EDVOTEK®

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.

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 µl) or Fixed Volume (10 µl)
- Small container for discarding used tips

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Measuring Small Volumes with Micropipets

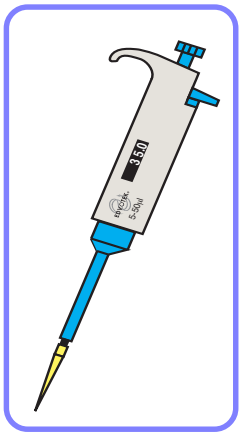
Background Information

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.

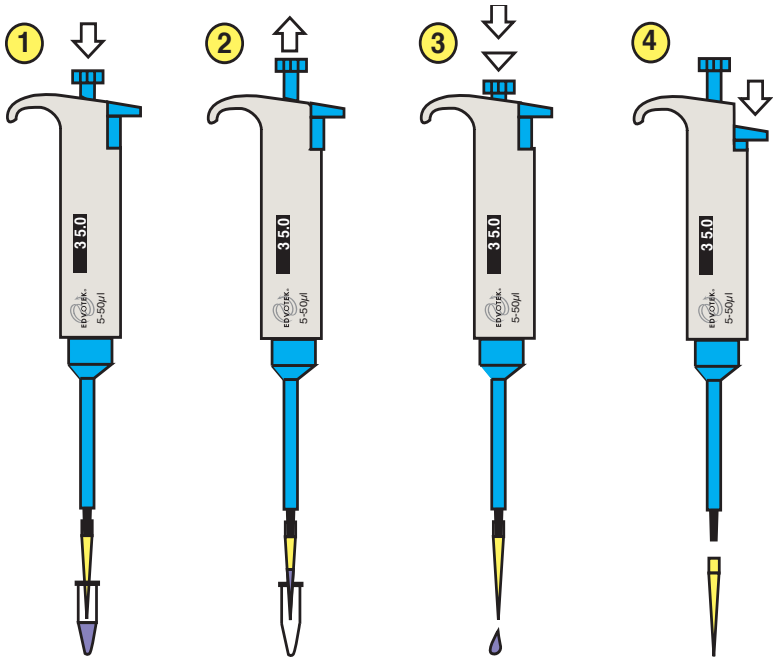
Pipeting is a critically important technique in life science experiments to ensure accurate experimental results. In typical biotechnology experiments, biologicals and reagents such as DNA, enzymes and buffers are transferred (by pipeting) into small microcentrifuge tubes which serve as reaction vessels. 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 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.

Background Information

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 µl = .000001 or 1 µl = 1×10^{-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? _____

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

A Taste of Chemistry Summer Workshop
Monell @ Springside School

Basic Microchemical Techniques: Micropipetting Tips

Micropipettors are precisely calibrated instruments that combine the functions of a pipette and a pump. Disposable plastic tips make micropipettors reusable. To measure small volumes accurately, you will use the **.5-10 ul and 20-200 ul adjustable micropipettors**. **The .5-10 should NEVER be used for volumes greater than 10 µl nor the 20-200 for volumes greater than 200 µl because this will damage the pipettes.**

The pushbutton at the top of the micropipettor has two working positions in addition to the fully upright position. **The first position is used for picking up liquid, and the second position is used to eject or deliver liquid from the tip.**

1. To reach the first position, push the button down using relatively light pressure with your index finger until you meet some resistance.

2. Reaching the second position requires significantly more pressure from your finger, so push the button down with more force to reach this position.

3. Put tips firmly onto the end of the pipettor by gently pushing the barrel of the pipettor into a tip in the rack. Using the pipettor to pick up tips is preferable to picking up a tip with your hand to avoid contaminating the tip with microbes on your hand. (If needed, you can then use your fingers to push the tip gently but firmly onto the barrel of the pipette without touching the end of the tip).

