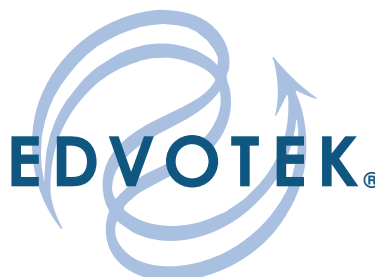


The Biotechnology Education Company®



270
EDVO-Kit #

**Antigen-Antibody
Interaction:
The Ouchterlony Procedure**

Storage:

Some components require refrigerator storage.
See page 3 for storage requirements.

EXPERIMENT OBJECTIVES:

The objective of this experiment is to introduce the principles of antigen-antibody interactions using the Ouchterlony procedure.

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.

The Biotechnology Education Company® • 1-800-EDVOTEK • www.edvotek.com

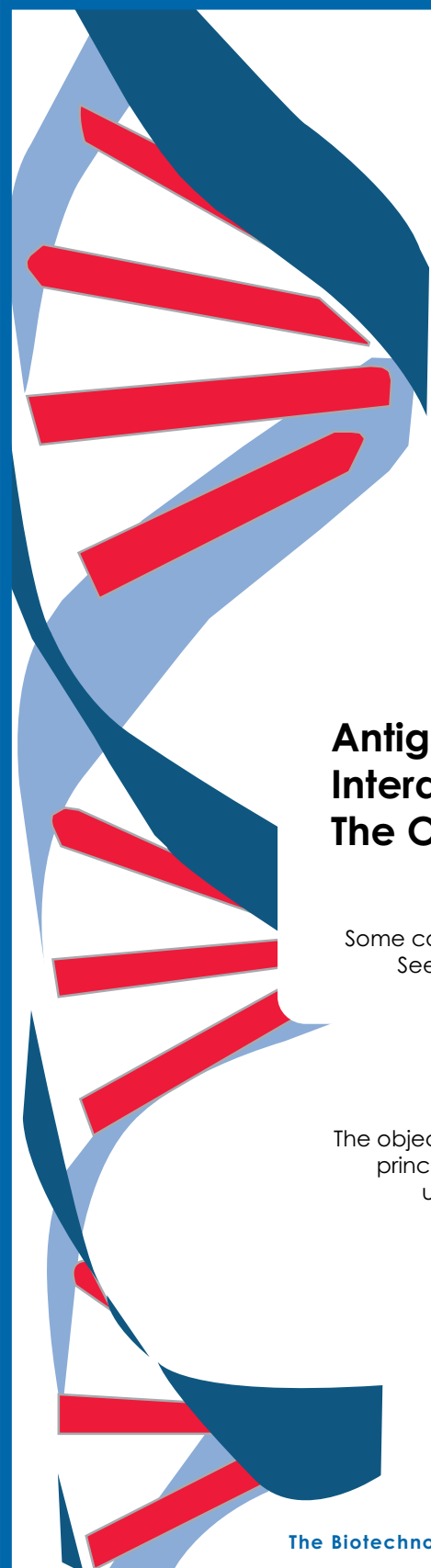


Table of Contents

	Page
Experiment Components	3
Experiment Requirements	3
Background Information	
Antigen-Antibody Interaction	4
Experiment Procedures	
Experiment Overview	7
Student Experimental Procedures	8
Study Questions	12
Instructor's Guidelines	
Notes to the Instructor	13
Pre-Lab Preparations	14
Avoiding Common Pitfalls	16
Idealized Schematic of Results	17
Study Questions and Answers	18
Material Safety Data Sheets	19



This experiment is designed for 10 groups.

Store components A - D in the refrigerator.

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.

This experiment does not contain components which have been prepared from human sources.

