The Biotechnology Education Company®

EDVOTEK®

269

EDVO-Kit #

Introduction to ELISA Reactions

Storage:
Some components require refrigerator storage. See page 3 for details.

EXPERIMENT OBJECTIVES:

This experiment introduces concepts and methodologies of enzyme linked immunosorbent assays (ELISA).

Table of Contents

	Page
Experiment Components	3
Experiment Requirements	3
Background Information	
Introduction to ELISA Reactions	4
Experiment Procedures	
Experiment Overview	7
General Instructions and Procedures	8
Study Questions	12
Instructor's Guidelines	
Notes to the Instructor	13
Pre-Lab Preparations	14
Quick Reference Tables	17
Avoiding Common Pitfalls	18
Expected Results	18
Study Questions and Answers	19
Material Safety Data Sheets	20

Experiment Components

A Antigens
B Primary antibody
C Anti-IgG-peroxidase conjugate (secondary antibody)
D Hydrogen peroxide, stabilized (for S1)
E Peroxide co-substrate (for S1)
F ABTS substrate (S2)
G Phosphate buffered saline concentrate

- 2 Microtiter plates
- Transfer pipets
- Microtest tubes with attached caps
- 15 ml plastic tubes

This experiment is designed for 10 groups.

Upon receipt, refrigerate Components A-G.

None of the components have been prepared from human sources.

Requirements

- Distilled or deionized water
- Beakers
- 37°C Incubation oven
- Disposable lab gloves
- Safety goggles
- Automatic micropipets (0 - 50 µl) and tips recommended

Make sure glassware is clean, dry and free of soap residue.
For convenience, additional disposable transfer pipets can be purchased for liquid removal and washing steps.

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.

Introduction to ELISA Reactions

Background Information

Principles of Enzyme linked immunosorbent assay (ELISA)

During an infection, an individual mounts an antibody response which eventually results in production of plasma IgG molecules that bind to various parts of the infectious agent. If these antibodies are present in the sample, they will bind to the adsorbed antigens in the well and remain there after washing, and will be detected by the ELISA technique.

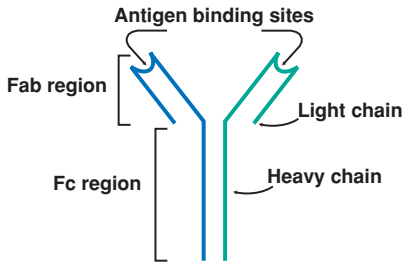


Figure 1:
General Structure of an antibody

All antibodies belong to a group of serum proteins known as globulins. Each antibody is made up of a heavy and light polypeptide chain (Figure 1). In general, antibodies are produced in response to the presence of a "non-self" antigenic response.

Antibodies obtained from animals, such as rabbits, in response to an antigen are known as polyclonal antibodies. Polyclonal antibodies are heterogeneous in structure and vary in their ability to bind to antigens. Antibodies that have a high affinity for non-specific antigens may give unwanted cross-reactions that can result in high backgrounds. Such antibodies can also give false negative results. By contrast, antibodies with weak binding constants may not be as sensitive.

Enzyme linked immunosorbent assay (ELISA) tests were originally developed for antibody measurement but have also been adapted to successfully detect samples that contain antigens. This ELISA experiment has been designed to detect an antibody directed against an antigen.

ELISAs (Figure 2) are done in microtiter plates usually made of polystyrene or polyvinyl chloride. The plates are somewhat transparent and contain many small wells, into which liquid samples are deposited. The following are the basic steps of the ELISA technique.

Step 1
The antigen is added to the wells where some remain adsorbed by hydrophobic association to the walls after washing away the excess. The antigens can be a lysate, a specific protein, or a mixture of the two. There is no specificity involved with the adsorption process, although some substances may exhibit low binding to the walls. In certain cases the antigens can be covalently cross-linked to the plastic using UV light.

Introduction to ELISA Reactions

Step 2
After washing away unadsorbed material, the unoccupied sites on the walls of the plastic wells are blocked with proteins, typically gelatin or bovine serum albumin.

Step 3
A solution that may or may not contain the primary antibody is added to the wells. If present in the solution, the primary antibody will bind to the adsorbed antigen in the well and remain after washing.

Step 4
A solution containing the secondary antibody is then added to the wells. If the primary antibody has remained bound to the well, then the secondary antibody will bind to it and remain attached after washing. Secondary antibodies are purified and covalently cross linked to enzymes such as horseradish peroxidase. This coupling does not significantly affect the binding specificity and affinity of the antibody or the enzymatic activity of the peroxidase.

