The Biotechnology Education Company®

EDVOTEK®

# 317

EDVO-Kit #

# Western Blot Analysis

**Storage:**
See page 2 for specific storage instructions.

## EXPERIMENT OBJECTIVES:

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

## Table of Contents

This experiment contains enough reagents for 6 lab groups.

Enough membrane is provided to accommodate blotting of three 8x10 cm gels.

The samples, controls and markers are ready for electrophoresis after boiling.

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

## Experiment Components

| Contents | | Storage |
|---|---|---|
| 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 | Powdered Milk | Room Temp. |
| K | 10x PBS | Room Temp. |

| | | |
|---|---|---|
| • | 10x Tris-Glycine-SDS Buffer (Chamber Buffer) | Room Temp. |
| • | 10x Tris-Glycine Buffer (for transfer buffer) | Room Temp. |
| • | Practice Gel Loading Solution | Room Temp. |
| • | Nylon Membrane | Room Temp. |
| • | Filter Paper | Room Temp. |
| • | Large Filter Paper | Room Temp. |

## Requirements

- 12% Denaturing Polyacrylamide gels (3)
- Vertical Gel Electrophoresis Apparatus* (Cat. #581) or equivalent
- D.C. Power Supply
- Shaker Platform (optional)
- Burners or Hot Plates
- Automatic Pipets with Tips
- Microtest (Microcentrifuge) Tubes
- Beakers
- Pipets
- Graduated Cylinders
- Plastic Wrap
- Scissors
- Metric Rulers
- Trays or Containers
- Several Packs of Paper Towels
- Latex or Vinyl Lab Gloves
- Safety Goggles
- Methanol
- Distilled Water

* EDVOTEK® Model MV10 (catalog #581) is recommended.

All 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. No components have been prepared from human sources.

## Western Blot Analysis

Background Information

Enzyme linked immunoadsorbent assay (ELISA) is an important diagnostic immunochemical method used for the detection of low levels of antigens. A positive reaction in the ELISA requires further, more definitive testing for verification. One reason for this problem is that antibodies sometimes exhibit cross reactivity. For example, it is possible that an individual possesses IgG molecules that were not raised against a particular virus but bind to the viral antigens. Cross reactivity is usually due to common or related sets of epitopes between two different antigens. There are also other technical and procedural problems that can give false positives. ELISA testing can only indicate the presence of certain antigens or antibodies. However, immunoblotting can be used to detect several protein antigens that are viral specific.

In Western Blot Analysis, the first step is a denaturing polyacrylamide gel analysis. Denaturing gel electrophoresis separates proteins based on their size. In most cases the protein's native conformation, charge and amino acid composition do not affect the electrophoretic migration rate in the presence of saturating SDS (sodium dodecylsulfate). SDS is a detergent which consists of a hydrocarbon chain bonded to a highly negatively charged sulfate group.

### PROPERTIES OF PROTEINS

SDS binds strongly to most proteins and causes them to unfold to a random, rod-like chain and also makes them net negative in charge. 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 their polypeptide chains remaining intact are called denatured. Proteins which contain several polypeptide chains that are associated only by non-covalent forces, but do not contain denaturing agents such as 2-mercaptoethanol, will be dissociated by SDS into separate, denatured polypeptide chains. Proteins can also contain 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. 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

Western Blot Analysis involves the direct transfer of protein bands from a polyacrylamide gel to a charged nylon sheet. Proteins are adsorbed to the membrane by hydrophobic bonds. After electrophoresis, transfer of proteins can be done electrophoretically in a specially designed chamber. Transfer can also be accomplished by capillary flow or suction. Protein bands are transfered on the surface of the nylon membrane. Membranes are much stronger and more pliable than gels and can undergo many manipulations without tearing. Total protein transferred can be visualized by staining the membrane with protein specific dyes. Specific proteins are detected by immunochemical methods.

Specific proteins cannot be detected by total protein staining because the amount of protein may be too low and due to the banding of other proteins that may block it from view. For immunological detection the unstained membrane is placed in blocking buffer which contains detergents and blocking proteins that bind to all unoccupied sites on the 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. Subsequent washings will remove excess, unbound antibody. A secondary antibody against the first antibody, linked to an enzyme such as alkaline phosphatase or horseradish peroxidase, is used for detection. The cross-linking of the enzyme to the secondary antibody is done under conditions that do not appreciably affect the antigen binding specificity, the affinity of the antibody, or the catalytic activity of the enzyme. The membrane is incubated in a solution of the secondary antibody where it will bind selectively to the antigen-antibody complex and washed to remove excess secondary antibody. In the next step, the membrane is incubated in a solution containing phosphatase or peroxidase substrates that yield chromogenic products. Areas containing antigen-antibody conjugates will develop color, depending on the type of substrate used and product formed.

