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.

Micropipeting Basics

Storage:
Store this experiment at room temperature

EXPERIMENT OBJECTIVES:
The objectives of this experiment are to become familiar with metric units of measurement and their conversions, to learn how to accurately pipet different microliter volumes using a micropipet and to practice micropipeting solutions of different viscosities.
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</table>
Experiment Components

- Red dye
- Blue dye
- Yellow dye
- Glycerol
- Alcohol
- Buffer
- Pipeting Cards
- Microtiter plates

Requirements

- Automatic micropipets with tips
  - Variable automatic (5-50 µl) or Fixed Volume (10 µl)
- Small container for discarding used tips

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.

Storage:
Store entire experiment at room temperature.
Measuring Small Volumes with Micropipets

Over the past several decades, advances in biotechnology have influenced many changes in experimental techniques and methods, including the volume of reagents and biological samples used. Depending upon the procedure being performed, biotechnology experiments can utilize a variety of volumes of biological samples and reagents, ranging from several hundreds of liters to very small microliter (µl) volumes.

Pipeting is a critically important technique in life science experiments to ensure accurate experimental results. In typical biotechnology experiments, biologicals and reagents such as DNA, enzymes and buffers are transferred (by pipetting) into small microcentrifuge tubes which serve as reaction vessels. For these type of reactions, microliter volumes are typically used. There are 1,000 microliters in 1 milliliter of a solution. To put it in perspective, a 50 microliter sample is approximately equal in size to a single raindrop. A raindrop-sized sample is relatively large when compared to experimental samples which often are 10 to 50 microliters in volume.

VARIABLE AUTOMATIC MICROPIPET

To measure microliter volumes, a special instrument called a micropipet is used. The variable automatic micropipet is the preferred instrument for delivering accurate, reproducible volumes of sample. These instruments are manufactured to deliver samples in various ranges (e.g., 0.5-10 µl, 5-50 µl, 200-1000 µl, etc.) and usually can be adjusted in one microliter increments. Typically, these instruments have an ejector button for releasing the tip after sample delivery. Variable automatic micropipets can also be multi-channeled, designed to uniformly deliver several samples at the sample time. However, for this experiment, only one sample will be delivered at a time.
Measuring Small Volumes with Micropipets

SAMPLE DELIVERY WITH VARIABLE AUTOMATIC MICROPIPETS

1. Set the micropipet to the appropriate volume and place a clean tip on the micropipetor. Press the top button down to the first stop and hold it in place while placing the tip into the sample tube.

2. Once the tip is immersed in the sample, release the button slowly to draw sample into the tip.

3. Deliver the sample by pressing the button to the first stop -- then empty the entire contents of the tip by pressing to the second stop.

   Note: After delivering the sample, do not release the top button until the tip is out of the tube or vessel to which the sample is delivered.

4. Press the ejector button to discard the tip. Obtain a new clean tip for the next sample.
Measuring Small Volumes with Micropipets

**FIXED VOLUME MICROPIPET**

Accurate pipetting can also be achieved using fixed volume micropipets. These types of micropipets are pre-set to a specific volume. Although the volume of each individual micropipet can not be changed, fixed volume micropipets operate similarly to the variable automatic micropipets. Most fixed volume pipets do not have ejector buttons, so the tips must be removed manually.
Experiment Overview

BEFORE YOU START THE EXPERIMENT

1. Read all instructions before starting the experiment.
2. Write a hypothesis that reflects the experiment and predict experimental outcomes.

EXPERIMENT CONTENT OBJECTIVE

The objective of this experiment is to learn how to accurately pipet different microliter volumes using a micropipet and to practice micropipeting solutions of different viscosities.

BRIEF DESCRIPTION OF THE EXPERIMENT

Activity One is a “Dry lab” exercise to familiarize students with the metric system in micropipeting.

In Activity Two, various dye samples will be diluted from concentrated solutions in microcentrifuge tubes and spotted in triplicate on a Pipet Card™.

- Option A of this experiment involves pipeting of different volumes and requires a variable automatic micropipet (5-50 µl).
- Option B requires a 10 µl fixed micropipet.

Samples with various viscosities, such as a solution containing glycerol and/or alcohol, will also be used to provide the opportunity to practice micropipeting solutions with different viscosities. The concentrated dyes will be diluted in an aqueous buffer solution. The glycerol solution, which has a higher viscosity than the buffer solution, will emulate protein or DNA solutions that tend to be more viscous than aqueous buffers. By contrast, alcohol will serve as the example of a solution that is less viscous than buffer.
Activity One: Volumetric Applications of the Metric System

The metric system is used in micropipeting. The milliliter (ml) and microliter (µl) are two very useful units of measure in molecular biology. “Milli” means one-thousandth and “Micro” means one-millionth. The symbol “µ” means micro, the prefix for $1 \times 10^{-6}$ (expressed in scientific notation) or 0.000001 (expressed in decimals). One microliter is abbreviated as “µl”. The two ways that this would be expressed is: 1 µl = .000001 or 1 µl = $1 \times 10^{-6}$. There are 1,000 µl in 1 milliliter, and 1,000 ml in one liter.