Experiment Components

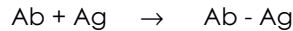
A	Antiserum (antibody)	Refrigerator
B	Whole serum (antigen)	Refrigerator
C	Albumin (antigen)	Refrigerator
D	IgG (antigen)	Refrigerator
E	Powdered buffer	Room temp.
1	Package UltraSpec-Agarose™	Room temp.
1	Tube practice loading solution	Room temp.
40	Transfer pipets	
40	Petri plates	
10	Well cutters	
1	Template for cutting wells	
40	Microtest tubes	

Requirements

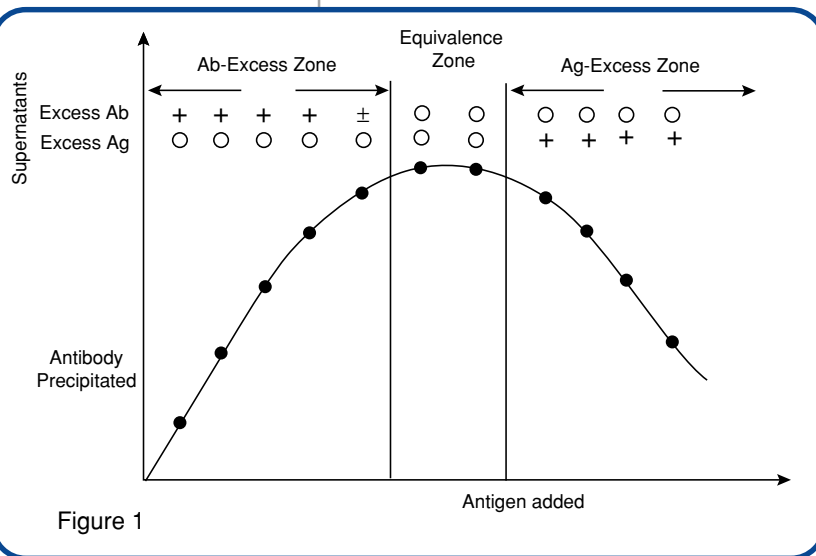
- Micropipet and tips
- Plastic container or Pyrex baking dish
- Plastic wrap
- Distilled Water
- Pipets - 5 ml or 10 ml
- Marking pen
- Measuring spatula or toothpicks
- Heat plate, Bunsen burner, or microwave
- Paper towels
- Waterbath

Antigen-Antibody Interaction: The Ouchterlony Procedure

The interactions of an antibody (Ab) with an antigen (Ag) is the fundamental reaction of immunology.

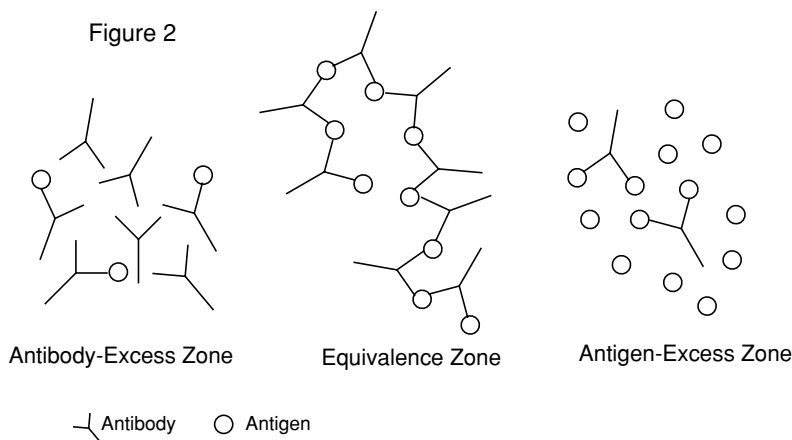


Most antigens are proteins. The exact identity of the groups that react with the antibody are usually not known. Macromolecular antigens and antibodies form complexes that become insoluble and precipitate from solution. This property makes it possible to perform qualitative and quantitative assays on the antibody-antigen system.



