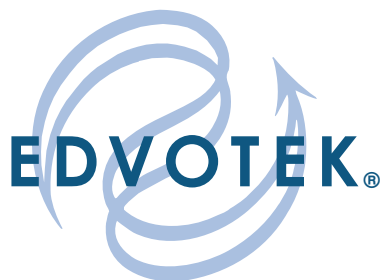


The Biotechnology Education Company®



**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.

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.

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## Western Blot Analysis

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.

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## 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 transferred 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.

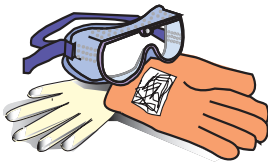
In this experiment, students will use Western Blot Analysis to detect a specific protein.

## Experiment Overview

### Experiment Procedures

#### 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.



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## Electrophoresis of Proteins

### PREPARING THE POLYACRYLAMIDE GEL FOR ELECTROPHORESIS

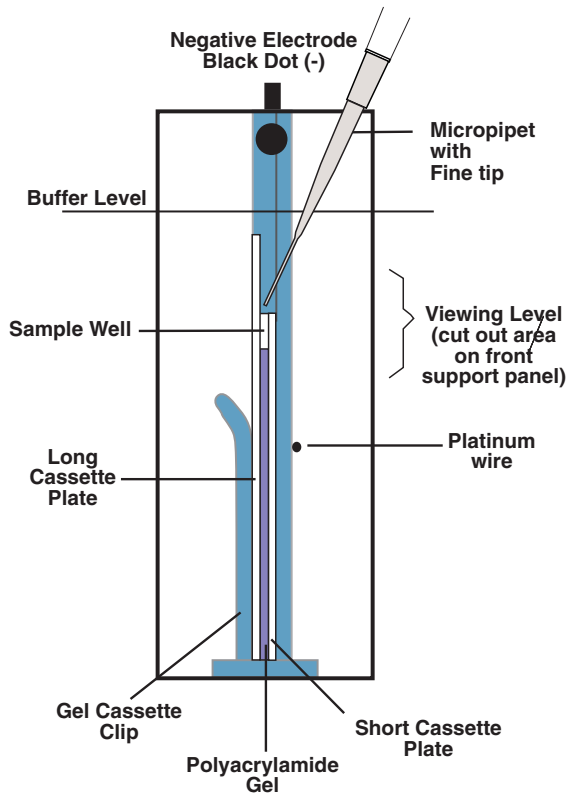
#### Pre-cast Polyacrylamide Gels:

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

### PRACTICE GEL LOADING

1. Place a fresh tip on the micropipet. Remove 20  $\mu$ l 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.



Wear gloves  
and safety  
goggles

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.



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## 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.*

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

#### Quick Reference:

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

#### Quick Reference:

Use an automatic micropipet to deliver samples. The amount of sample that should be loaded is 20  $\mu$ l.

## Electrophoresis of Proteins

### 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



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## Western Blot Procedure

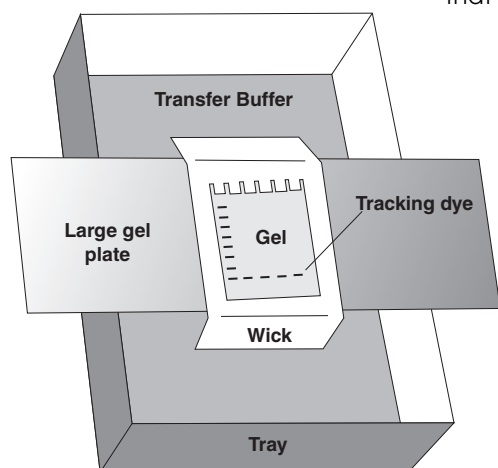
### Remember!



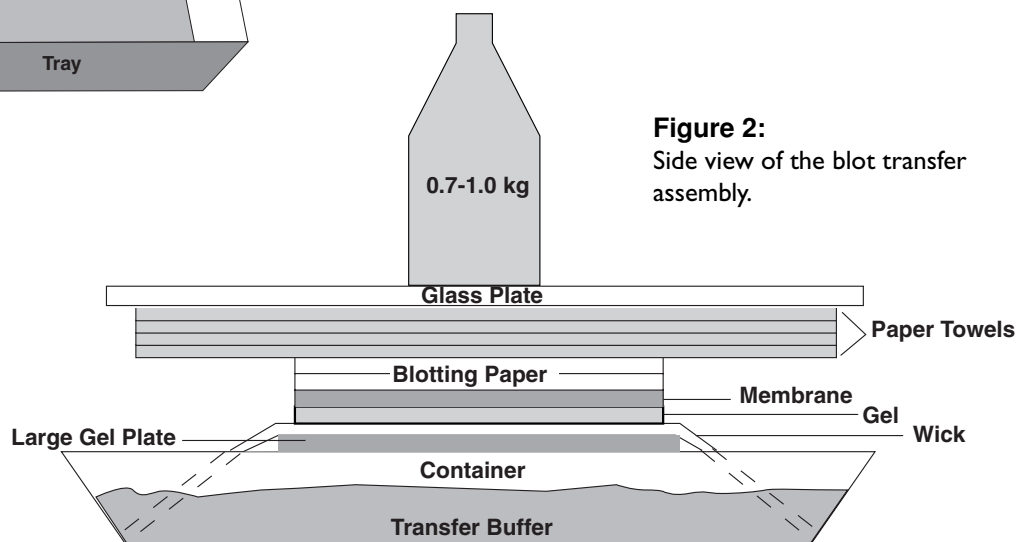
Wear gloves when handling nylon membrane to avoid transferring oil from your skin which will interfere with the protein transfer.