1. Perform the following conversions:

<table>
<thead>
<tr>
<th>In decimals</th>
<th>In scientific notation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ml = _____ liter</td>
<td>1 ml = _____ liter</td>
</tr>
<tr>
<td>1 liter = _____ ml</td>
<td>1 liter = _____ ml</td>
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<tr>
<td>1 ml = _____ µl</td>
<td>1 ml = _____ µl</td>
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<tr>
<td>1 µl = _____ ml</td>
<td>1 µl = _____ ml</td>
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<tr>
<td>10 µl = _____ ml</td>
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<td>20 µl = _____ ml</td>
<td>20 µl = _____ ml</td>
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<tr>
<td>50 µl = _____ ml</td>
<td>50 µl = _____ ml</td>
</tr>
<tr>
<td>100 µl = _____ ml</td>
<td>100 µl = _____ ml</td>
</tr>
</tbody>
</table>

2. How many times greater is a ml than a µl? ______________

3. How many times greater is a liter than a ml? ______________

4. How many times greater is a liter than a µl? ______________

5. Write an application sentence about each of the words in the following vocabulary list:

- Micropipet
- Metric system
- Microliter
- Viscosity
- Scientific notation

6. Discuss the importance of the following in scientific experimentation:

- Using accurate and precise laboratory techniques
- Making careful observations
- Recording results in a concise and accurate manner
- Drawing valid interpretations of results
Activity Two - Option A: Micropipeting Using a Variable Micropipet

OPTION A: USING A VARIABLE MICROPIPET

In the activity which follows, you will use a variable micropipet to prepare (mix) seven different dye mixtures in the wells of a microtiter plate. Each dye mixture will be prepared in triplicate. You will then pipet 10 µl from each well of the microtiter plate onto the Pipet Card.

1. Place the microtiter plate as shown in the figure below, and carefully mark the plate with your initials or lab group number.

2. Using a permanent marker, label the rows 1 – 7 down the side of the plate.

3. Refer to Table A below to prepare seven dye mixtures, with each dye mixture prepared in triplicate wells of the microtiter plate.

4. After preparing the seven dye mixtures, pipet 10 µl in triplicate from each well of the microtiter plate onto the appropriate circles on the Pipet Card™. Pipet the dye mixture in the center of each circle in the appropriate row.

CAUTION: To avoid cross contamination and false results, always remember to use a fresh pipet tip for each dye mixture.

<table>
<thead>
<tr>
<th>Option A</th>
<th>Pipeting Chart A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Using Variable (5-50 µl) Automatic Micropipets</td>
<td>Wells</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
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<td>6</td>
<td>6</td>
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<tr>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>
Activity Two - Option B: Micropipeting Using a Fixed Volume Micropipet

OPTION B: USING A FIXED VOLUME MICROPIPET

In the following activity, you will use one or more fixed volume micropipet to prepare (mix) seven different dye mixtures in the wells of a microtiter plate. Each dye mixture will be prepared in triplicate. You will then pipet 10 µl from each well of the microtiter plate onto the Pipet Card™.

1. Place the microtiter plate as shown in the figure below, and carefully mark the plate with your initials or lab group number.

2. Using a permanent marker, label the rows 1 – 7 down the side of the plate.

3. Refer to Table B below to prepare seven dye mixtures, with each dye mixture prepared in triplicate wells of the microtiter plate.

4. After preparing the seven dye mixtures, pipet 10 µl in triplicate from each well of the microtiter plate onto the appropriate circles on the Pipet Card™. Pipet the dye mixture in the center of each circle in the appropriate row.

<table>
<thead>
<tr>
<th>Option B Using Fixed Volume (10 µl) Micropipets</th>
<th>Pipetting Chart B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wells</td>
<td>Red (µl)</td>
</tr>
<tr>
<td>-------</td>
<td>----------</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
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<tr>
<td>3</td>
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<td>10</td>
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<td>6</td>
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<tr>
<td>7</td>
<td>10</td>
</tr>
</tbody>
</table>
Critical Thinking and Hypothesis Development

Record the following in your Laboratory Notebook or as instructed by your teacher.

