EDVOTEK®

THE BIOTECHNOLOGY EDUCATION COMPANY®

Edvo-Kit #

S-44

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Micropipetting Basics

Experiment Objective:

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

See page 3 for storage instructions.

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Table of Contents

Safety Data Sheets can be found on our website: **www.edvotek.com/Safety-Data-Sheets**

Experiment Components

Contents	Check (✓)
• Red dye	❑
• Blue dye	❑
• Yellow dye	❑
• Glycerol	❑
• Alcohol	❑
• Buffer	❑
• Pipeting Cards	❑
• Microtiter plates	❑
• Microcentrifuge tubes	❑

Experiment #S-44 is designed for 10 groups.

Storage:
Experiment is stored at room temperature

Requirements

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

All experiment 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.

Background Information

ACCURACY AND PRECISION IN BIOTECHNOLOGY

Accuracy describes how close a measurement is to the true value of a given quantity. Precision describes the reproducibility of the measurement. Accordingly, measurements can be categorized as follows (summarized in Figure 1):

- Neither accurate nor precise – measurements do not match accepted value, nor are they reproducible.
- Accurate but not precise – the average of the measurements matches the accepted value, but their values vary greatly.
- Precise but not accurate – the value of the measurements match one another, but the average deviates from accepted value.
- Both accurate and precise – the measurements agree with one another and with the accepted value.

Accuracy and precision of measurements ensure that your experiments are both successful and reproducible. 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. Advances in biotechnology have resulted in the development and equipment that make these measurements both accurate and precise.

Pipeting is a critically important technique in life science experiments to ensure accurate experimental results. For example, small differences in primer or template concentration can make a big difference in the results of PCR experiments. To address this concern, scientists use carefully calibrated micropipets to measure the volume of each component.

In most biotechnology experiments, reagents such as DNA, enzymes, and buffers are transferred by pipeting into small microcentrifuge tubes that serve as reaction vessels. For these types 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 that often are 10 to 50 microliters in volume.

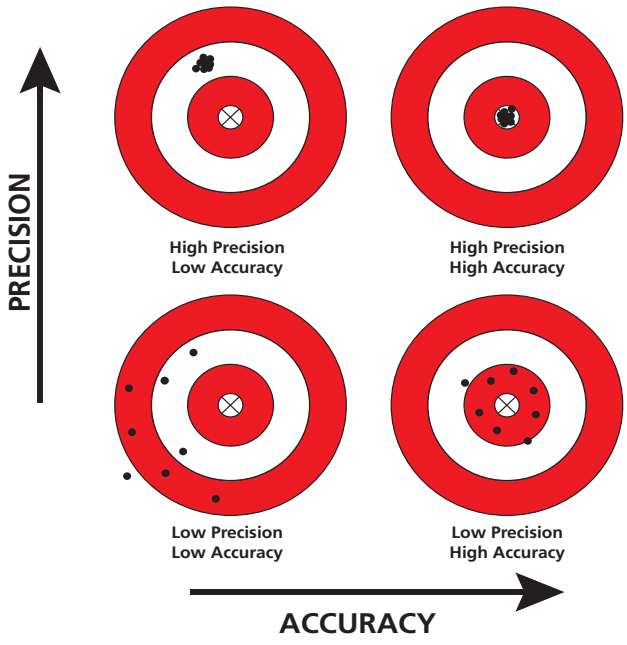


Figure 1: Accuracy and Precision

Experiment Overview

EXPERIMENT OBJECTIVE:

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

LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

Wear gloves and safety goggles

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

PRE-LAB DISCUSSION:

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

Micropipet	Metric system	Microliter
Viscosity	Scientific notation	

2. 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 One: Volumetric Applications of the Metric System

The metric system is used in micropipetting. 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 x 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 x 10^{-6}. There are 1,000 µl in 1 milliliter, and 1,000 ml in one liter.