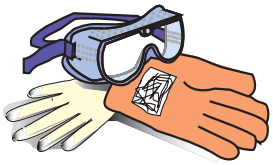
In this experiment, students will use Western Blot Analysis to detect a specific protein.

Experiment Procedures

## Experiment Overview

### EXPERIMENT OBJECTIVE:

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



Wear gloves and safety goggles

### LABORATORY SAFETY

Gloves and goggles should be worn routinely throughout the experiment as good laboratory practice.

## Electrophoresis of Proteins



### PREPARING THE POLYACRYLAMIDE GEL FOR ELECTROPHORESIS

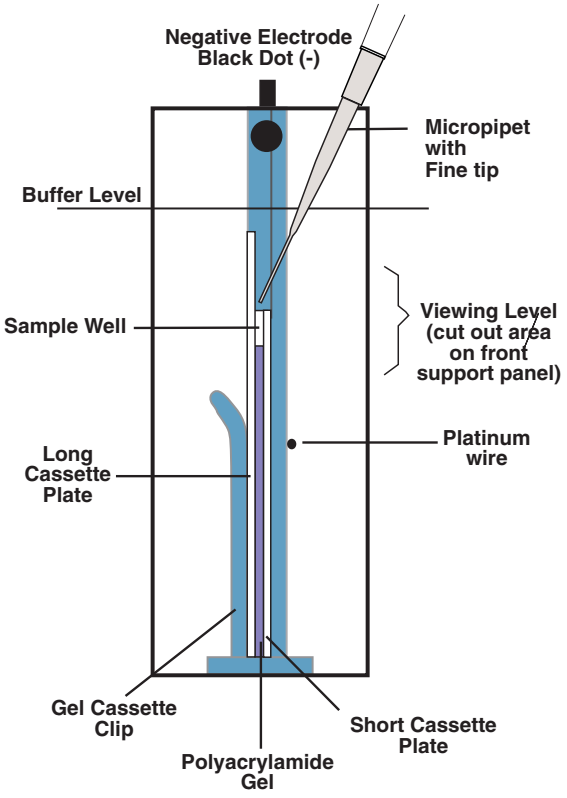
#### Pre-cast Polyacrylamide Gels:

If you are using pre-cast polyacrylamide gels, depending upon the manufacturer, they will vary slightly in design. Procedures for their use will be similar.

1. Open the pouch containing the gel cassette with scissors. Remove the cassette and place it on the bench top with the front facing up.

   *Note: The front plate is smaller (shorter) than the back plate.*

**The figure below shows a polyacrylamide gel cassette in the EDVOTEK® Vertical Electrophoresis Apparatus, Model #MV10**




2. Some cassettes will have tape at the bottom of the front plate. Remove all of the tape to expose the bottom of the gel to allow electrical contact.

#### Proper Orientation of the Gel in the Electrophoresis Unit

1. Place the gel cassette in the electrophoresis unit in the proper orientation. The proteins samples will not separate in gels that are not oriented correctly. Follow the directions accompanying the specific apparatus.

2. Remove the comb by placing your thumbs on the ridges and pushing (pressing) upwards.

3. Add the diluted buffer into the chamber. The sample wells and the back plate of the gel cassette should be submerged under buffer.

#### Well Preparation

1. Use a transfer pipet to rinse each well with the electrophoresis buffer in the chamber.

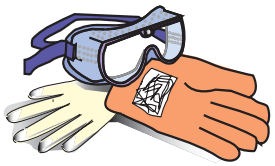
2. Use a syringe or transfer pipet to carefully straighten any wells which may have been distorted during comb removal or rinsing.

   The gel is now ready for practice gel loading and/or samples.

## Electrophoresis of Proteins

Experiment Procedures

### PRACTICE GEL LOADING



Wear gloves and safety goggles

1. Place a fresh tip on the micropipet. Remove 20 µl of practice gel loading solution.

2. Place the lower portion of the pipet 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, as illustrated on page 7.

   Do not try and jam the pipet tip in between the plates of the gel cassette.

3. Eject all the sample by steadily pressing down on the plunger of the automatic pipet.

   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 micropipet tip. Release the pipet plunger after the sample has been delivered and the pipet tip is out of the buffer.

4. Before loading protein samples for the actual experiment, the practice gel loading solution must be removed from the sample wells. Do this by filling a transfer pipet with buffer and squirting 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.

## Electrophoresis of Proteins



### PREPARATION OF SAMPLES FOR ELECTROPHORESIS

#### Protein Denaturation (Day One)

*Samples may have been reconstituted by your instructor. If not, you will need to perform this step before the samples are loaded on the gel.*

**Quick Reference:**

The heating (Steps 1-3) disrupts aggregates of denatured proteins. Denatured proteins tend to form super-molecular aggregates and insoluble particulates.