1. What is the variable in this experiment?
2. What is the control in this experiment?
3. What could one change in the experiment if this experiment was repeated?
4. Write a hypothesis that would reflect a change.

Study Questions

Record the answers to the following Study Questions in your Laboratory Notebook or as instructed by your teacher.

1. Describe a good technique for withdrawing samples using a variable automatic micropipet or fixed volume micropipet.
2. How does the pipetting exercise help you understand the importance of accurate pipetting using microliter volumes?
3. Why did you practice pipetting samples with various viscosities?
Notes to the Instructor

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students. These guidelines include Suggestions for Lesson Plan Content which can be adapted to fit your specific set of circumstances.

APPROXIMATE TIME REQUIREMENTS

Activity One: Approximately 30 minutes are required for students complete the dry lab exercise.

Activity Two: Approximately 40 minutes are required for students complete the pipeting exercise.

RESOURCES AND TECHNICAL SERVICE

The EDVOTEK web site provides a variety of resources which are continuously being updated and added. Several suggestions and reminders for various aspects of biotechnology education are available.

Online Ordering now available

Visit our web site for information about EDVOTEK’s complete line of experiments for biotechnology and biology education.

EDVO-Kit # S-44
Micropipeting Basics

Technical Service Department

Mon - Fri
9:00 am to 6:00 pm ET

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

If you do not find the answers to your questions in this section or at the EDVOTEK web site, 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).

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
Optional Activity - Practice Gel Loading for Agarose Gel Electrophoresis

Electrophoresis trays and well former templates (combs) required. Samples and reagents not included.

PRACTICE GEL LOADING

Accurate sample delivery technique ensures the best possible gel results. Pipeting mistakes can cause the sample to become diluted with buffer, or cause damage to the wells with the pipet tip while loading the gel. The agarose gel is sometimes called a "submarine gel" because it is submerged under buffer for sample loading and electrophoretic separation. In this activity, students can practice gel loading in a gel placed under water to simulate gel loading in the electrophoresis apparatus under buffer.

1. Obtain a tube of practice gel loading solution and a gel section with wells submerged under water in a small tray or petri plate.
2. Practice delivering the practice gel loading solution to the sample wells. Take care not to damage or puncture the wells with the pipet tip.
   - If you are using a variable automatic micropipet, load the sample well with 35-38 microliters of sample.
   - If using fixed volume pipets for sample delivery, load each sample well with 40 microliters of sample.
3. If you need additional practice, squirt water into the wells with a transfer pipet to remove the practice gel loading solution and practice loading samples again.

Optional Activity - Practice Pipeting Accurate Amounts of Sample

Samples and reagents not included.

1. Place a strip of laboratory parafilm paper on the lab bench
2. Set the pipet to 1 µl and pipet the sample onto the parafilm paper.
3. Repeat step 2.
4. Compare the sizes of the two drops. They should be the same size. If not, repeat steps 2 and 3 again.
5. Set the pipet to 5 µl and pipet two times. Compare. Repeat if the drops are not the same size.
6. Repeat in duplicate for the following volumes: 10 µl, 20 µl, 30 µl, 50 µl, 100 µl, 200 µl, 400 µl, 500 µl, 1000 µl.
7. Compare the sizes of the drops as you go from lowest to the highest volume. What relationship do you observe if you have pipeted accurately?
Connections to National Content Standards

1. Students will understand how to use equipment and learn proper techniques for conducting an experiment.

2. Students will understand the relationship between the metric system and English system in the measurement of small volumes (microliters).

3. Students will develop an understanding through inquiry. They will:
   - Develop a logical hypothesis
   - Make careful observations.
   - Interpret results correctly.
   - Formulate explanations from results.

Connections to National Skill Standards

Students will be able to:

1. Use micropipets for metric measurements of small liquid volumes of various viscosities.

2. Make careful observations and record results.

3. Compare and evaluate results.

4. Make metric conversions in decimals and scientific notation.
Pre-Lab Preparations

For the Pipeting Exercise (Activity Two), dispense reagents for each student/group. The quantities listed below are sufficient for performing either Option A (Variable automatic micropipets) or Option B (Fixed volume micropipets). Each student/group should receive the following:

- Red dye 150 µl
- Blue dye 150 µl
- Yellow dye 150 µl
- Glycerol 150 µl
- Alcohol 100 µl
- Buffer 0.8 ml

Pipeting Card
Microtiter plate section/strip for mixing dyes
Micropipet and tips
Small container for discarding used tips
## Experiment Results