1. Perform the following conversions:

		In decimals			In scientific notation
1 ml	=	_____ liter	1 ml	=	_____ liter
1 liter	=	_____ ml	1 liter	=	_____ ml
1 ml	=	_____ µl	1 ml	=	_____ µl
1 µl	=	_____ ml	1 µl	=	_____ ml
10 µl	=	_____ ml	10 µl	=	_____ ml
20 µl	=	_____ ml	20 µl	=	_____ ml
50 µl	=	_____ ml	50 µl	=	_____ ml
100 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? _____

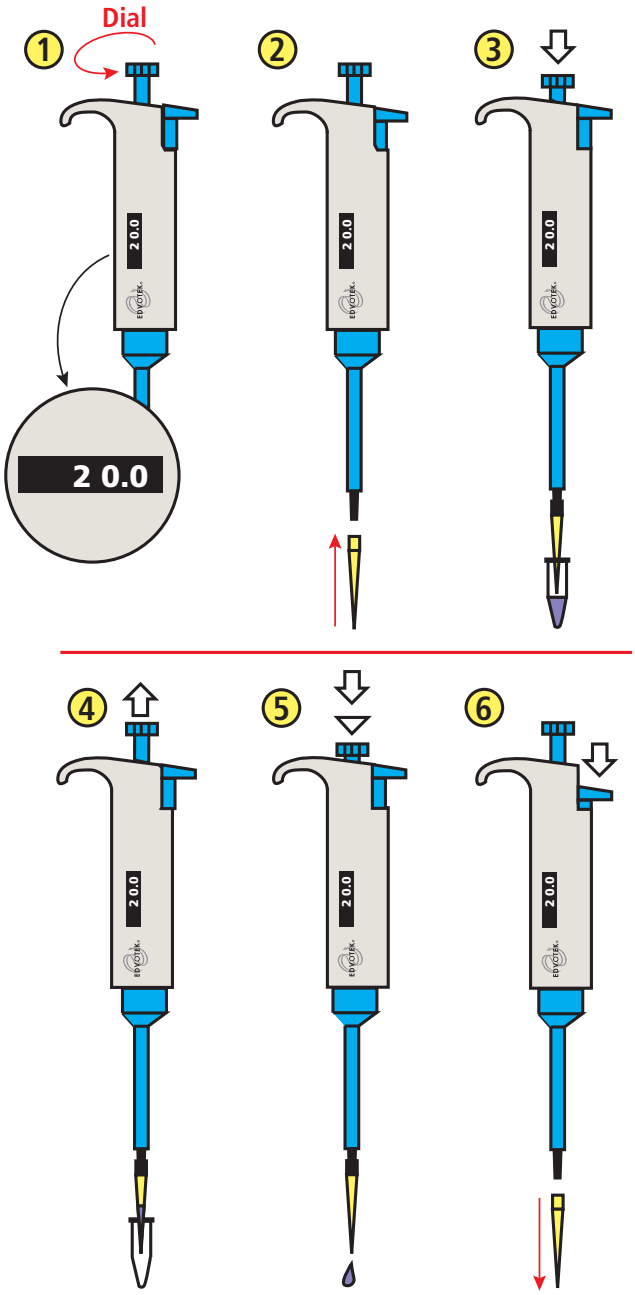
Metric System Prefixes

Prefix	Abbreviation	Meaning
Giga-	G	10^{9}
Mega-	M	10^{6}
Kilo-	k	10^{3}
Deci-	d	10^{-1}
Centi-	c	10^{-2}
Milli-	m	10^{-3}
Micro-	µ	10^{-6}
Nano-	n	10^{-9}
Pico-	p	10^{-12}
Femto-	f	10^{-15}

Activity Two - Option A: Micropipetting Using a Variable 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.

1. **SET** the micropipet to the appropriate volume by adjusting the dial.
2. **PLACE** a clean tip on the micropipet.
3. **PRESS** the plunger down to the first stop. **HOLD** the plunger down while placing the tip beneath the surface of the liquid.
4. Slowly **RELEASE** the plunger to draw sample into the pipette tip.
5. **DELIVER** the sample by slowly pressing the plunger to the first stop. Depress the plunger to the second stop to expel any remaining sample. **DO NOT RELEASE** the plunger until the tip is out of the sample container.
6. **DISCARD** the tip by pressing the ejector button. Use a new tip for the next sample.



Activity Two - Option A: Micropipetting Using a Variable Micropipet, continued

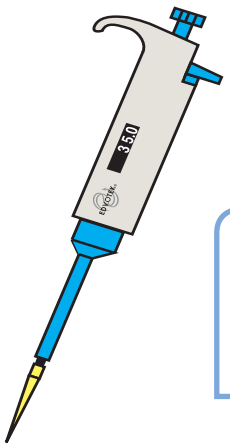
In the activity which follows, you will use a variable micropipet to prepare (mix) seven different dye mixtures in the wells of a microtiter plate. Each dye mixture will be prepared in triplicate. You will then pipet 10 µl from each well of the microtiter plate onto the Pipet Card.