### 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.

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## Western Blot Procedure

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



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## 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.

Useful Hint!

The lid from a micropipet rack (200 $\mu$ l size) works well for a tray to incubate the membrane.



**NOTE:**

Substrate is prepared by your instructor just prior to use.

## Experiment Results and Study Questions

### 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?



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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)  
**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 <sub>2</sub> O <sub>2</sub>	No data			1.2%
CAS # 7722-84-1				

**Section III - Physical/Chemical Characteristics**

Boiling Point	No data	Specific Gravity (H <sub>2</sub> O = 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)	No data	Flammable Limits	LEL	UEL
			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.			

**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 <sub>2</sub> O = 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)	No data	Flammable Limits	LEL	UEL
			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	X	Conditions to Avoid
	Stable		Excessive heat
Incompatibility	Strong acids, aluminum, steel		
Hazardous Decomposition or Byproducts	Toxic oxides of phosphorous		
Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	None

**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.			

**Section V - Reactivity Data**

Stability	Unstable		Conditions to Avoid
	Stable	X	Incompatibles
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			

 <p align="center"><b>Material Safety Data Sheet</b> May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.</p>			
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.	
<b>Section I</b>			
Manufacturer's Name <b>EDVOTEK, Inc.</b>		Emergency Telephone Number <b>(301) 251-5990</b>	
Address (Number, Street, City, State, Zip Code) <b>14676 Rothgeb Drive Rockville, MD 20850</b>		Telephone Number for information <b>(301) 251-5990</b>	
		Date Prepared 09-19-2002	
		Signature of Preparer (optional)	
<b>Section II - Hazardous Ingredients/Identify Information</b>			
Hazardous Components [Specific Chemical Identity, Common Name(s)] OSHA PEL ACGIH TLV Other Limits Recommended % (Optional)			
N/A Blend			
<b>Section III - Physical/Chemical Characteristics</b>			
Boiling Point	100°C	Specific Gravity (H <sub>2</sub> O = 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			
<b>Section IV - Physical/Chemical Characteristics</b>			
Flash Point (Method Used) Noncombustible		Flammable Limits	LEL UEL
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			

 <p align="center"><b>Material Safety Data Sheet</b> May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.</p>			
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.	
<b>Section I</b>			
Manufacturer's Name <b>EDVOTEK, Inc.</b>		Emergency Telephone Number <b>(301) 251-5990</b>	
Address (Number, Street, City, State, Zip Code) <b>14676 Rothgeb Drive Rockville, MD 20850</b>		Telephone Number for information <b>(301) 251-5990</b>	
		Date Prepared 09-15-2002	
		Signature of Preparer (optional)	
<b>Section II - Hazardous Ingredients/Identify Information</b>			
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			
<b>Section III - Physical/Chemical Characteristics</b>			
Boiling Point	No data	Specific Gravity (H <sub>2</sub> O = 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			
<b>Section IV - Physical/Chemical Characteristics</b>			
Flash Point (Method Used) No data		Flammable Limits	LEL UEL
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.			

<b>Section V - Reactivity Data</b>			
Stability	Unstable		Conditions to Avoid
	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	May Occur		Conditions to Avoid
	Will Not Occur	X	
<b>Section VI - Health Hazard Data</b>			
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 toxicological properties have not been thoroughly investigated.			
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			
<b>Section VII - Precautions for Safe Handling and Use</b>			
Steps to be Taken in case Material is Released for Spilled Mop up with absorbent 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			
<b>Section VIII - Control Measures</b>			
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			

<b>Section V - Reactivity Data</b>			
Stability	Unstable		Conditions to Avoid
	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	May Occur		Conditions to Avoid
	Will Not Occur	X	
<b>Section VI - Health Hazard Data</b>			
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			
<b>Section VII - Precautions for Safe Handling and Use</b>			
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