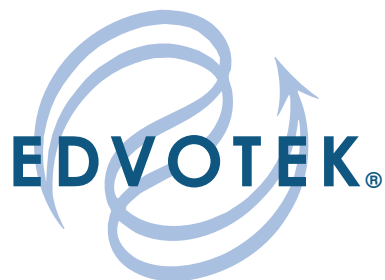




The Biotechnology Education Company®



273
EDVO-Kit #

Radial Immunodiffusion

Storage:

Store the entire experiment
in the refrigerator.

EXPERIMENT OBJECTIVES:

Radial Immunodiffusion is a sensitive quantitative technique that is often used clinically to detect patient levels of blood proteins. In this experiment, students will learn to quantitatively determine the unknown concentration of an antigen.

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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Experiment Components

- A Antibody Solution
 - B Standard Antigen Solution
 - C UltraSpec-Agarose™
 - D Buffer Powder
 - E Unknown Concentration of Antigen
-
- 1 Sleeve Petri Dishes
 - 2 10 ml Pipets
 - 10 Well Cutters
 - 80 Transfer Pipets
 - 70 Microtest Tubes
 - 1 Graph Paper Template
 - 1 Practice Loading Solution

This experiment is
designed for
10 groups.

Store entire
experiment in
the refrigerator.

Requirements

- Automatic Micropipets and Tips (5-50 μ l)
- Pipet Pumps (for 10 ml pipets)
- Ruler
- Plastic Box or Dish
- Plastic Wrap
- Foil
- Paper Towels
- Distilled Water
- Heat plate, Bunsen burner, or microwave
- 400 to 600 ml beaker or Erlenmeyer flask
- 150 ml beaker or flask
- Water bath
- 250 ml Graduated Cylinder
- 37°C Incubation Oven

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Radial Immunodiffusion

The fundamental reaction of immunology involves the interaction of antibodies (Ab) and antigens (Ag). These interactions are useful in the defense of the body against bacterial and viral infections and toxins. The defense capabilities are dependent upon the recognition of antigens by humoral components of the immune system. Specific antibodies are then produced in response to exposure to the antigen.

The formation of antigen-antibody complexes is the first step in removing infectious agents from the body. Because each antibody can bind more than one antigen and each antigen can be bound by more than one antibody molecule, very large macromolecular complexes can form. These complexes form precipitates which can be cleared from the body through various means. These precipitates are also useful for laboratory and diagnostic tests.

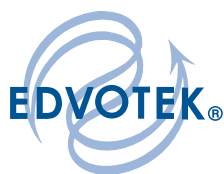
When antibodies and antigens are inserted into different areas of an agarose gel, they diffuse toward each other and form opaque bands of precipitate at the interface of their diffusion fronts. Precipitation reactions of antibodies and antigens in agarose gels provide a method of analyzing the various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

Double diffusion in two dimensions is a simple procedure invented by the Swedish scientist, 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.

Radial immunodiffusion (RID) is a technique that can quantitatively determine the concentration of an antigen. Unlike many gel and liquid precipitation techniques which qualitatively detect antigen, RID is a sensitive quantitative technique that is often used clinically to detect patient levels of blood proteins.

Antibody is incorporated into molten agarose which is poured into a Petri dish and allowed to solidify. Small wells are cut into the agarose and are filled with known concentrations of antigen which corresponds to the antibody in the agarose. Samples of unknown concentrations are placed in similar wells. The antigens in solution then diffuse outwards from the well in a circular pattern surrounding the well. Antibody is present in excess and diffusion of the antigen will continue until a stable ring of antigen-antibody precipitate forms. There are antigen-antibody complexes

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Radial Immunodiffusion

throughout the zone surrounding the well within the precipitin line. At the precipitin line is where the greatest number of complexes can be found because the antigen and antibody are present in roughly equal proportions. This is known as the equivalence zone or equivalence point. Generally, it takes 24 to 48 hours for optimal diffusion to occur and precipitation to become apparent.

For each antigen, an endpoint precipitation ring of a certain diameter will form. From the known standard concentrations, a standard curve can be drawn by plotting antigen concentration versus the diameter squared measurements of the rings. From this linear calibration curve the concentration of the unknown antigen samples may be determined.

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Experiment Overview

EXPERIMENT OBJECTIVE:

Radial Immunodiffusion is a sensitive quantitative technique that is often used clinically to detect patient levels of blood proteins. In this experiment, students will learn to quantitatively determine the unknown concentration of an antigen.