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

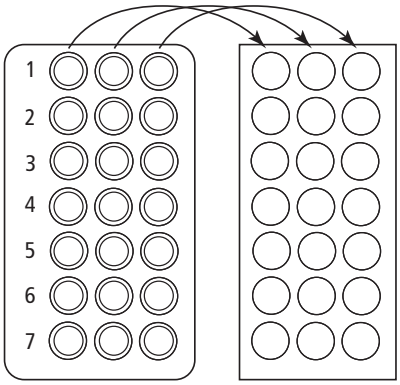
2. Using a permanent marker, label the rows 1 – 7 down the side of the plate.

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.



Pipeting Chart A

Option A
Using variable (5–50µl) Automatic Micropipets

Wells	Red (µl)	Blue (µl)	Yellow (µl)	Glycerol (µl)	Alcohol (µl)	Buffer (µl)	Total Volume (µl)
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

Activity Two - Option B: Using a Fixed Volume Micropipet

Accurate pipeting can 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.

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™.

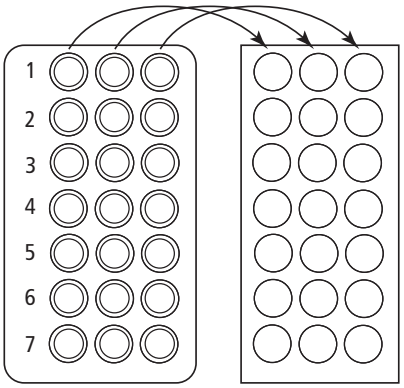
1. Place the microtiter plate as shown in the figure below, and carefully mark the plate with your initials or lab group number.

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.

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



Option B Using Fixed Volume (10µl) Micropipets — **Pipeting Chart B**

Wells	Red (µl)	Blue (µl)	Yellow (µl)	Glycerol (µl)	Alcohol (µl)	Buffer (µl)	Total Volume (µl)
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

Study Questions

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?

Instructor's Guide

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 800 µl
- Pipeting Card
- Microtiter plate section/strip for mixing dyes
- Micropipet and tips
  For Option A, a 5 - 50 µl variable volume micropipet (Cat. #590) is required.
  For Option B, a 10 µl fixed volume micropipet (Cat. #586) is required. If available, students can also use 20, 30, or 40 µl micropipets (Cat. #586-1, #587-1, and #588, respectively).
- Small container for discarding used tips

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 pipetted accurately?

Optional Activity - Practice Gel Loading for Agarose Gel Electrophoresis

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

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.

If you do not wish to pour agarose gels, Edvotek® DuraGels™ (Cat. S-43) can be used as a substitute. Edvotek® DuraGels™ are reusable polymer gel models that allows students to gain experience with gel loading before performing agarose gel electrophoresis. The use of DuraGels™ eliminates the preparation time, expense, and waste of pouring agarose practice gels

Experiment Results

RESULTS FOR ACTIVITY ONE

1. Perform the following conversions:

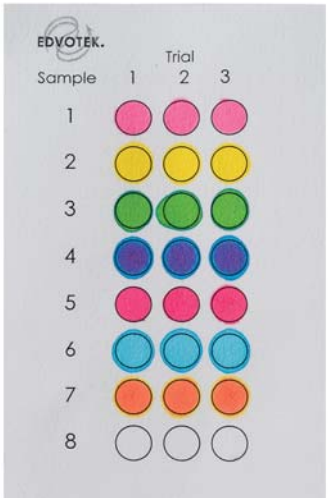
		In decimals			In scientific notation	
1 ml	=	**.001**	liter	1 ml	=	**1×10^{-3}** liter
1 liter	=	**1,000**	ml	1 liter	=	**1×10^{3}** ml
1 ml	=	**1,000**	µl	1 ml	=	**1×10^{3}** µl
1 µl	=	**.001**	ml	1 µl	=	**1×10^{-3}** ml
10 µl	=	**.01**	ml	10 µl	=	**1×10^{-2}** ml
20 µl	=	**.02**	ml	20 µl	=	**2×10^{-2}** ml
50 µl	=	**.05**	ml	50 µl	=	**5×10^{-5}** ml
100 µl	=	**.1**	ml	100 µl	=	**1×10^{-1}** ml

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**

RESULTS FOR ACTIVITY TWO



Sample	Color
1	Light Pink
2	Yellow
3	Green
4	Dark Blue
5	Dark Pink
6	Aqua
7	Orange

Please refer to the kit insert for the Answers to Study Questions