



Sci-On[®] Biology

S-44

EDVO-Kit #

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.

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

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

Storage:
Store entire
experiment at
room
temperature.

Requirements

- Automatic micropipets with tips
Variable automatic (5-50 μ l) or Fixed Volume (10 μ l)
- 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.

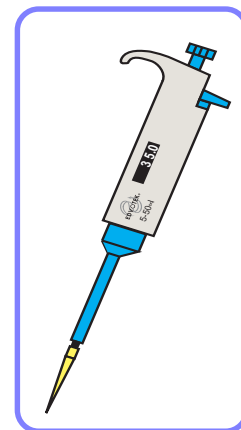
Measuring Small Volumes with Micropipets

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 pipeting) 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

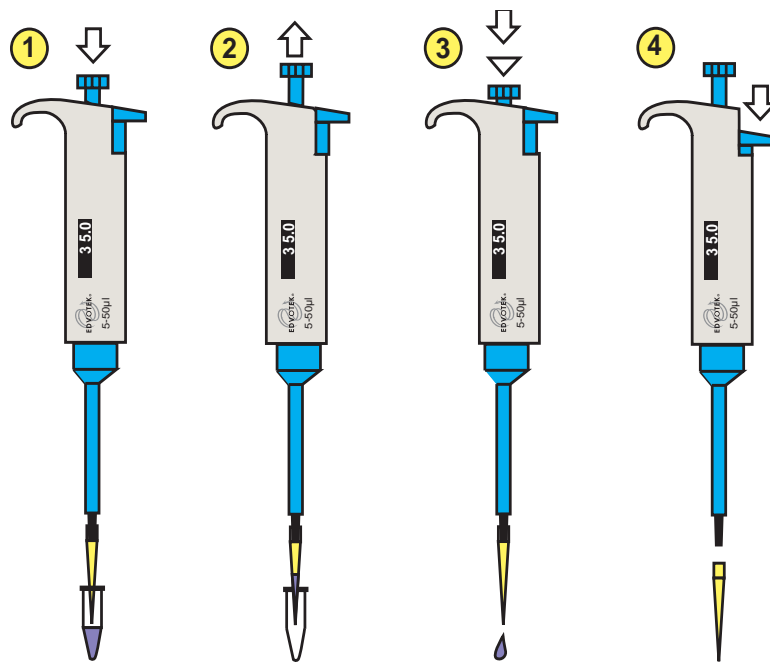
To measure microliter volumes, a special instrument called a micropipet is used. The variable automatic micropipet is the preferred instrument for delivering accurate, reproducible volumes of sample. These instruments are manufactured to deliver samples in various ranges (e.g., 0.5-10 μl , 5-50 μl , 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 same time. However, for this experiment, only one sample will be delivered at a time.



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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 micropipet. 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.

Background Information

Measuring Small Volumes with Micropipets

Background Information

FIXED VOLUME MICROPIPET

Accurate pipeting 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.



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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**Experiment Procedures**

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 1×10^{-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 $1 \mu\text{l} = .000001$ or $1 \mu\text{l} = 1 \times 10^{-6}$. There are 1,000 μl in 1 milliliter, and 1,000 ml in one liter.

1. Perform the following conversions:

In decimals

1 ml = _____ liter
 1 liter = _____ ml
 1 ml = _____ μl
 1 μl = _____ ml
 10 μl = _____ ml
 20 μl = _____ ml
 50 μl = _____ ml
 100 μl = _____ ml

In scientific notation

1 ml = _____ liter
 1 liter = _____ ml
 1 ml = _____ μl
 1 μl = _____ ml
 10 μl = _____ ml
 20 μl = _____ ml
 50 μl = _____ ml
 100 μl = _____ ml

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
Viscosity

Metric system
Scientific notation

Microliter

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



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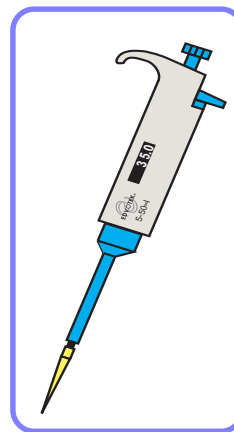
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Activity Two - Option A: Micropipeting Using a Variable Micropipet

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. You will then dispense 5 microliters (5 μ l) of each diluted dye mixture on a Pipet card in triplicate.