Precipitation occurs with most antigens because the antigen is multivalent, i.e., has several antigenic determinants per molecule to which antibodies can bind. Antibodies have at least two antigen binding sites, thus large aggregates or lattices of antigen and antibody are formed. Experimentally, an increasing amount of antigen is added to a constant amount of antibody in solution. Initially at low antigen concentration, all of the antigen is contained in the precipitate. This is called the antibody-excess zone. As more antigen is added, the amount of protein precipitated increases until the antigen and antibody molecules are at an optimal ratio. This is called the equivalence zone, or equivalence point, where maximum precipitation occurs. When the amount of antigen in solution exceeds the amount of antibody, the amount of precipitation will decrease. This is known as the antigen-excess zone (Figures 1 and 2).

Figure 2



Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1989, 1992, 1995, 1998, 2005 EDVOTEK, Inc., all rights reserved.

EVT 004185AM

Antigen-Antibody Interaction: The Ouchterlony Procedure

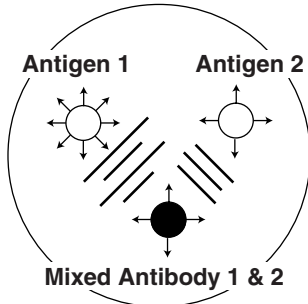
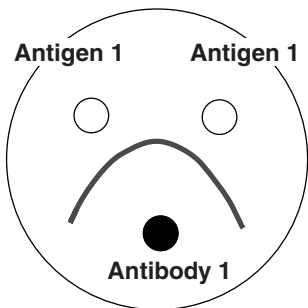


Figure 3

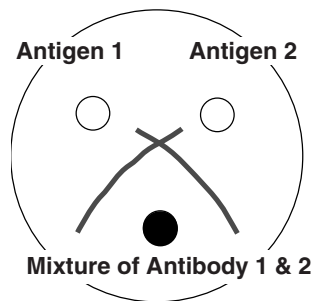
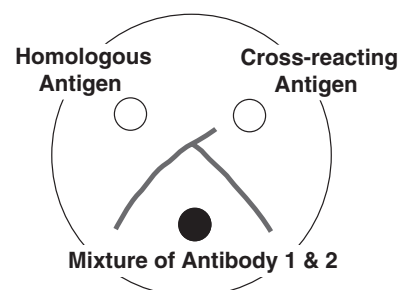
Figure 4:
Reaction of Identity

When antibodies and antigens are inserted into different areas of an agarose gel, they diffuse toward each other and form opaque bands of precipitate at the interface of their diffusion fronts. Precipitation reactions of antibodies and antigens in agarose gels provide a method of analyzing various antibody-antigen reactions.

THE OUCHTERLONY PROCEDURE

Double diffusion in two dimensions is a simple procedure invented by and named after the Swedish scientist, Örjan Ouchterlony. Antigen and antibody solutions are placed in separate wells cut in an agarose plate. The reactants diffuse from the wells toward each other and precipitate where they meet at equivalent proportions. A single antigen will combine with its homologous antibody to form a single precipitation line. When two antigens are present, each behaves independently of each other. Thus, the number of precipitin bands indicates there are at least that many antibody-antigen pairs present (see Figure 3). Arrows indicate diffusion patterns of antigens and antibodies.

Double diffusion in two dimensions is a useful technique for comparing antigens for the number of identical or cross-reacting determinants. If a solution of antigen is placed in two adjacent wells and the homologous antibody is placed in the center well, the two precipitin bands that form will join at their closest ends and fuse. This is known as a reaction of identity (Figure 4).

Figure 5
Reaction of Non-identityFigure 6
Reaction of Partial Identity

EDVOTEK and The Biotechnology Education Company are registered trademarks of EDVOTEK, Inc.

The patterns shown in Figures 3 - 6 are the ideal representation. Under experimental conditions, the spurs are often difficult to visualize.