### ANSWERS TO ACTIVITY ONE

1. Perform the following conversions:

<table>
<thead>
<tr>
<th>In decimals</th>
<th>In scientific notation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ml = ______.001 liter</td>
<td>1 ml = $1 \times 10^{-3}$ liter</td>
</tr>
<tr>
<td>1 liter = ______1,000 ml</td>
<td>1 liter = $1 \times 10^3$ ml</td>
</tr>
<tr>
<td>1 ml = ______1,000 µl</td>
<td>1 ml = $1 \times 10^3$ µl</td>
</tr>
<tr>
<td>1 µl = ______.001 ml</td>
<td>1 µl = $1 \times 10^{-3}$ ml</td>
</tr>
<tr>
<td>10 µl = ______.01 ml</td>
<td>10 µl = $1 \times 10^{-2}$ ml</td>
</tr>
<tr>
<td>20 µl = ______.02 ml</td>
<td>20 µl = $2 \times 10^{-2}$ ml</td>
</tr>
<tr>
<td>50 µl = ______.05 ml</td>
<td>50 µl = $5 \times 10^{-2}$ ml</td>
</tr>
<tr>
<td>100 µl = ______.1 ml</td>
<td>100 µl = $1 \times 10^{-1}$ ml</td>
</tr>
</tbody>
</table>

2. How many times greater is a ml than a µl? ______1,000______

3. How many times greater is a liter than a ml? ______1,000______

4. How many times greater is a liter than a µl? ______1,000,000______
Study Questions and Answers

1. Describe a good technique for withdrawing samples using a variable automatic micropipet or fixed volume micropipet.

   Hold the sample tube at eye level, depress the top button to the first stop and hold. Put the end of the tip into the fluid and gradually depress the top button to draw up the sample. Be sure to keep the tip below the sample while pipeting. Check to see that there are no air bubbles at the tip or in the sample.

2. How does the pipeting exercise help you understand the importance of accurate pipeting using microliter volumes?

   By comparing the amounts of sample delivered when pipeting various volumes ranging from 5 to 50 µl, one can see only a small difference between each succeeding increase in volume. Therefore, in order to have reliable experimental results, it is important to pipet each sample accurately.

3. Why did you practice pipeting samples with various viscosities?

   It is helpful to feel the small differences in pipeting small samples of higher viscosity, such as samples containing glycerol, compared to less viscous solutions containing alcohol. In order to have reliable experimental results, it is important to pipet samples accurately.
## Material Safety Data Sheet

**IDENTITY**

**Ethyl Alcohol**

**Section I - Identification**

- **Manufacturer's Name:** EDVOTEK
- **Address:** 1121 5th Street, NW.
- **City, State, Zip Code:** Washington, DC 20001
- **Telephone Number for Information:** 301-251-5990
- **Emergency Telephone Number:** (301) 251-5990
- **Date Prepared:** 08-12-11
- **Preparer:** [Signature]

**Section II - Hazardous Ingredients/Identity Information**

- **Hazardous Components (Specific Chemical Identity, Common Name(s), CAS, OSHA PEL)**
  - CAS # 64-17-5

- **Other Limits Recommended (Optional):**

**Section III - Physical/Chemical Characteristics**

- **Boiling Point:** 78°C
- **Specific Gravity (d10/4°C):** 0.794
- **Vapor Pressure (mm Hg):** N.D.
- **Vapor Density (Air = 1):** N.D.
- **Evaporation Rate (Styrene Acetate):** N.D.

**Solubility in Water**

- **Appearance and Odor:** Clear, colorless liquid

**Section IV - Fire and Explosion Hazard Data**

- **Flash Point (Method Used):** 62°F/16°C
- **Flammable Limits:** LEL,UEL
- **Extinguishing Media:** Carbon dioxide, dry chemical powder or appropriate foam
- **Special Fire Fighting Procedures:** Wear SCBA and protective clothing to prevent contact with skin and eyes
- **Unusual Fire and Explosion Hazards:** Use water spray to cool exposed containers

**Section V - Reactivity Data**

- **Stability:** Unstable
- **Conditions to Avoid:** Stable
- **Incompatibility (Materials to avoid):** Strong oxidizing agents, strong bases, protect from moisture
- **Hazardous Decomposition or Byproducts:** CO, CO2
- **Hazardous Polymerization:** May Occur
- **Will Not Occur:** X

**Section VI - Health Hazard Data**

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

**Health Hazards (Acute and Chronic):**

- **Vapor or Mist is irritating to the eyes, mucous membrane and upper respiratory tract causes skin irritation, can cause CNS depression, nausea, headache and vomiting, narcotic effect.