Denatured proteins tend to form super-molecular aggregates and insoluble particulates. Heating disrupts metastable aggregates of denatured proteins.

1. Bring a beaker of water, covered with aluminum foil, to a boil. Remove from heat.
2. Resuspend the lyophilized proteins (Tubes A-D) in 125 µl distilled water. Vortex well.
3. Make sure the sample tubes A through D are tightly capped and thawed. Label the tops of the tubes. Tap the tubes until all the sample is at the bottom. The bottom of the tubes should be pushed through the foil and immersed in the hot water for 10 minutes. The tubes should be kept suspended by the foil.
4. Proceed to loading samples while they are still warm.

#### Loading Samples

Load 20 µl each of the samples in tubes A - D into wells 1 - 4 in consecutive order. The other group sharing the gel should load 20 µl each of the samples in tubes A - D into wells 7 - 10 in consecutive order.

**Quick Reference:**

Use an automatic micropipet to deliver samples. The amount of sample that should be loaded is 20 µl.

## Electrophoresis of Proteins

Experiment Procedures

### RUNNING THE GEL

1. After the samples are loaded, carefully snap the cover all the way down onto the electrode terminals. On EDVOTEK® electrophoresis units, the black plug in the cover should be on the terminal with the black dot.

2. Insert the plug of the black wire into the black input of the power supply (negative input). Insert the plug of the red wire into the red input of the power supply (positive input).

3. Set the power supply at the required voltage and run the electrophoresis for the length of time as determined by your instructor. When the current is flowing, you should see bubbles forming on the electrodes. The sudsing is due to the SDS in the buffer.

4. After the electrophoresis is finished, turn off power, unplug the unit, disconnect the leads and remove the cover.

5. Remove the gel cassette from the electrophoresis apparatus and blot off excess buffer with a paper towel.

6. Lay the cassette down and remove the front plate by placing a spatula or finger at the top edge, near the sample wells, and lifting 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.

7. Carefully place the gel in transfer buffer and soak for 10 minutes.

**Time and Voltage**

| Volts | Recommended Time | |
|---|---|---|
| | Minimum | Optimal |
| 125 | 45 min | 60 min |
| 70 | 60 min | 1.5 hrs |

## Western Blot Procedure


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

### BLOTTING PROCEDURE

1. Cut two pieces of blotting paper and one nylon membrane to fit gel.
2. Cut wick to width of gel and length to overhang from edge of tray to within 1/8" edge of tray bottom (Figure 2).
3. Pre-soak wick, blotting paper, and membrane in transfer buffer (20 mM Tris-Cl pH 8.0, 150 mM glycine, 20% methanol) for 5-10 minutes.
4. Soak gel for 10 minutes in transfer buffer.
5. Place larger gel plate on top of a container of dimensions of approximately 16 cm x 9 cm 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 1).