Antigen-Antibody Interaction: The Ouchterlony Procedure

When unrelated antigens are placed in adjacent wells and the center well is filled with antibodies for each antigen, the precipitin bands will form independently of each other and will cross. This is known as a reaction of non-identity (Figure 5).

If two purified antigens cross-react, then placing them in adjacent peripheral wells with antibody to one in the central well will give a single band with the homologous and cross-reacting antigen. Since the cross-reacting antigen lacks some of the antigenic determinants present in the homologous antigen, it is not able to precipitate all of the antibody. The remaining antibody will diffuse beyond the line of cross-reacting precipitate to react with the homologous antigen to produce a spur. The spur that forms projects toward the antigen with the fewer determinants, i.e., the cross-reacting antigen. This is called a reaction of partial identity. Since these non-cross-reacting antibodies often are only a fraction of the total antibody involved in the homologous precipitin reaction, the spur is usually less dense (often difficult to visualize) than the precipitin band from which it projects (See Figure 7).

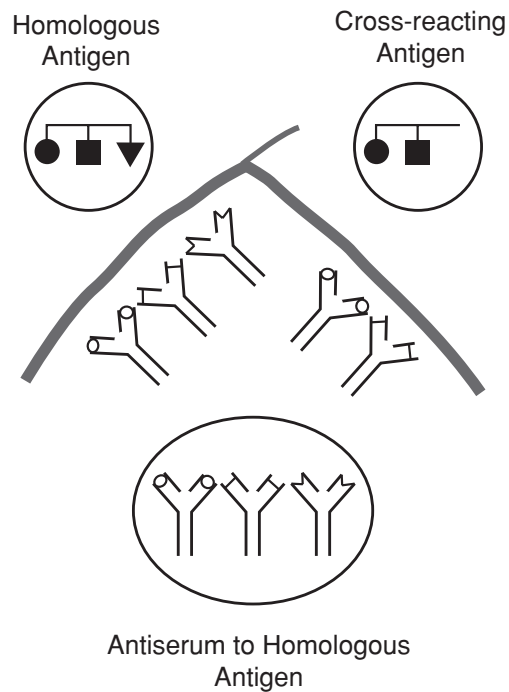


Figure 7



Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1989,1992,1995,1998,2005 EDVOTEK, Inc., all rights reserved. EVT 004185AM

Experiment Overview

EXPERIMENT OBJECTIVE:

The objective of this experiment is to introduce the principles of antigen-antibody interactions using the Ouchterlony procedure.



Wear gloves
and safety
goggles

LABORATORY SAFETY

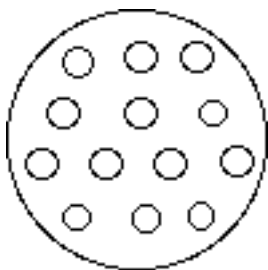
Gloves and goggles should be worn routinely as good laboratory practice.

Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1989,1992,1995,1998,2005 EDVOTEK, Inc., all rights reserved.

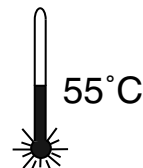
EVT 004185AM



Student Experimental Procedures

**A. PREPARATION OF AGAROSE AND POURING OF OUCHTERLONY PLATES**

1. Each group requires 4 plates: 1 practice loading plate and 3 experimental plates. Using a 5 ml or a 10 ml pipet, carefully pipet 5 ml of the cooled agarose (55°C) into each plate, rotating the plate to cover the bottom with agarose. Repeat with the remaining plates.
2. If the molten agarose contains bubbles, gently swirl to remove the bubbles.
3. Allow the agarose to solidify. This will take approximately 10-15 minutes, at which time the gel will appear slightly opaque.
4. If the plates are not to be used that day, the plates can be wrapped with plastic wrap and stored inverted in the refrigerator for two weeks.

**B. PRACTICE WELL LOADING (OPTIONAL)**

This experiment contains practice loading solution. This solution is included to allow instructors and students to practice loading the sample wells before performing the actual experiment. Use a micropipetting device, or one of the plastic transfer pipets included in your experiment kit to practice loading the sample wells with the practice loading solution.

