

How to Select and Use a Micropipet

Introduction

Measuring tiny volumes with precision and accuracy requires a micropipet. In the biology lab, micropipets are used for preparing and loading DNA samples, microscale experiments and the preparation of many types of samples. These applications rely on good technique to reduce error. This guide explains how to choose the proper micropipet for the application and techniques to help ensure that measurements are accurate and precise.

Concepts

- Forward pipetting
- Repetitive pipetting

Background

A well-stocked laboratory may have several sizes of micropipets that overlap in range (e.g., 2–20 μL , 10–100 μL , 20–200 μL , 100–1000 μL). The accuracy of the pipet is highest when the pipetting volume is in the mid-range of its capacity. For example, when transferring 15 μL , it is best to use the 2–20 μL pipet instead of the 10–100 μL pipet.

When considering which micropipets to purchase, consider the application and if the data are highly quantitative or if slight errors can be tolerated. For DNA, a 2–20 μL may work best, but a 10–100 μL may be more practical if the pipets will be used for slightly larger quantities for which the exact quantities are not as critical. As a rule of thumb, the greater the range of the pipet, the less precise the measurement.

Two techniques are presented in the activity that follows—forward pipetting and repetitive pipetting. Forward pipetting is the most common type in an educational setting. The exact volume is drawn up by releasing the plunger from the first stop, and the entire sample is expelled by depressing the plunger to the second stop. This technique is used when the pipet tip is changed after each sample is transferred (e.g., when loading electrophoresis gels). Repetitive pipetting is used when the same type of sample is transferred multiple times. An excess is drawn up the first time by releasing the plunger from the second stop, then the exact volume is expelled by depressing the plunger to the first stop and drawn up and expelled over and over again.

Materials

Food colouring dyes	Micropipet, variable-volume, 10–100 μL
Water	Micropipet tips
Beaker, 100-mL	Reaction plate, 96-well

Safety Precautions

The materials used in this activity are considered nonhazardous. Please follow all laboratory safety guidelines.

Procedure

1. Add 1–2 drops of food colouring to 50 mL of water. *Note:* Food dye is used to help visualize the water in the micropipet tip.
2. Place the beaker of coloured water and the reaction plate next to each other.
3. Open the micropipet tip box. Insert the end of the micropipet into the micropipet tip and push down to secure the tip.
4. Set the volume of the micropipet to 70 μL by turning the plunger. *Note:* Different brands of pipets will have slightly different mechanisms for changing the volume.

5. Practice depressing the plunger, noticing that there are two points of resistance. These are called the first stop and the second stop. *Note:* Avoid pressing the release, which will eject the tip (see Figure 1).

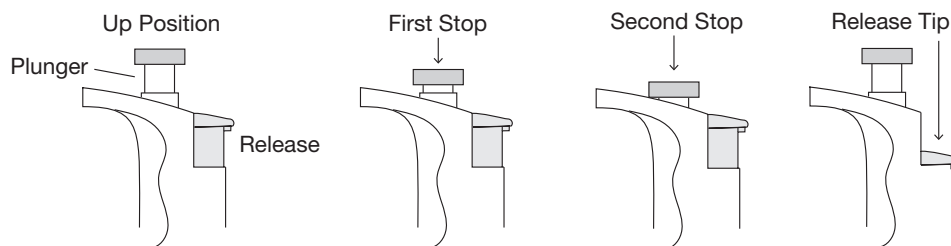


Figure 1.

6. First practice forward pipetting.
- With the pipet out of the solution, depress the plunger to the first stop.
 - While keeping the pipet vertical, submerge the tip 1 cm into the beaker of coloured water.
 - Slowly release the plunger.
 - Remove the pipet from the water, and verify the presence of water in the tip. Ensure that there are no bubbles in the water or a void at the end of the tip.
 - Transfer the water to the reaction plate by depressing the plunger to the second stop. Raise the pipet away from the reaction plate before releasing the plunger.
 - In order to conserve tips, keep the same tip on the micropipet. (When working with DNA samples or field samples, it is required to use a new tip for each sample. To eject the tip, depress the release button over a waste container.)
7. Repeat step 6 four more times with the micropipet set to the following volumes—60 μL , 50 μL , 40 μL and 30 μL . Note the size of the drops created in the well plate for each volume. Continue to practice until comfortable with forward pipetting.
8. Next practice repetitive pipetting.
- Set the volume to 50 μL , and secure a new tip.
 - With the pipet out of the solution, depress the plunger to the second stop.
 - While keeping the pipet vertical, submerge the tip 1 cm into the beaker of coloured water.
 - Slowly release the plunger.
 - Remove the pipet from the water, and verify the presence of water in the tip. Ensure that there are no bubbles in the water or a void at the end of the tip.
 - Transfer the water to the reaction plate by depressing the plunger to the first stop. Some liquid will remain in the tip.
 - Keep the plunger depressed to the first stop, and submerge the tip into the beaker of coloured water.
 - Repeat steps d–g until ten wells are filled or until comfortable with reverse pipetting. The drops should all be equal in size.
 - To eject the tip, depress the release button over a waste container.

Tips

- Any size micropipet can be used for this activity. The volumes dispensed in the activity may be adjusted as long as they are in the mid-range of the pipet.
- Practicing micropipetting technique is recommended before pipetting costly reagents or samples.
- Pipetting Practice—Super Value Laboratory Kit* (Catalogue No. FBJ1649) is a good follow-up activity to practice using micropipets to load gels for electrophoresis.
- Micropipets should be calibrated regularly, depending on amount of use. To calibrate, use an analytical balance that has been calibrated to mass precise volumes of water. Follow the instructions from the manufacturer of your micropipet to make any adjustments.

Materials for *How to Select and Use a Micropipet* are available from Flinn Scientific Canada, Inc.

Catalogue No.	Description
AP8088	Micropipet, Variable-Volume, 0.5–10 μL
AP8089	Micropipet, Variable-Volume, 2–20 μL
AP8090	Micropipet, Variable-Volume, 10–100 μL
AP8091	Micropipet, Variable-Volume, 20–200 μL
AP8092	Micropipet, Variable-Volume, 100–1000 μL
AP8077	Micropipet Tips, Sterile, Racked, 0.5–200 μL
AP8080	Micropipet Tips, Sterile, Racked, 100–1000 μL
AP1448	Reaction Plates, 96-well
V0003	Food Coloring Dyes, Set

Consult www.flinnsci.ca or your *Flinn Scientific Canada Catalogue/Reference Manual* for current prices.