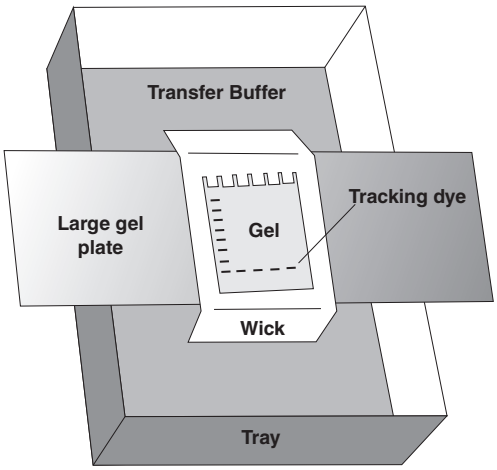



**Figure 1:**
Top view of the blot transfer assembly.

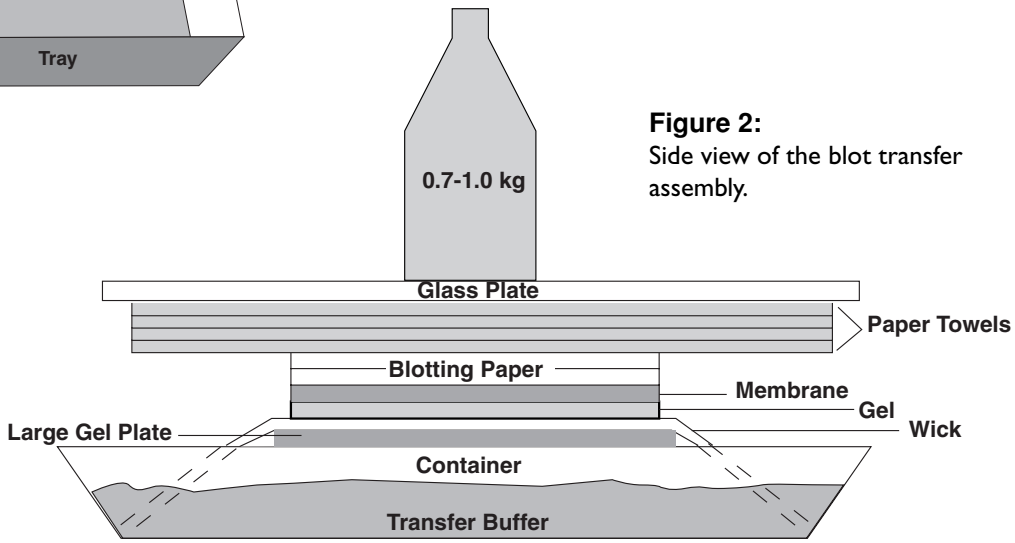



**Figure 2:**
Side view of the blot transfer assembly.

## Western Blot Procedure

Experiment Procedures

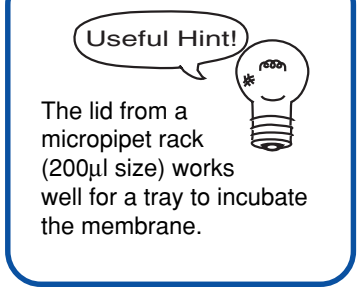
6. Place gel flat on top of wick. Smooth over top of gel to remove air bubbles.
7. Place nylon membrane on top of gel. Smooth over top to remove air bubbles.
8. Place the two pieces of blotting paper (from step 1) on top of the membrane.
9. Place a 6 cm stack of paper towels on top of blotting paper. Finally, place a 1 kg weight on top of stack to complete assembly, as shown in Figure 2.
10. Allow transfer to take place overnight (12-15 hrs).


**STOPPING POINT**

## Western Blot Procedure



### IMMUNODETECTION




1. Dismantle stack above membrane. Carefully remove membrane from gel with spatula. Verify that transfer occurred by the presence of prestained protein markers on membrane.
2. Carefully cut membrane in half. Each group will then proceed independently.
3. Transfer membrane to a small tray or small sealable plastic bag containing 10ml blocking solution (membrane should be submerged) and occasionally agitate for 5 minutes.
4. Discard the blocking solution. Place the membrane in 10ml fresh blocking solution containing the primary antibody at a dilution of 1:1000. Place on a rotating or shaking platform for one hour at room temperature.
5. Discard antibody-containing solution and wash membrane for 5 minutes in 10 ml blocking buffer.
6. Discard blocking wash buffer and repeat washing with 10 ml fresh blocking buffer.
7. Incubate with 10 ml peroxidase-conjugated secondary antibody (1:2000) in blocking buffer for one hour, with shaking.
8. Discard solution and wash membrane for five minutes with PBS. Repeat wash.
9. Add substrate solution which has been prepared by your instructor. Place membrane in 12 ml substrate solution and incubate until color development is observed.
10. Wash membrane with water and then air dry. Alternatively, you can blot the edge of the membrane onto a paper towel.
11. Compare the size of the samples containing the various concentrations relative to the protein standard markers.

**NOTE:**

Substrate is prepared by your instructor just prior to use.

## Experiment Results and Study Questions

Experiment Procedures

### LABORATORY NOTEBOOK RECORDINGS:

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.

**Following 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.

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

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

## Notes to the Instructor

This experiment requires three **12%** Denaturing Polyacrylamide Gels to be shared by the 6 student groups. 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.

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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 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

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9:00 am to 6:00 pm ET

FAX: (301) 340-0582
web: www.edvotek.com
email: edvotek@aol.com

Please have the following information:

- The experiment number and title
- Kit Lot number on box or tube
- The literature version number (in lower right corner)
- Approximate purchase date

## PreLab Preparations



### TRANSFER BUFFER (REQUIRED FIRST DAY)

1. To 700 ml of distilled water, add 100 ml of 10X Tris-Glycine concentrate.

2. Add 200 ml of 95 - 100% methanol. Mix. If the final volume is less than 1 liter, bring up to volume with distilled water. Mix. Keep tightly covered.

### ELECTROPHORESIS BUFFER, TRIS-GLYCINE-SDS BUFFER

1. Add 1 part EDVOTEK® 10X buffer to every 9 parts distilled water.

2. Make enough 1X buffer for the 3 electrophoresis units (2 liters for three EDVOTEK® units).

### PREPARATION OF MEMBRANES
**(Any time before the lab - required first day)**

*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.