1. Label a microtiter plate with your initials and number seven of the wells 1 to 7. Refer to Table A to prepare 7 dye solutions.



Option A Using Variable (5-50 μ l) Automatic Micropipets		Pipeting Chart A					
Tube	Red (μ l)	Blue (μ l)	Yellow (μ l)	Glycerol (μ l)	Alcohol (μ l)	Buffer (μ l)	Total Volume (μ l)
1	5	-	-	-	-	40	45
2	-	-	10	10	10	15	45
3	-	10	10	-	-	25	45
4	5	15	-	-	-	25	45
5	13	-	-	-	10	22	45
6	-	10	-	10	-	25	45
7	6	-	6	13	-	20	45

Dye Mixture Preparation Example:

- To the well labeled # 1, add 5 μ l of Red dye and 40 μ l of buffer for a total volume of 45 μ l.
2. After preparing the dye mixtures, pipet 10 μ l of each mixture in the corresponding numbered row of circles on the Pipet Card™. Pipet the dye mixture in the center of each of the three circles in the appropriate row.

Activity Two - Option B: Micropipeting Using a Fixed Volume Micropipet**OPTION B: USING A FIXED VOLUME MICROPIPET**

In the activity which follows, you will use one or more fixed volume micropipet to prepare (mix) seven different dilute dye mixtures in the wells of a microtiter plate. You will then dispense 10 microliters (10 μ l) of each diluted dye mixture on a Pipet card in triplicate.



1. Label a microtiter plate with your initials and number seven of the wells 1 to 7. Refer to Table A to prepare 7 dye solutions.

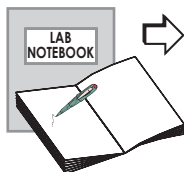
Option B Using Fixed Volume (10 μ l) Micropipets			Pipeting Chart B				
Tube	Red (μ l)	Blue (μ l)	Yellow (μ l)	Glycerol (μ l)	Alcohol (μ l)	Buffer (μ l)	Total Volume (μ l)
1	10	-	-	-	-	40	50
2	10	20	-	-	-	20	50
3	-	10	10	-	10	20	50
4	-	10	-	-	10	30	50
5	10	-	-	10	-	30	50
6	-	-	20	10	-	20	50
7	10	-	10	20	-	10	50

Dye Mixture Preparation Example:

- To the well labeled # 1, add 10 μ l of Red dye and 40 μ l of buffer for a total volume of 50 μ l.
2. After preparing the dye mixtures, pipet 10 μ l of each dye mixture in the corresponding numbered row of circles on the pipet card. Pipet the dye mixture in the center of each of the three circles.



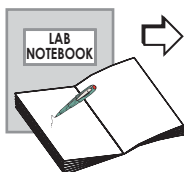
Critical Thinking and Hypothesis Development



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 pipeting exercise help you understand the importance of accurate pipeting using microliter volumes?
3. Why did you practice pipeting samples with various viscosities?

 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) Ethyl Alcohol	
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	Emergency Telephone Number 301-251-5990
Address (Number, Street, City, State, and ZIP Code) 14676 Rothgeb Drive Rockville, MD 20850	Telephone Number for information 301-251-5990
	Date Prepared 06/01/04
	Signature of Preparer (optional)
Section II - Hazardous Ingredients/Identify Information	
Hazardous Components (Specific Chemical Identity; Common Name(s)) CAS # 64-17-5	OSHA PEL ACGIH TLV Other Limits Recommended % (Optional)
Section III - Physical/Chemical Characteristics N.D. = No data	
Boiling Point 78 C	Specific Gravity (H ₂ O = 1) .794
Vapor Pressure (mm Hg.) N.D.	Melting Point N.D.
Vapor Density (AIR = 1) N.D.	Evaporation Rate (Butyl Acetate = 1) 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 clothing with skin and eyes	
Unusual Fire and Explosion Hazards Vapor may travel considerable distance to source of ignition and flash back	