Wear gloves
and safety
goggles

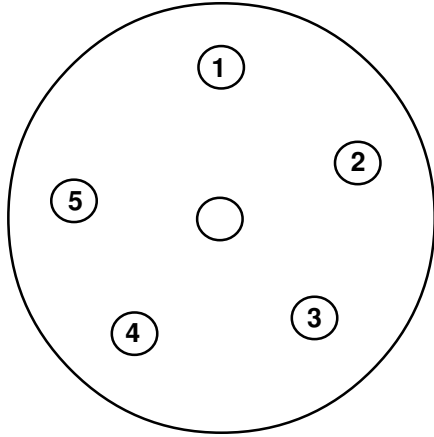
LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS OR BULBS.
3. Dispose of RID plates through proper laboratory waste disposal procedures.



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Student Experimental Procedures



Circle shown actual size.

Dilution	Concentration
Undiluted	2 mg/ml
1:2	1 mg/ml
1:4	0.5 mg/ml
1:8	0.25 mg/ml
1:16	0.125 mg/ml

A. PREPARATION OF AGAROSE PLATES

1. Place the template under the plate so the pattern is centered.
2. Cut the 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.

B. PREPARING THE STANDARDS (SERIAL DILUTION)

1. Label four microtest tubes: 1:2, 1:4, 1:8, and 1:16.
2. Using a micropipet, add 50 microliters of Buffer to each tube.
3. With a fresh pipet tip, add 50 microliters of "Standard" to the tube labeled 1:2. Mix.
4. With a fresh pipet tip, transfer 50 microliters of the 1:2 dilution to the tube labeled 1:4. Mix.
5. With a fresh pipet tip, transfer 50 microliters of the 1:4 dilution to the tube labeled 1:8. Mix.
6. With a fresh pipet tip, transfer 50 microliters of the 1:8 dilution to the tube labeled 1:16. Mix.
7. There are now five antigen samples for the standard curve (see chart).

Student Experimental Procedures

C. 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 the experiment to practice loading the sample wells with the practice loading solution. Make enough copies of the template for each lab group.

1. One practice plate should be prepared for every two groups. Enough reagents have been provided for this purpose.
2. Using the well cutters provided, cut several wells in the agarose as shown in the template below. Refer to Student Instructions for preparation of sample wells.
3. Practice loading the sample wells with the Practice Loading Solution using a micropipetting device. Load 5 μ l per well and make sure the sample covers the entire surface of the well. If a micropipetting device is not available, use the transfer pipets provided, taking care not to overfill the wells. If using transfer pipets, put in just enough sample to cover the bottom of the well.

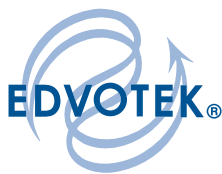
D. LOADING THE SAMPLES

1. On the bottom of the plate, number the wells on the perimeter of the plate 1 through 5. Leave the center well unlabeled.
2. Load wells 1 through 5 using the same pipet tip or transfer pipet. In well #5, load 5 μ l of the 1:16 antigen dilution. Make sure the sample covers the entire surface of the well by carefully spreading it with the pipet tip.
3. In well #4, load 5 μ l of the 1:8 antigen dilution.
4. In well #3, load 5 μ l of the 1:4 antigen dilution.
5. In well #2, load 5 μ l of the 1:2 antigen dilution.
6. In well #1, load 5 μ l of the undiluted antigen.
7. With a fresh pipet tip or microtipped transfer pipet, load 5 μ l of your unknown in the center well.
8. Label the cover of the Petri dish with your lab group number or your initials. Place the cover on the dish, place the dish right side up (do not invert) inside the incubation chamber on the paper toweling. Cover the incubation chamber and place in a 37°C incubation oven or at room temperature for 24 to 48 hours.

Remember!



You may use the same pipet tip or transfer pipet to load wells #1 through #5, starting with the most dilute antigen dilution and ending with the most concentrated. Use a fresh tip for the unknown.