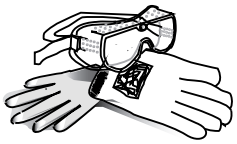
2. If you are using gels that are smaller or larger than the 8 x 10 cm, you must adjust the dimensions of your membrane squares accordingly. You may also have to alter the sizes of the filter paper and towels the students prepare. Larger gels may necessitate less groups.

3. Cut three membranes for the 6 groups to share.

### SPECIFIC REQUIREMENT FOR THIS EXPERIMENT

This experiment requires three 12% Denaturing Polyacrylamide Gels to be shared by the 6 student groups. Each group requires 4 sample wells. The protein standard marker and components B, C, and D must be reconstituted.

## Pre-Lab Preparations



Wear Safety Goggles and Gloves

### RECONSTITION OF LYOPHILIZED PROTEINS

1. Add 125 µl distilled water to each of the tubes, A-D. Vortex each tube for 30 seconds each, or until completely dissolved.
2. Wear safety goggles and bring a beaker of water, covered with aluminum foil, to a boil. Remove from heat.
3. Make sure the tube lids are securely fastened. Suspend the tubes in a boiling water bath for 10 minutes.
4. Remove and have students load gels while proteins are still warm Tap or briefly microcentrifuge to get condensate at the top of the tubes back into the sample.

   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 Instructions".

### 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 the table to the left.

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

**Time and Voltage**

| Volts | Time |
|---|---|
| 125 | 45 - 60 min. |
| 70 | 1 - 1.5 hours |

### PREPARATION OF REAGENTS FOR IMMUNODETECTION

**(On the day of the lab - required second day)**

1. Dilute 10x blocking buffer by adding 540 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. Prepare complete blocking buffer by adding the powdered milk (J) to 600 ml diluted blocking buffer (I).
3. Dilute the Anti-BSA Antibody 1:1000 by adding the entire contents of tube E to 65 ml complete blocking buffer. Rinse tube E to ensure you have all of the Anti-BSA. Refrigerate.
4. Dilute the secondary antibody 1:2000 by adding the entire contents of tube F to 65 ml complete blocking buffer. Rinse tube F to ensure you have all of the secondary antibody. Refrigerate.
5. Dilute the 10x PBS solution by adding the contents of bottle K (30 ml) to 270 ml distilled water. Dispense 25 ml for each group.


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

Instructor's Guide

## Pre-Lab Preparations




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

### PREPARATION OF PEROXIDASE SUBSTRATE DURING THE LAB EXPERIMENT

Prepare 15 - 30 minutes before the last incubation:

1. Dispense 68 ml of diluted phosphate buffered saline (PBS) to a clean flask or beaker.
2. Add peroxide co-substrate (H) to the 68 ml of PBS. Cap and mix thoroughly by shaking and /or vortexing. There is usually undissolved material remaining.
3. Then add 7.5 ml of Hydrogen peroxide (G). Cap and mix.
4. Dispense 12 ml of the peroxidase substrate for each group.

**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).

### INDIVIDUAL GROUP REAGENT REQUIREMENTS:

**First Day (Reagents for Two Groups)**

- Boiled components A - D (aliquots if desired)
- Practice gel loading solution (optional)
- Diluted electrophoresis buffer
- 100 ml of diluted transfer buffer
- 1 nylon membrane
- 5 filter paper pieces
- Wick
- Paper towels and plastic wrap
- Small plastic boxes for soaking membranes and gels
- Pipet
- 0.7 - 1.0 kg weight
- Small dish (less than width of gel) for transfer

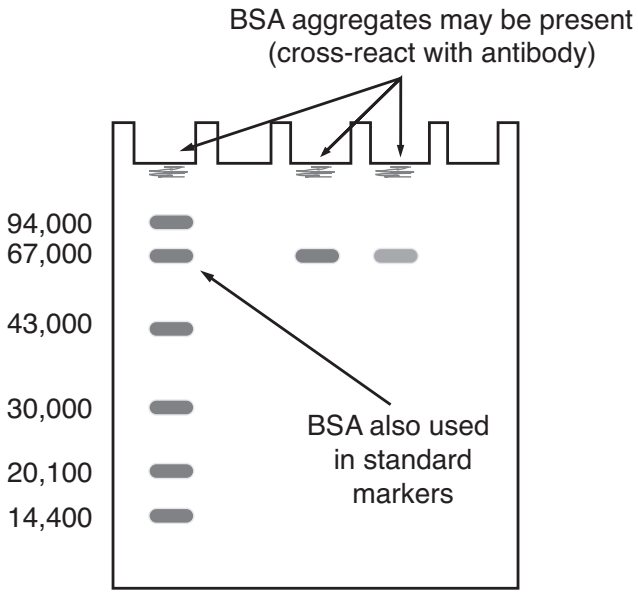
**Second Day (Individual Groups)**

- Approximately 60 ml complete blocking buffer
- 25 ml diluted PBS
- 70 ml distilled water
- 10 ml diluted secondary antibody conjugate
- 10 ml diluted Anti-BSA Antibody
- 12 ml prepared substrate