**Carcinogenicity:**

- **NTP:** No data
- **IARC Monographs:** No data
- **OSHA Regulation:**

**Signs and Symptoms of Exposure:**

- **CNS depression, irritation to eyes, mucous, damage to heart, narcotic effect, nausea, headache and vomiting, diarrhea,

**Medical Conditions Generally Aggravated by Exposure:**

**Emergency and First Aid Procedures:**

- **If swallowed wash out thoroughly with water provided person is conscious.

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

- **Storage:** Store in a cool, dry area, away from heat, water, and other incompatible materials.
- **Handling:** Use Safety Glasses, Chemical resistant gloves, Eye Protection, Safety goggles.
- **Other Precautions:** Use only in a well-ventilated area.

**Section VIII - Control Measures**

- **Respiratory Protection (Space Type):** NIOSH/MSHA approved respirator
- **Ventilation:** Local Exhaust
- **Protective Gloves:** Chemical resistant gloves
- **Other Protective Clothing or Equipment:**

- **Work/Hygienic Practices**

**Section IX - Other Information**

- **Material Safety Data Sheet**
  - **Manufacturer's Name:** EDVOTEK
  - **Address:** 1121 5th Street, NW.
  - **City, State, Zip Code:** Washington, DC 20001
  - **Telephone Number:** 301-251-5990

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

- **Date Prepared:** 08-12-11
- **Preparer:** [Signature]

---

**Material Safety Data Sheet**

**IDENTITY**

**50% Glycerol**

**Section I - Identification**

- **Manufacturer's Name:** EDVOTEK, Inc.
- **Address:** 1121 5th Street, NW.
- **City, State, Zip Code:** Washington, DC 20001
- **Telephone Number for Information:** 301-251-5990

**Section II - Hazardous Ingredients/Identity Information**

- **Hazardous Components (Specific Chemical Identity, Common Name(s), CAS, OSHA PEL)**
  - CAS # 62:31-5

- **Other Limits Recommended (Optional):**

---

**Section III - Physical/Chemical Characteristics**

- **Boiling Point:** 182°C
- **Specific Gravity (d10/4°C):** 1.28
- **Vapor Pressure (mm Hg):** 20°C < 1.15
- **Making Point:** 20°C
- **Vapor Density (Air = 1):** 3.3
- **Evaporation Rate (Styrene Acetate):** N/A
- **Solubility in Water:**

**Appearance and Odor:** Viscous colorless liquid

**Section IV - Fire and Explosion Hazard Data**

- **Flash Point (Method Used):** 62°F/16°C
- **Flammable Limits:** LEL
- **Extinguishing Media:** Water spray, CO2, Dry chemical powder or appropriate foam
- **Special Fire Fighting Procedures:** Wear SCBA and protective clothing to prevent contact with skin and eyes

**Section V - Reactivity Data**

- **Stability:** Stable
- **Conditions to Avoid:**

**Incompatibility:** Strong oxidizing agents, strong bases, protect from moisture

**Hazardous Decomposition or Byproducts:** CO, CO2

**Hazardous Polymerization:** May Occur

**Will Not Occur:** X

**Section VI - Health Hazard Data**

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

**Health Hazards (Acute and Chronic):** May cause skin irritation prolonged exposure can cause headache, nausea and vomiting

**Carcinogenicity:**

- **NTP:** Yes
- **IARC Monographs:** Yes
- **OSHA Regulation:**

**Signs and Symptoms of Exposure:**

- **CNS depression, irritation to eyes, mucous, damage to heart, narcotic effect, nausea, headache and vomiting, diarrhea,

**Medical Conditions Generally Aggravated by Exposure:**

**Emergency and First Aid Procedures:**

- **Flush with copious amounts of H2O for at least 15 minutes. Remove to fresh air & remove contaminated clothing.

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

- **Steps to be Taken in Case Material is Released or Spilled:**
  - Absorb on sand or vermiculite and place in closed container for disposal.
  - Ventilate area and wash spill site

- **Waste Disposal Method:**
  - Dispose of the material with a combustible solvent and burn in a chemical incinerator equipped with adequate controls for fine particles.
  - Precautions to be taken in Handling and Storage:
    - Don’t breathe vapor, avoid contact with eyes, skin and clothing wash thoroughly after handling.
  - Keep safety data sheets

**Other Precautions:**

**Section VIII - Control Measures**

- **Respiratory Protection (Space Type):**
  - **Ventilation:** X
  - **Local Exhaust:** Special
  - **Mechanical (General):** Mechanical exhaust
  - **Other:**

- **Protective Gloves:** Chemical resistant
- **Eye Protection:** Safety goggles

- **Other Protective Clothing or Equipment:**

- **Work/Hygienic Practices**

- **Wash thoroughly after handling**