4. Change the volume setting of the scale on the front of the .5-10 to read 7.5 ul

5. Depress the button at the top of the pipettor until you reach the first stop position and feel considerable resistance. **Be sure not to push the button down to the second stop position when picking up liquid or your measurement will be grossly incorrect.**

6. Keep the button at this position and insert the tip into the red solution. Do not insert the tip too far into the solution or extra drops will cling to the tip and make your measurement inaccurate.

7. With the tip still in the red liquid, SLOWLY release the pressure of your finger and allow the button to return to a full upright position. The red solution will enter the tip. If you release the button too quickly, air bubbles could enter the tip, and the volume of liquid picked up will not be correct.

8. **To dispense the liquid:** Place the bottom of the tip just at or just above the meniscus of the solution to which you are transferring (or near the bottom of a tube if the tube is empty).

9. Depress the button all the way to the second stop position until all the red liquid is expelled.

10. Use new graduated tips to transfer 5 µl and 1 µl of the red solution to the 1.5 ml microtube.

11. Always use a new tip for each transfer because residual liquid on or in the tip from a previous transfer could contaminate the new solution or cause inaccurate measurements.

12. There are three maneuvers that you should NEVER try with these pipettors:

a. Never measure a volume greater than the maximum volume of a micropipettor, the volume indicated on the pushbutton.

b. Never use the micropipettor without a tip.

c. Never lay down a pipettor with a tip containing liquid attached. The liquid could run back into the pipette and damage it.

Micropipetting Small Volumes: Each person should perform the exercises described below.

1. Use a black marker to label three clear 1.5 ml microtubes with the letter "A", "B", or "C".

2. Set your .5-10 micropipettor to 4 ul and add 4 ul of Solution I (blue) to each microtube, as shown in the chart below. Be sure you use a new tip each time you pipette.

Tube	Solution I (Blue)	Solution II (Red)	Solution III (Green)	Solution IV (Yellow)	Total Volume
A	4 µl	3 µl	1 µl	--	
B	4 µl	2 µl	1 µl	--	
C	4 µl	1 µl	--	.5 µl	

3. Use new tips to add the indicated volumes of Solution II (red) to each reaction tube.

4. Use new tips again to add the indicated volumes of Solutions III (green) and IV (yellow) to the reaction tubes.

5. Close the tops of the tubes and mix the reagents by flicking each tube with your fingers.

6. To bring the contents to the bottom of each tube, place your tubes and your partner's tubes in a microfuge and pulse spin them for a few seconds as described to the right. Be sure there is a tube across the rotor from each of your tubes for balance. To operate the microfuge, screw or press the top of the rotor on, close the lid, and spin the tubes for three seconds. Centrifuging for short times is accomplished easily by holding down the button on the front panel of the microfuge for the required time and then releasing it to terminate the spin.

7. Remove the tubes from the microfuge.

8. Check the volumes by setting your pipette to the total volume that should be present in the first tube. Then put a new tip on your pipettor, depress the pushbutton to the first stop, put the tip to the bottom of

the tube and release the button slowly so all the liquid in the tube enters the tip. The tip should be completely full if the volume is correct. Repeat this for your other tubes.

Micropipetting Large Volumes: **Each person** will each now practice pipetting using his/her 20-200ul micropipettor.

1. Use a black marker to label three new (1.5 ml) microtubes "D", "E", or "F".

2. Pipette the volumes shown in the chart below into the appropriate tubes. **Use a new tip for each transfer.**

Tube	Solution I	Solution II	Solution III	Solution IV	Total Volume
D	20 µl	30 µl	40 µl	50 µl	
E	20 µl	20 µl	20 µl	20 µl	
F	20 µl	40 µl	50 µl	70 µl	

3. Close the tops of the tubes and mix the reagents by flicking each tube with your finger.

4. Spin the contents to the bottoms of the tubes for 3 sec in the microfuge.

5. Calculate what the volumes of the tubes and check the volumes in these tubes yourself like you did above, to assess your accuracy.