1. One practice plate should be prepared for each group. Enough reagents have been provided for this purpose.
2. Using the well cutters provided, cut several rows of wells as shown in the diagram at left.
3. Practice loading the sample wells with the plastic, disposable transfer pipets. (See "Sample Loading of Wells with Transfer Pipets").
4. If you are using an automatic micropipetting device, the amount of sample that should be loaded is 30 microliters.



Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1989,1992,1995,1998,2005 EDVOTEK, Inc., all rights reserved.
EVT 004185AM

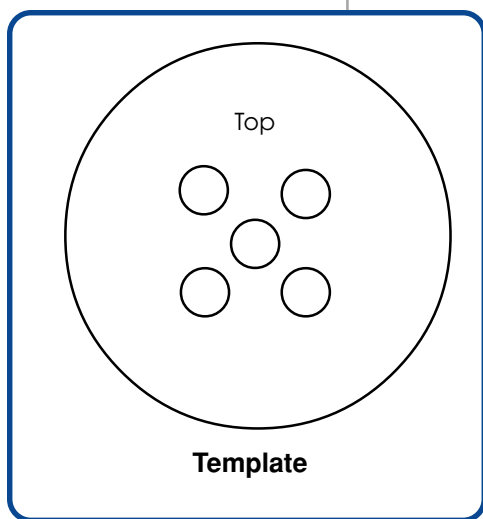
Student Experimental Procedures

Sample Loading of Wells With Transfer Pipets

1. Squeeze the pipet stem, not the bulb, to slowly draw a portion of the sample up into the pipet. The sample should remain in the lower portion of the pipet.

If the sample is overdrawn and becomes lodged in the bulb or on the walls, tap until the sample moves down into the lower stem of the pipet. Eject it back into the tube. Try step 1 again.

2. While holding the pipet tip above the tube, **slowly** squeeze the pipet stem until the sample is nearly at the opening of the pipet tip.
3. Place the pipet tip just over, **not** inside, the sample well. Maintain steady pressure on the pipet stem to prevent sample from being drawn back up into the pipet.
4. **Slowly** squeeze the pipet bulb to eject two (2) drops of sample. The well should appear full, but be careful not to overfill the wells and cause spillage on the agarose surface. Put any remaining sample in the pipet back into the tube.



C. PREPARATION OF SAMPLE WELLS

1. Make several copies of the template (at left) for your lab group.
2. Place the template under one of the plates so that the pattern is in the center of the plate. The distances between the wells is important. Try to follow the template as accurately as possible.
3. Cut the five wells using the well cutter (provided in the kit) in a gentle punching motion. Remove the agarose plugs with a flat-edged toothpick or spatula.
4. If well placement is not accurate, there should be enough room on the plate to re-cut the wells using the template.
5. Repeat steps 2 and 3 with the remaining two plates.



WEAR SAFETY GOGGLES AND GLOVES

D. LOADING THE SAMPLES

1. Orient your lab number or group designation at the top before loading samples.
2. Using the same pipet, fill the center wells of all three plates with 30 microliters (2 drops with a transfer pipet) of antiserum (antibody) from Tube A. Wells should appear full, but be careful not to overfill the wells and cause spillage on the agarose surface. This may affect your results.
3. Fill the outer wells with 30 microliters of antigen using a clean pipet tip for each antigen as follows:

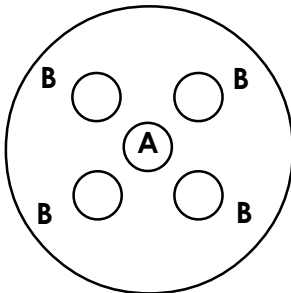


Plate 1 Center well: antiserum to the fluid containing antibodies (Tube A)