## Idealized Schematic of Results

The positive control and patient sample 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 Standard Protein Markers |
| 2 | B Negative Control |
| 3 | C BSA low concentration |
| 4 | D BSA high concentration |



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

## Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): **Hydrogen Peroxide, Stabilized**

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

### Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive, Rockville, MD 20850**

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 09-17-2002

Signature of Preparer (optional):

### Section II - Hazardous Ingredients/Identify Information

| Hazardous Components [Specific Chemical Identity; Common Name(s)] | OSHA PEL | ACGIH TLV | Other Limits Recommended | % (Optional) |
|---|---|---|---|---|
| Hydrogen peroxide, $H_2O_2$ | No data | | | 1.2% |
| CAS # 7722-84-1 | | | | |

### Section III - Physical/Chemical Characteristics

| | | | |
|---|---|---|---|
| Boiling Point | No data | Specific Gravity ($H_20$ = 1) | 1.110 |
| Vapor Pressure (mm Hg.) at 30°C | 22.3 | Melting Point | No data |
| Vapor Density (AIR = 1) | 1 | Evaporation Rate (Butyl Acetate = 1) | No data |

Solubility in Water: Soluble

Appearance and Odor: Colorless liquid, no odor

### Section IV - Physical/Chemical Characteristics

N.D. = No data

| Flash Point (Method Used) | Flammable Limits | LEL | UEL |
|---|---|---|---|
| No data | | N.D. | N.D. |

Extinguishing Media: Waterspray

Special Fire Fighting Procedures: Wear SCBA and protective clothing to prevent contact with skin and eyes.

Unusual Fire and Explosion Hazards: Strong oxidizer, contact with other material may cause fire. Container explosion may occur under fire conditions.

### Section V - Reactivity Data

| | | | |
|---|---|---|---|
| Stability | Unstable | X | Conditions to Avoid: Excessive heat |
| | Stable | | |

Incompatibility: Strong acide, aluminum, steel

Hazardous Decomposition or Byproducts: Toxic oxides of phosphorous

| | | | |
|---|---|---|---|
| Hazardous Polymerization | May Occur | | Conditions to Avoid: None |
| | Will Not Occur | X | |

### Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Acute: Irritates mucous membranes upperrespiratory tract, eyes, skin
Chronic: May have mutagenic affect

Carcinogenicity: No data NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: Inhalation: burning sensation, coughing, wheezing, laryngitis, shortness of breath, headache, nausea. Irritation.

Medical Conditions Generally Aggravated by Exposure: No data

Emergency First Aid Procedures: Ingestion: Wash mouth out with water. Contact physician
Eyes: Flush with water Inhalation: Move to fresh air Skin: Flush with water

### Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Mop up with absorbent material and dispose of properly

Waste Disposal Method: Cautiously acidify to pH2 with Sulfuric acid. Add a 50% excess of aqueous sodium bisulfate with stirring (heat generated). If no heat is evident, cautiously add until heat is liberated.

Precautions to be Taken in Handling and Storing: Observe federal, state, and local laws

Other Precautions: Store away from incompatibilities

### Section VIII - Control Measures

Respiratory Protection (Specify Type): NIOSH/MSHA approved

| | | | | |
|---|---|---|---|---|
| Ventilation | Local Exhaust | No | Special | No |
| | Mechanical (General) | No | Other | Chemical fume hood |

Protective Gloves: Rubber Eye Protection: Safety goggles

Other Protective Clothing or Equipment: Rubber boots

Work/Hygienic Practices: Avoid inhalation. Keep away from incompatibilities and combustible material.

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## Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Peroxide Co-substrate

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

### Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive, Rockville, MD 20850**

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 09-18-2002

Signature of Preparer (optional):

### Section II - Hazardous Ingredients/Identify Information

| Hazardous Components [Specific Chemical Identity; Common Name(s)] | OSHA PEL | ACGIH TLV | Other Limits Recommended | % (Optional) |
|---|---|---|---|---|
| 5-aminosalicylic acid | ----------No data---------- | | | |
| CAS# 89-57-6 | | | | |

### Section III - Physical/Chemical Characteristics

| | | | |
|---|---|---|---|
| Boiling Point | No data | Specific Gravity ($H_20$ = 1) | No data |
| Vapor Pressure (mm Hg.) | No data | Melting Point | 280°C |
| Vapor Density (AIR = 1) | No data | Evaporation Rate (Butyl Acetate = 1) | No data |