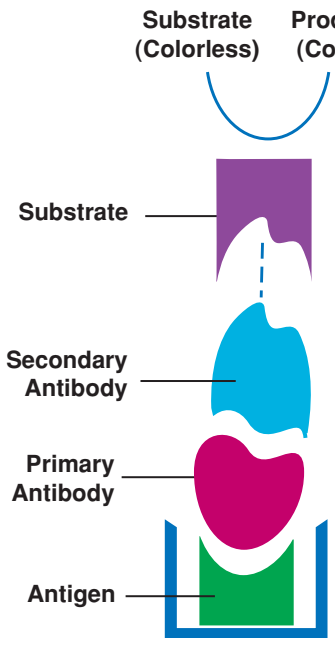


Figure 2:
Schematic of the ELISA Reaction

Step 5
The wells are washed with buffer to remove unbound secondary antibody.

Step 6
After washing the wells, substrate 1 (S1) will be added to all the wells in rows 1 and 2. Substrate 2 (S2) will be added to rows 3 and 4. The enzyme attached to the secondary antibody is a peroxidase. Peroxidase possesses a high catalytic activity and can exceed turnover rates of 10^6 per second. Consequently, amplification of a positive sample can occur over several orders of magnitude. Many hydrogen donor co-substrates can be used by peroxidase. These co-substrates include o-diansidine, aminoantipyrine, aminosalicylic acid and numerous phenolic compounds that develop color upon oxidation.

Substrate 1 (S1) contains hydrogen peroxide and amino salicylate. The substrate solution added is nearly colorless. Peroxidase converts the peroxide to $H_2O + O_2$ using the salicylate as the hydrogen donor. The oxidized salicylate is brown and can be easily observed in wells that have received each of the components required for completion of the reaction.

Introduction to ELISA Reactions

Background Information

Substrate 2 (S2) contains hydrogen peroxide and azino-di-ethylbenzthiazoline sulfonate (ABTS). The substrate solution added is nearly colorless. Peroxidase converts the peroxide to $H_2O + O_2$ using the ABTS as the hydrogen donor. The oxidized ABTS is green and can be easily observed in positive wells.

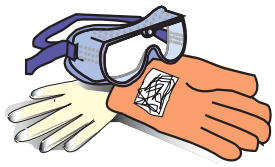
It should be noted that polyclonal antibody preparations to a given antigen can have variable binding affinities due to differences in the immunological responses between animals. Different immunizations with the same antigen in animals can also produce antibodies with variable binding affinities. The use of monoclonal antibodies directed against a single epitope eliminates this variability. Western blot analysis is usually used to confirm the ELISA results and to quantitate the size and amount of antigen. Western Blots and ELISA-based tests are used as diagnostic tools.

This experiment demonstrates two important concepts. The first is the effect of the absence of the antigen or the primary antibody which results in the disruption of the ELISA reaction. The second is the demonstration that the substrate utilized by the enzyme attached to the secondary antibody can result in different positive well colors.

Experiment Overview

EXPERIMENT OBJECTIVE:

The objective of this experiment is to understand the experimental concepts and methodology involved with enzyme linked immunosorbent (ELISA) assays .



Wear gloves and safety goggles

LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment which is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS OR BULBS.

Experiment Procedures

General Instructions and Procedures

Equilibrate a 37°C incubation oven before starting the experiment.

LABELING THE MICROTITER PLATE

1. Orient the microtiter plate as shown in figure 3. Carefully mark the microtiter plate with your initials or lab group number.
2. If your microtiter plate is pre-labeled by the manufacturer, mark out the letters or numbers and re-label the plate as instructed as follows.
3. Label the microtiter plate A, B and C across the top.
4. Label the rows of wells consecutively 1, 2, 3 and 4 down the left side of the microtiter plate.

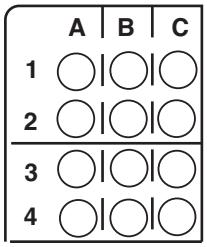


Figure 3

LABELING THE PLASTIC TRANSFER PIPETS

Label 10 transfer pipets as follows:

- PBS (Phosphate Buffered Saline)
- Ag (Antigen)
- 1°Ab (Primary Antibody)
- 2°Ab (Secondary Antibody)
- Sub 1 (Substrate 1)
- Sub 2 (Substrate 2)
- Row 1
- Row 2
- Row 3
- Row 4

Use the appropriately labeled plastic transfer pipet for sample additions, removals, and washes as outlined in the experimental procedures starting on page 9.

General Instructions and Procedures

If available, reagents should be dispensed with an automatic micropipet using disposable tips.