- Left upper well: Whole serum (Tube B)
- Right upper well: Whole serum (Tube B)
- Left lower well: Whole serum (Tube B)
- Right lower well: Whole serum (Tube B)

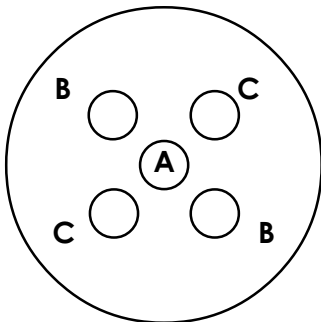
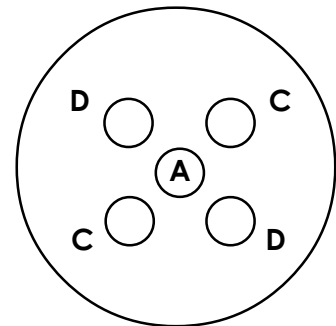


Plate 2 Center well: antiserum to the fluid containing antibodies (Tube A)

- Left upper well: Whole serum (Tube B)
- Right upper well: albumin (Tube C)
- Left lower well: albumin (Tube C)
- Right lower well: Whole serum (Tube B)

Plate 3 Center well: antiserum to the fluid containing antibodies (Tube A)

- Left upper well: IgG (Tube D)
- Right upper well: albumin (Tube C)
- Left lower well: albumin (Tube C)
- Right lower well: IgG (Tube D)



Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/ laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1989,1992,1995,1998,2005 EDVOTEK, Inc., all rights reserved. EVT 004185AM

Student Experimental Procedures

E. INCUBATION

Replace lids onto plates. Carefully place the covered plates in the incubation chamber on top of the wet paper towel layer. Do not invert the plates. Cover the chamber with plastic wrap and let incubate at room temperature 24-48 hours to allow precipitin lines to form or the chamber can be placed in a 37°C incubation oven.

F. READING THE RESULTS

The precipitin lines will be visible in 24-48 hours. Carefully hold a plate up so that the overhead room lights shine through it. You should be able to see opaque white arcs in each side of the plate where the antibody and antigen precipitated. A drawing of the results should be made.

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. Explain how qualitative observations can be performed on the antigen-antibody system.
2. What is the equivalence zone or equivalence point?
3. When would you observe the antigen-excess zone? What effect does this have on the amount of precipitation?
4. What would cause two or more precipitin bands to form in an antigen-antibody experiment?




Duplication of this document, in conjunction with use of accompanying reagents, is permitted for classroom/laboratory use only. This document, or any part, may not be reproduced or distributed for any other purpose without the written consent of EDVOTEK, Inc. Copyright © 1989,1992,1995,1998,2005 EDVOTEK, Inc., all rights reserved.
EVT 004185AM


 <p>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.</p>			
IDENTITY (As Used on Label and List) Agarose			
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) This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard. CAS #9012-36-6			
Section III - Physical/Chemical Characteristics			
Boiling Point For 1% solution	194° F	Specific Gravity (H ₂ 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 Insoluble - cold			
Appearance and Odor White powder, no odor			
Section IV - Physical/Chemical Characteristics N.D. = No data Flash Point (Method Used) No data Flammable Limits LEL N.D. UEL N.D.			
Extinguishing Media Water spray, dry chemical, carbon dioxide, halon or standard foam			
Special Fire Fighting Procedures Possible fire hazard when exposed to heat or flame			
Unusual Fire and Explosion Hazards None			