Solubility in Water: Soluble

Appearance and Odor: Light tan-gray powder with clumps

### Section IV - Physical/Chemical Characteristics

N.D. = No data

| Flash Point (Method Used) | Flammable Limits | LEL | UEL |
|---|---|---|---|
| No data | | N.D. | N.D. |

Extinguishing Media: Water spray, carbon dioxide, dry chemical powder or appropriate foam

Special Fire Fighting Procedures: Wear protective clothing and SCBA to prevent contact with skin & eyes

Unusual Fire and Explosion Hazards: Emits toxic fumes under fire conditions

### Section V - Reactivity Data

| | | | |
|---|---|---|---|
| Stability | Unstable | | Conditions to Avoid: Incompatibles |
| | Stable | X | |

Incompatibility: Acids, acid chlorides, acid anhydrides, chloroformates, strong oxidizing agents

Hazardous Decomposition or Byproducts: Nitrogen oxides, carbon monoxide, and carbon dioxide

| | | | |
|---|---|---|---|
| Hazardous Polymerization | May Occur | | Conditions to Avoid |
| | Will Not Occur | X | |

### Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Irritating to mucous membranes and upper respiratory tract

Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?
----------No data----------

Signs and Symptoms of Exposure: Eye and skin irritation

Medical Conditions Generally Aggravated by Exposure: No data

Emergency First Aid Procedures: Ingestion: Rinse mouth with water
Eyes/Skin: Flush with water Inhalation: Move to fresh air

### Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear suitable protective clothing. Sweep up and place in container for disposal. Avoid raising dust. Ventilate area.

Waste Disposal Method: Mix with combustible solvent and burn in chemical incinerator with afterburner and scrubber. Observe federal, state, and local laws.

Precautions to be Taken in Handling and Storing: Avoid contact or raising dust.

Other Precautions: None

### Section VIII - Control Measures

Respiratory Protection (Specify Type):

| | | | | |
|---|---|---|---|---|
| Ventilation | Local Exhaust | No | Special | None |
| | Mechanical (General) | Yes | Other | None |

Protective Gloves: Chemical resistant Eye Protection: Chemical safety goggles

Other Protective Clothing or Equipment: Lab coat

Work/Hygienic Practices: Avoid contact

## Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): 10x PBS

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

### Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive, Rockville, MD 20850**

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 09-19-2002

Signature of Preparer (optional):

### Section II - Hazardous Ingredients/Identify Information

| Hazardous Components [Specific Chemical Identity; Common Name(s)] | OSHA PEL | ACGIH TLV | Other Limits Recommended | % (Optional) |
|---|---|---|---|---|
| N/A Blend | | | | |

### Section III - Physical/Chemical Characteristics

| | | | |
|---|---|---|---|
| Boiling Point | 100°C | Specific Gravity ($H_20$ = 1) | 1.017 |
| Vapor Pressure (mm Hg.) | No data | Melting Point | No data |
| Vapor Density (AIR = 1) | No data | Evaporation Rate (Butyl Acetate = 1) | No data |

Solubility in Water: soluble

Appearance and Odor: colorless liquid

### Section IV - Physical/Chemical Characteristics

| Flash Point (Method Used) | Flammable Limits | LEL | UEL |
|---|---|---|---|
| Noncombustible | | | |

Extinguishing Media: Use extinguishing media appropriate to surrounding fire

Special Fire Fighting Procedures: Wear SCBA and protective clothing to prevent contact with skin and eyes

Unusual Fire and Explosion Hazards: Emits toxic fumes under fire conditions

### Section V - Reactivity Data

| Stability | | | Conditions to Avoid |
|---|---|---|---|
| | Unstable | | |
| | Stable | | |

Incompatibility: Strong acids

Hazardous Decomposition or Byproducts: Nature of decomposition products not known

| Hazardous Polymerization | | | Conditions to Avoid |
|---|---|---|---|
| | May Occur | | |
| | Will Not Occur | | |

### Section VI - Health Hazard Data

| Route(s) of Entry: | Inhalation? | Skin? | Ingestion? |
|---|---|---|---|
| | Yes | Yes | Yes |

Health Hazards (Acute and Chronic): Cause eye & skin irritation, material is irritating to mucous membranes and upper respiratory tract. The toxocological properties have not been thoroughly investigated.

| Carcinogenicity: | NTP? | IARC Monographs? | OSHA Regulation? |
|---|---|---|---|

Signs and Symptoms of Exposure

Medical Conditions Generally Aggravated by Exposure

Emergency First Aid Procedures: Swallowed - wash out mouth with water provided person is conscious. Skin/eye contact - flush with water Inhalation - remove to fresh air

### Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear respirator, chemical safety goggles, rubber boots and heavy rubber gloves, sweep up, place in a bag and hold for waste disposal.