INSTRUCTIONS FOR ADDING LIQUIDS AND WASHING WELLS

Adding Reagents to wells:

For adding reagents to the wells, use the appropriately labeled transfer pipets or use an automatic micropipet and disposable tips.

Liquid Removal and Washes:

1. When instructed in the experimental procedures to remove liquid reagents (Antigen, Primary Antibody and Secondary Antibody), use the appropriately labeled transfer pipet designated for each row.

2. To wash the wells, do the following:

   A. Use the transfer pipet labeled "PBS", to add PBS buffer to the wells. Add PBS buffer until each well is almost full.

   The capacity of each well is approximately 0.2 ml. Do not allow the liquids to spill over into adjacent wells.

   B. Remove all the PBS from each of the wells with the transfer pipet designated for each row.

WEAR SAFETY GOGGLES AND GLOVES

EXPERIMENTAL STEPS FOR THE ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

Antigen:

1. Add 50 µl or 3 drops of Antigen (Ag) to all the wells in Rows 2, 3, and 4. Do not add antigen to the wells in Row 1 (Figure 4).

2. Incubate for 5 minutes at room temperature.

3. Remove all the liquid with the transfer pipet labeled "Ag".

4. Wash all 12 wells once with PBS buffer as described in the previous section "Liquid Removal and Washes". If stopping at this point, leave PBS in wells - see "Optional Stopping Point" on page 9. If continuing with experiment, remove PBS from each well using the transfer pipet designated for each row.

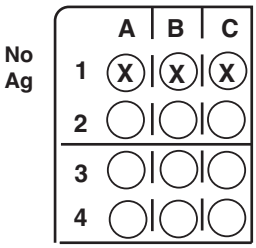


Figure 4

In research labs, following addition of antigen, all sites on the microtiter plate are saturated with a blocking solution consisting of a protein mixture, such as BSA. This experiment is designed to eliminate this step to save time.

Experiment Procedures

General Instructions and Procedures

Optional Stopping Point: The experiment can be stopped after step 4, but requires that PBS be left in all the wells for overnight storage at room temperature. The experiment can be resumed during the next lab period. Remove the PBS and continue with step 5.

REMINDERS:

ADDING REAGENTS:

Be sure to use a fresh tip for the addition of each reagent (Steps 1, 5, 9, & 13). Alternatively, use the appropriately labeled transfer pipet for each reagent.

LIQUID REMOVALS:

Use the appropriately labeled transfer pipet to remove all liquid from each of the wells (Steps 3, 7, & 11) and after washes (Steps 4, 8 & 12).

Dispose the liquid into a beaker labeled "waste".

WASHES:

For all wells, use the transfer pipet labeled "PBS" to add PBS until each well is almost full (Steps 4, 8, & 12).

Primary Antibody:

5. Add 50 µl or 3 drops of the primary antibody (1°Ab) to all the wells in Rows 1, 2, and 4. Do not add Primary Antibody to the wells in Row 3 (Figure 5).
6. Incubate for 5 minutes at 37°C.
7. Remove all the liquid using the transfer pipet designated for each row.

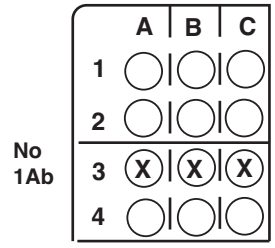


Figure 5

8. Wash each well once with PBS buffer as described in the section "Liquid Removal and Washes" on page 8. Remove PBS from each well using the transfer pipet designated for each row.

Secondary Antibody:

9. Add 50 µl or 3 drops of Secondary Antibody (2°Ab) to all the wells in Rows 1, 2, 3, and 4.
10. Incubate for 5 minutes at 37°C.
11. Remove all the liquid using the transfer pipet designated for each row.
12. Wash each well once with PBS buffer as described in the section "Liquid Removal and Washes" on page 8. Remove PBS from each well using the transfer pipet designated for each row.

General Instructions and Procedures

Substrate:

13. Add 0.1 ml or 5 drops of the substrate S1 to each of the wells in rows 1 and 2 (Figure 6).

14. Add 0.1ml or 5 drops of substrate S2 to each of the wells in rows 3 and 4 (Figure 6).

15. Incubate for 5 minutes at 37°C.

16. Remove the plate for analysis.

17. If color is not fully developed after 5 minutes, incubate at 37°C for a longer period of time.

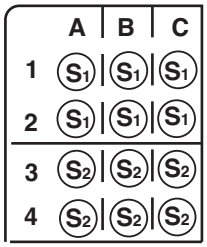


Figure 6

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 is there a 37°C incubation step after the addition of the substrate?