Section V - Reactivity Data			
Stability Unstable Stable	Conditions to Avoid X		
Incompatibility (Materials to avoid) Oxidizing agents, peroxides, acids, acid chlorides, acid anhydrides, alkali metal			
Hazardous Decomposition or Byproducts Toxic fumes of carbon monoxide, carbon dioxide			
Hazardous Polymerization Will Not Occur	May Occur X		
Section VI - Health Hazard Data			
Route(s) of Entry: 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 depressing, nausea, headache and vomiting, narcotic effect.			
Carcinogenicity: NTP? IARC Monographs? OSHA Regulation? No data			
Signs and Symptoms of Exposure CNS depression, irritating to eyes, mucous, damage to heart, narcotic effect, nausea, headache and vomiting, diarrhea.			
Medical Conditions Generally Aggravated by Exposure Chronic Pulmonary Edema, Ataxia, dyspnea Damage to the heart Target organ(s) Nerves, liver			
Emergency and First Aid Procedures If inhaled remove to fresh air --In case of contact with eyes flush with copious amounts of water If swallowed wash out mouth with water provided person is conscious.			
Section VII - Precautions for Safe Handling and Use			
Steps to be Taken in case Material is Released or Spilled Evacuate area -- shut off all sources of ignition. Wear SCBA, rubber boots and heavy rubber gloves cover with dry-lime, sand, or soda ASH place in covered containers.			
Waste Disposal Method Burn in a chemical incinerator equipped with an afterburner and scrubber but exert extra care in igniting as this material is highly flammable.			
Precautions to be taken in Handling and Storing Ventilate area and wash spill site after material pickup is complete			
Other Precautions Use only in a chemical fume hood, do not breathe vapors, safety shower and eye bath			
Section VIII - Control Measures			
Respiratory Protection (Specify Type) NIOSH/MSHA approved respirator			
Ventilation X	Local Exhaust Mechanical (General)	Chemical fume hood yes	Special Other
Protective Gloves Chemical resistant	Eye Protection Safety goggles		
Other Protective Clothing or Equipment			
Work/Hygienic Practices			

 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) 50% Glycerol	
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.	Emergency Telephone Number (301) 251-5990
Address (Number, Street, City, State, Zip Code) 14676 Rothgeb Drive Rockville, MD 20850	Telephone Number for information (301) 251-5990
	Date Prepared 06/01/04
	Signature of Preparer (optional)
Section II - Hazardous Ingredients/Identify Information	
Hazardous Components (Specific Chemical Identity; Common Name(s)) CAS # 56-81-5	OSHA PEL ACGIH TLV Other Limits Recommended % (Optional)
Section III - Physical/Chemical Characteristics	
Boiling Point 182°C	Specific Gravity (H ₂ O = 1) 1.26
Vapor Pressure (mm Hg.) 20°C <1MM	Melting Point 20°C
Vapor Density (AIR = 1) 3.1	Evaporation Rate (Butyl Acetate = 1) N/A
Solubility in Water	
Appearance and Odor Viscous colorless liquid	
Section IV - Physical/Chemical Characteristics	
Flash Point (Method Used)	Flammable Limits LEL UEL
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	
Unusual Fire and Explosion Hazards Emits toxic fumes under fire conditions.	

Section V - Reactivity Data			
Stability Unstable Stable	Conditions to Avoid X		
Incompatibility Strong oxidizing agents, strong bases, protect from moisture			
Hazardous Decomposition or Byproducts CO, CO2			
Hazardous Polymerization Will Not Occur	May Occur X	Conditions to Avoid	
Section VI - Health Hazard Data			
Route(s) of Entry: 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? IARC Monographs? OSHA Regulation?			
Signs and Symptoms of Exposure			
Medical Conditions Generally Aggravated by Exposure			
Emergency 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 for Spilled Absorb on sand or vermiculite and place in closed container for disposal. Ventilate area and wash spill site			
Waste Disposal Method Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with afterburner			
Precautions to be Taken in Handling and Storing Don't breathe vapor, avoid contact with eyes, skin and clothing wash thoroughly after handling. Keep tightly closed.			
Other Precautions			
Section VIII - Control Measures			
Respiratory Protection (Specify Type)			
Ventilation X	Local Exhaust Mechanical (General)	Chemical exhaust Mechanical exhaust	Special Other
Protective Gloves Chemical resistant	Eye Protection Safety goggles		
Other Protective Clothing or Equipment			
Work/Hygienic Practices Wash thoroughly after handling			