Waste Disposal Method: For small quantities - cautiosly add to a large stirred excess of water. Adjust pH to neutral

Precautions to be Taken in Handling and Storing: Wear appropriate NIOSH/MSHA approved respirator, chemical resistant gloves, safety goggles safety shower and eye bath.

Other Precautions

### Section VIII - Control Measures

Respiratory Protection (Specify Type): NIOSH/MSHA approved respirator

| Ventilation | | | | |
|---|---|---|---|---|
| | Local Exhaust | N/A | Special | N/A |
| | Mechanical (General) | N/A | Other | N/A |

| Protective Gloves | Yes | Eye Protection | Yes |
|---|---|---|---|

Other Protective Clothing or Equipment

Work/Hygienic Practices: Do not ingest. Avoid contact with skin, eyes and clothing. Wash thoroughly after handling.

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## Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Antibody Samples/317

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

### Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive, Rockville, MD 20850**

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 09-15-2002

Signature of Preparer (optional):

### Section II - Hazardous Ingredients/Identify Information

| Hazardous Components [Specific Chemical Identity; Common Name(s)] | OSHA PEL | ACGIH TLV | Other Limits Recommended | % (Optional) |
|---|---|---|---|---|
| CAS # 139-33-3 | | | | |
| CAS # 26628-22-8 Very dilute | | | | |

### Section III - Physical/Chemical Characteristics

| | | | |
|---|---|---|---|
| Boiling Point | No data | Specific Gravity ($H_20$ = 1) | No data |
| Vapor Pressure (mm Hg.) | No data | Melting Point | No data |
| Vapor Density (AIR = 1) | No data | Evaporation Rate (Butyl Acetate = 1) | No data |

Solubility in Water: Soluble

Appearance and Odor: Clear liquid, no odor

### Section IV - Physical/Chemical Characteristics

| Flash Point (Method Used) | Flammable Limits | LEL | UEL |
|---|---|---|---|
| No data | | | |

Extinguishing Media: Dry chemical, carbon dioxide, halon, water spray or standard foam

Special Fire Fighting Procedures: Move container from fire area if possible. Dike fire control water for later disposal

Unusual Fire and Explosion Hazards: Thermal decomposition products may include toxic and hazardous oxides of carbon, nitrogen, and sodium.

### Section V - Reactivity Data

| Stability | | | Conditions to Avoid |
|---|---|---|---|
| | Unstable | | |
| | Stable | X | Excessive heat, sparks or open flame, protein denaturants |

Incompatibility: Acids, aluminum, metals, oxidizers (strong)

Hazardous Decomposition or Byproducts: Thermal decomposition products of toxic & hazardous oxides of Carbon and nitrogen

| Hazardous Polymerization | | | Conditions to Avoid |
|---|---|---|---|
| | May Occur | | |
| | Will Not Occur | X | |

### Section VI - Health Hazard Data

| Route(s) of Entry: | Inhalation? | Skin? | Ingestion? |
|---|---|---|---|
| | Yes | Yes | Yes |

Health Hazards (Acute and Chronic): Moderately toxic by ingestion. Systematic toxicity may result. May chelate lead, magnesium, zinc, trace metals if present in intestine. Sensitivity reactions-anaphylactic shock

| Carcinogenicity: | NTP? | IARC Monographs? | OSHA Regulation? |
|---|---|---|---|
| None | No data | No data | No data |

Signs and Symptoms of Exposure: Mucous membrane irritation, eye/skin irritation, irritating to gastrointestinal system.

Medical Conditions Generally Aggravated by Exposure: Renal or heart disease, potassium deficiency, insulin-dependent, diabetes, seizures or intracranial lesions

Emergency First Aid Procedures: Treat symptomatically and supportively

### Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Mop up with absorptive material. Containerize to dispose of properly.

Waste Disposal Method: Observe all federal, state and local regulations

Precautions to be Taken in Handling and Storing: Store away from strong oxidizers or heat. Avoid eye/skin contact.

Other Precautions: NONE

### Section VIII - Control Measures

Respiratory Protection (Specify Type): Chemical cartridge respirator with full facepiece and organic vapor cartridge

| Ventilation | | | | |
|---|---|---|---|---|
| | Local Exhaust | No | Special | None |
| | Mechanical (General) | Gen. dilution vent sys. | Other | None |

| Protective Gloves | Yes | Eye Protection | Splash proof goggles |
|---|---|---|---|

Other Protective Clothing or Equipment: Impervious clothing to prevent contact.

Work/Hygienic Practices: Emergency eye wash should be available