2. What is the effect of not including the antigen or the primary antibody in the ELISA reaction?

3. Why is it important to wash all the wells between the additions of the various components?

4. Can nucleic acids be detected by the ELISA format?

Notes to the Instructor

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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APPROXIMATE TIME REQUIREMENTS FOR PRE-LAB AND EXPERIMENTAL PROCEDURES

1. Pre-lab preparation of biologicals and reagents takes approximately one and one-half hours.
2. The student experimental activity requires approximately 60 minutes.

EDVO-TECH SERVICE
1-800-EDVOTEK
(1-800-338-6835)
Mon - Fri 9 am - 6 pm ET

Technical Service Department

Mon - Fri
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

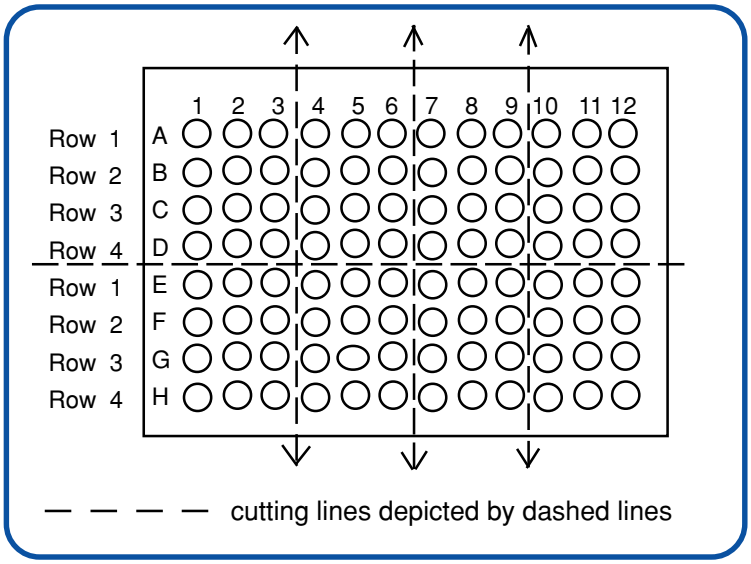
Instructor's Guide

PREPARATIONS BEFORE THE LAB

Microtiter Plates

1. As shown in the figure below, orient the microtiter plates so that the numbers 1-12 are at the top and the letters A-H are on your left.

2. Cut each plate on the dotted lines as shown in the figure. Each piece will be 3 wells on one axis and 4 wells on the other axis. Each lab group will receive one piece.



Pre-Lab Preparations

PREPARATIONS OF REAGENTS ON THE DAY OF THE LAB

Dispensing Components A and B:

1. Use a FRESH 1 ml pipet for dispensing the Antigen (A) directly from the component tubes provided in this experiment kit. Label 10 microtest tubes "Ag" and dispense 0.6 ml per tube.

2. Use a FRESH 1 ml pipet for dispensing the Primary Antibody (B) directly from the component tubes provided. Label 10 microtest tubes "1°Ab" and dispense 0.6 ml to each tube.

Preparation of Phosphate Buffered Saline

1. Add all of the Phosphate Buffered Saline concentrate **(G)** to 135 ml of distilled water. Mix.

2. Label this diluted Phosphate Buffered saline as "PBS".

3. Dispense 12 ml into 10 small beakers or tubes for each lab group.

Preparation of Anti-IgG Peroxidase Conjugate (Secondary Antibody)

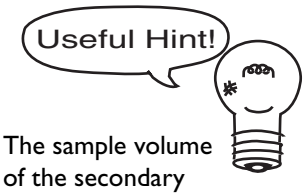
Note: *Prepare on same day as needed for the experiment.*

1. Add 0.3 ml of diluted Phosphate Buffered Saline (PBS) to the tube of concentrated Anti-IgG peroxidase conjugate **(C)**. Mix thoroughly by tapping and inverting the tube.

2. Transfer 6 ml of diluted Phosphate Buffered Saline (PBS) to one of the 15 ml plastic tubes provided.

3. Transfer the entire contents of tube "C" prepared in step 1 to the 15 ml tube containing 6 ml of PBS. Label the tube "2°Ab" (Secondary Antibody). Mix.

4. Dispense 0.6 ml of the diluted Anti-IgG peroxidase conjugate for each group.



The sample volume of the secondary antibody is very small - the tube can be centrifuged to collect the sample at the bottom.