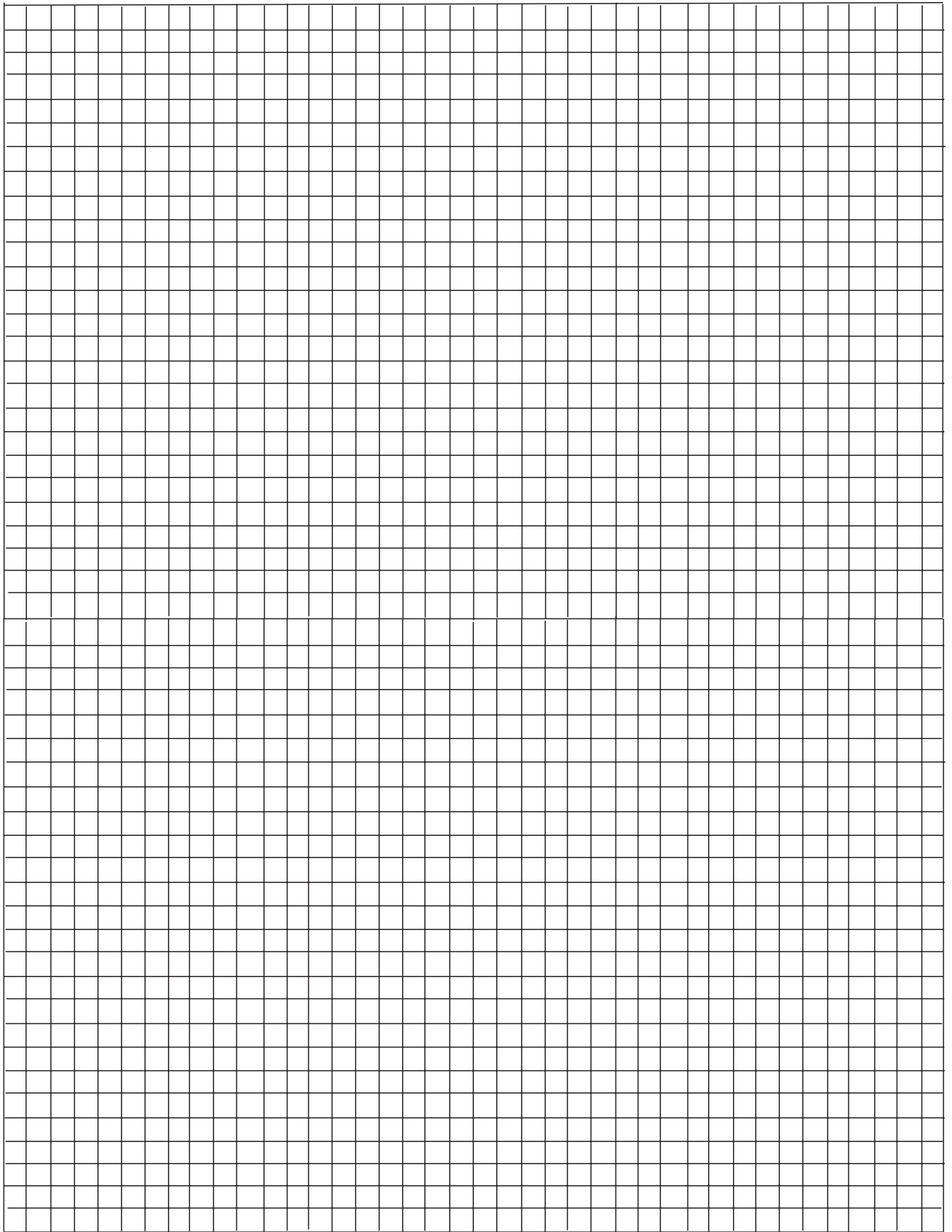
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Student Experimental Procedures

E. READING THE RESULTS

The precipitin rings will be visible in 24 to 48 hours. Carefully hold a plate up so that the overhead room lights shine through it. You should be able to see opaque circles around each well where antigen and antibody have precipitated.

With a ruler, measure the diameter (through the centers of the wells) of the precipitin ring in millimeters. To plot the standard curve, square the diameter value and plot antigen concentration on the X-axis and the diameter squared on the Y-axis. Draw the best fit line through these points. Calculate the value of the unknown antigen concentration from this graph.



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. What do the circular precipitin rings represent?
2. Why do the ring sizes change until equilibrium is reached?
3. Predict the results if a very low concentration of antigen were loaded into a well. What would happen if not enough antibody was incorporated into the agarose?
4. Compare and contrast Radial Immunodiffusion with its close relative, the Ouchterlony plate technique.

 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) 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		Conditions to Avoid
	Stable	X	None
Incompatibility No data available			
Hazardous Decomposition or Byproducts			
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) 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			

 Material Safety Data Sheet May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.			
IDENTITY (As Used on Label and List) 10x PBS		Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.	
Section I			
Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, Zip Code) 14676 Rothgeb Drive Rockville, MD 20850		Emergency Telephone Number (301) 251-5990 Telephone Number for information (301) 251-5990 Date Prepared 09-19-2002 Signature of Preparer (optional)	
Section II - Hazardous Ingredients/Identify Information			
Hazardous Components [Specific Chemical Identity; Common Name(s)] OSHA PEL ACGIH TLV Other Limits Recommended % (Optional)			
N/A Blend			
Section III - Physical/Chemical Characteristics			
Boiling Point	100°C	Specific Gravity (H 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 colorless liquid			
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		
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? Yes Skin? Yes Ingestion? 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.			