE: Do not use Methanol with acrylic materials. Methanol will destroy acrylic.

1. **ADD** the contents of the Tris-Glycine SDS Buffer to a flask or beaker (larger than one liter).
2. **ADD** 800 mL distilled or deionized water to the buffer concentrate. **SWIRL** and stir fully mix (a stir plate, if available may be useful).
3. **ADD** 200 mL of 95-100% methanol. **MIX, COVER** tightly, and **STORE** in the refrigerator until ready to use.

Nitrocellulose Membranes

NOTE: Wear rinsed and dried lab gloves. Powders from gloves will interfere with the procedure.

1. **KEEP** both upper and lower protective cover sheets around the membranes and make sure the cover sheets and membrane are all aligned. **KEEP** the membrane covered this way during all the following steps.
2. If you are using gels that are smaller or larger than the 8 x 10 cm, you must **ADJUST** the dimensions of your membrane squares accordingly. You may also have to alter the sizes of the filter paper and towels the students prepare. Larger gels may necessitate less groups.
3. **CUT** six membranes for the groups to share. Each membrane should be roughly the size of half a gel.

Additional Blotting Items

1. **CUT** two pieces of blotting paper to fit each gel.
2. **CUT** wicks to the width of each gel with enough length to overhang from the edge of tray to within 1/8" edge of tray bottom.

REQUIREMENTS FOR WESTERN BLOT
(Reagents and Supplies for Two Groups)

- 100 mL of diluted transfer buffer
- 2 nitrocellulose membranes
- 2 pieces of blotting paper
- Wick
- Paper towels and plastic wrap
- Small plastic boxes for soaking membranes and gels
- Pipette
- 0.7 - 1.0 kg weight
- Small dish (less than width of gel) for transfer
- Gel plates from Module I

Pre-Lab Preparations - Module III: Immunodetection

Reagents for Immunodetection

(Prepare on the Day of the Lab - Required Second Day)

1. **DILUTE** 10x Blocking Buffer (Component I) by adding 360 mL distilled water. The blocking buffer will likely precipitate during storage. **WARM** at 37° C for 5-10 minutes or until the precipitate has dissolved.

2. **DILUTE** the Anti-BSA Antibody (Component E) 1:200 by adding its entire contents to 65 mL diluted blocking buffer. **RINSE** Tube E to ensure you have all of the Anti-BSA. **REFRIGERATE** until use. **ALIQUOT** 10 mL to each group before the lab.

3. **DILUTE** the Secondary Antibody (Component F) 1:700 by adding its entire contents to 65 mL diluted blocking buffer. **RINSE** Tube F to ensure you have all of the secondary antibody. **REFRIGERATE** until use. **ALIQUOT** 10 mL to each group before the lab.

4. **PREPARE** the complete blocking buffer by adding the Powdered milk (Component K) to the remaining diluted blocking buffer (approximately 270 mL). **MIX** well to ensure the milk is fully dissolved. **ALIQUOT** 20 mL for each group before the lab.

5. **DILUTE** the 10x Wash Buffer (Component J) by adding its contents (40 mL) to 360 mL distilled water. **DISPENSE** 50 mL for each group and label as "Wash Buffer". **SAVE** the remaining diluted wash buffer to create the Peroxidase Substrate.

NOTE:
The blocking buffer will likely precipitate during storage. Warm at 37°C for 5-10 minutes or until the precipitate has dissolved.

QUICK REFERENCE:
The substrate is prepared for the peroxidase enzyme, which is attached to the anti-IgG peroxidase conjugate (secondary antibody).

Prepare the substrate 15-30 minutes before students require it for plate development (last incubation).

Peroxidase Substrate

(Prepare During the Lab Experiment, 15-30 minutes before the last incubation)

1. **DISPENSE** 75 mL of the diluted Wash Buffer (Step 5 above) to a clean flask or beaker.

2. **ADD** Peroxide co-substrate (Component H) to the 75 mL of PBS-T. **MIX** thoroughly by swirling or using a magnetic stir bar. There is usually undissolved material remaining.

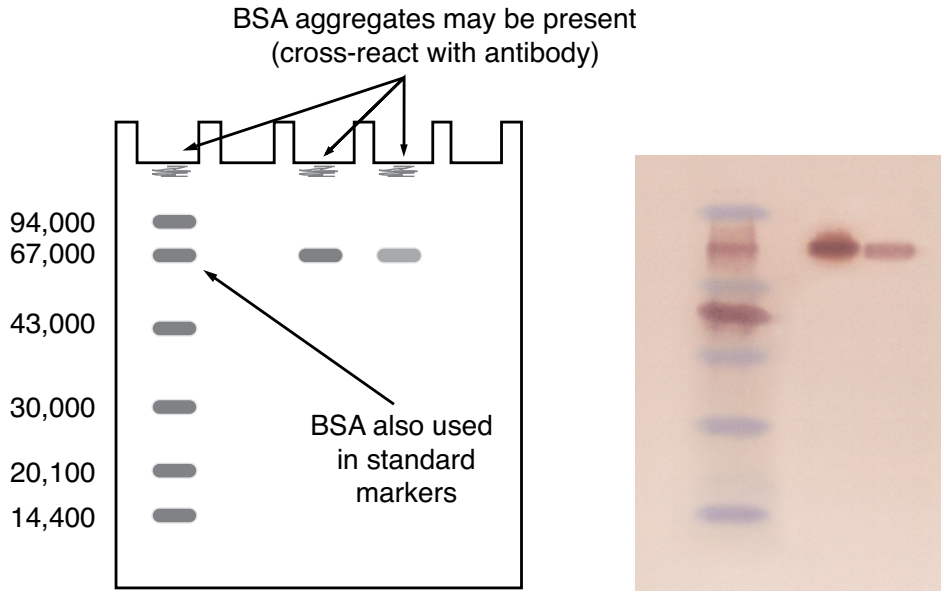
3. Then, **ADD** 7.5 mL of Hydrogen Peroxide (Component G). **MIX** well.

4. **DISPENSE** 12 mL of the peroxidase substrate for each group.

REQUIREMENTS FOR IMMUNODETECTION
(Reagents for Individual Groups)

- 10 mL of diluted Anti-BSA Antibody
- 10 mL of diluted Secondary Antibody Conjugate
- 20 mL complete blocking buffer
- 50 mL of wash buffer
- 12 mL of prepared peroxidase substrate

Expected Results and Analysis



The positive control (ladder) and BSA samples should show an immunoreactive band. The immunoreactive bands roughly correspond to a molecular weight of 67 KD. The negative control will not have immunoreactive bands.

Lane	Contents	
1	A	Protein Standard Markers
2	B	Negative Control
3	C	BSA high concentration
4	D	BSA low concentration

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