Section V - Reactivity Data Stability Unstable Stable X Conditions to Avoid None			
Incompatibility No data available			
Hazardous Decomposition or Byproducts			
Hazardous Polymerization May Occur Will Not Occur X Conditions to Avoid None			
Section VI - Health Hazard Data Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes			
Health Hazards (Acute and Chronic) Inhalation: No data available Ingestion: Large amounts may cause diarrhea			
Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?			
Signs and Symptoms of Exposure No data available			
Medical Conditions Generally Aggravated by Exposure No data available			
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 Sweep up and place in suitable container for disposal			
Waste Disposal Method Normal solid waste disposal			
Precautions to be Taken in Handling and Storing None			
Other Precautions None			
Section VIII - Control Measures Respiratory Protection (Specify Type) Chemical cartridge respirator with full facepiece.			
Ventilation		Local Exhaust	Special
		Mechanical (General)/Gen. dilution ventilation	Other
Protective Gloves Yes		Eye Protection	Splash proof goggles
Other Protective Clothing or Equipment Impervious clothing to prevent skin contact			
Work/Hygienic Practices None			

 <p>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.</p>			
IDENTITY (As Used on Label and List) Practice Gel Loading Solution			
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) This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.			
Section III - Physical/Chemical Characteristics			
Boiling Point	No data	Specific Gravity (H ₂ 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 Blue liquid, no odor			
Section IV - Physical/Chemical Characteristics Flash Point (Method Used) No data Flammable Limits LEL No data UEL No data			
Extinguishing Media Dry chemical, carbon dioxide, water spray or foam			
Special Fire Fighting Procedures Use agents suitable for type of surrounding fire. Keep upwind, avoid breathing hazardous sulfur oxides and bromides. Wear SCBA.			
Unusual Fire and Explosion Hazards Unknown			

Section V - Reactivity Data Stability Unstable Stable X Conditions to Avoid None			
Incompatibility None			
Hazardous Decomposition or Byproducts Sulfur oxides, and bromides			
Hazardous Polymerization May Occur Will Not Occur X Conditions to Avoid None			
Section VI - Health Hazard Data Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes			
Health Hazards (Acute and Chronic) Acute eye contact: May cause irritation. No data available for other routes.			
Carcinogenicity: No data available NTP? IARC Monographs? OSHA Regulation?			
Signs and Symptoms of Exposure May cause skin or eye irritation			
Medical Conditions Generally Aggravated by Exposure None reported			
Emergency First Aid Procedures Treat symptomatically and supportively. Rinse contacted area with copious amounts of water.			
Section VII - Precautions for Safe Handling and Use Steps to be Taken in case Material is Released for Spilled Wear eye and skin protection and mop spill area. Rinse with water.			
Waste Disposal Method Observe all federal, state, and local regulations.			
Precautions to be Taken in Handling and Storing Avoid eye and skin contact.			
Other Precautions None			
Section VIII - Control Measures Respiratory Protection (Specify Type)			
Ventilation		Local Exhaust	Special
		Mechanical (General)	Other
Protective Gloves Yes		Eye Protection	Splash proof goggles
Other Protective Clothing or Equipment None required			
Work/Hygienic Practices Avoid eye and skin contact			

 <p style="text-align: center;">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.</p>			
IDENTITY (As Used on Label and List) A,B,C and D/270		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 03-02-05 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)			
Ethylendiaminetetraacetic acid ----- No data ----- C10-H14-08-N2.2Na CAS# 139-33-3			
Section III - Physical/Chemical Characteristics			
Boiling Point	No data	Specific Gravity (H ₂ 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		
Section IV - Physical/Chemical Characteristics			
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.		

 <p style="text-align: center;">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.</p>			
IDENTITY (As Used on Label and List) Powdered Buffer		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 03-02-05 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)			
Section III - Physical/Chemical Characteristics			
Boiling Point	100C	Specific Gravity (H ₂ 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	solid		
Section IV - Physical/Chemical Characteristics			
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		

Section V - Reactivity Data			
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	
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?
	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			

Section V - Reactivity Data			
Stability	Unstable	Conditions to Avoid	
	Stable		
Incompatibility	Strong acids		
Hazardous Decomposition or Byproducts Nature of decomposition products not known			
Hazardous Polymerization	May Occur	Conditions to Avoid	
	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 toxicological 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 - cautiously 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.			