Pre-Lab Preparations

Instructor's Guide

PREPARATION OF PEROXIDASE SUBSTRATE
(During the lab experiment)

Prepare 15 - 30 minutes before the last incubation:

1. Dispense 9 ml of diluted Phosphate buffered saline (PBS) to the second 15 ml tube provided.
2. Add Peroxide co-substrate **(E)** to the 9 ml of PBS. Cap and mix thoroughly by shaking and/or vortexing. There is usually undissolved material remaining.
3. Add 1 ml of Hydrogen peroxide **(D)**. Cap and mix.
4. Dispense 0.75 ml peroxidase substrate (S1) for each of the 10 groups.

Dispensing Component F

1. Dispense 0.75 ml ABTS substrate for each of the 10 groups.

Quick Reference:

Substrate 1 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).

Quick Reference Tables

Preparation of Experiment Reagents

		Label	Dispense for each group
A*	Antigen	Ag	0.6 ml
B*	Primary Antibody	1°Ab	0.6 ml
C + PBS	Anti-IgG-peroxidase-conjugate	2°Ab	0.6 ml
PBS + D + E	Peroxidase-enzyme substrate**	S1	0.75 ml
F	ABTS Substrate	S2	0.75 ml
G + water	Phosphate Buffered Saline	PBS	12 ml

* **Components A and B** can be dispensed before the actual day of the lab and stored in the refrigerator. If dispensed on the day of the lab, leave at room temperature.

** **Peroxidase-enzyme substrate** should be prepared 15-20 minutes before the last incubation.

Each Lab Group Should Receive:

- 1 Microtiter section
- 1 Tube labeled "Ag"
- 1 Tube labeled "1°Ab"
- 1 Tube labeled "2°Ab"
- 1 Automatic micropipet with tips (optional)
- 10 Transfer pipets
- 1 Beaker or tube containing PBS
- 1 Empty beaker labeled "waste"
- 1 Tube labeled "S1" (just before the last incubation)
- 1 Tube labeled "S2" (just before the last incubation)

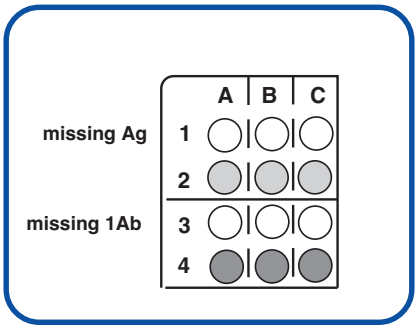
Instructor's Guide

Avoiding Common Pitfalls

1. Students should be advised to be very careful when transferring solutions into and out of the microtiter plate wells.
2. Use only clean or appropriately labeled pipets and avoid contaminating adjacent wells.
3. Do not attempt to empty the microtiter wells by shaking them out. This will not work - it will result in contaminating adjacent wells.
4. Wash the wells gently and slowly, without force.

Expected Results

Color should appear only in Rows 2 and 4. Rows 1 and 3 are each missing a critical component for the ELISA procedure. Row 2 will be a brown color and row 4 will be green in color.



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			Conditions to Avoid
	Unstable	X	Excessive heat
	Stable		

Incompatibility: Strong acide, aluminum, steel

Hazardous Decomposition or Byproducts: Toxic oxides of phosphorous

Hazardous Polymerization			Conditions to Avoid
	May Occur		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			Special	
	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.

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): ABTS 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: 03/14/00

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)
2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) Diammonium salt	-----No data-----			
CAS# 30931-67-0				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_20 = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	>300°C
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Soluble

Appearance and Odor: colorless, odorless liquid

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 powder or appropriate foam

Special Fire Fighting Procedures: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Unusual Fire and Explosion Hazards: No data

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		Incompatibles
	Stable		

Incompatibility: Strong oxidizing agents, strong acids

Hazardous Decomposition or Byproducts: Toxic fumes of carbon monoxide, carbon dioxide, nitrogen oxides sulfure oxides.

Hazardous Polymerization			Conditions to Avoid
	May Occur		No Data
	Will Not Occur		

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): Irritant

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

Signs and Symptoms of Exposure: Eye, skin and respiratory system irritation

Medical Conditions Generally Aggravated by Exposure: No data

Emergency First Aid Procedures: Eyes/Skin: Flush with copious amounts if water. If swallowed, wash out mouth with water, call a physician.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear suitable protective clothing and mop up

Waste Disposal Method: Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburrner and scrubber. Observe federal, state, and local laws.

Precautions to be Taken in Handling and Storing: Avoid contact. Wear self-contained breathing apparatus, rubber boots and rubber gloves.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type)

Ventilation			Special	
	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, NIOSH/MSHA approved respirator

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): 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 CAS# 89-57-6	----------No data----------			

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 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			Conditions to Avoid
	Unstable		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			Conditions to Avoid
	May Occur		
	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