

Micropipetting: Manipulating Small Volumes

Background

In order to perform procedures in a laboratory, one has to work with tiny amounts of samples (i.e. DNA, chemical solutions/ buffers). The instruments used to measure, aliquote or transfer these small amounts are known as **micropipettes**. For most of the biology experiments, a scientist can obtain the desired amount of information using very small volumes of specimens/samples.

Examples include: A forensic scientist/criminologist/genetic engineer/researcher isolate a microscopic amount of DNA from a very small amount of sample e.g., a drop of blood left at the scene of a crime.

A micropipette comes in many different models and volume ranges (microliters- to millilitres).

Purpose and principle:

This laboratory activity introduces micropipetting technique. Micropipettes measure small volume by air displacement.

Directions for using a micropipette:

1. Set pipette volume only within the range specified for that micropipette. Do not attempt to set a volume beyond the pipette's minimum or maximum values
2. When using a micropipette, first apply a tip. Forgetting to do this would cause liquid to be sucked into the nose cone. Since a micropipette works by air displacement, its internal mechanism must remain dry.
3. Always keep a micropipette in a vertical position when there is fluid in the tip. Do not allow liquid to accidentally run back into the nose cone.
4. Use your thumb to control the speed at which the plunger rises after taking up or ejecting fluid. Releasing the plunger too abruptly will cause liquid to pop up into the nose cone and damage the piston.

Procedure:

A. Setting and Preparing the Micropipette

1. Check that you have the right micropipette. There may be three sizes in the lab -- a "P-20" (for 2 to 20 μL), a "P-200" (for 20-200 μL), and a "P-1000" (for 200-1000 μL).

2. Dial the desired volume.

3. Push the end of the pipette into the proper-size tip. The yellow tips are for P-20's and P-200's.

B. How to Take Up Sample with a Micropipette

4. Before picking up the micropipette, open the cap or lid of the tube from which you are taking fluid (or, have your lab partner do this and hand the tube to you).

5. Hold the micropipette in one hand, almost vertical; hold the tube in your other hand. Both should be almost at eye-level. NOTE: the person holding the micropipette is also the person who should be holding the tube!

6. Before lowering the tip into the liquid, push down the plunger to the first stop and hold in this position. (DO NOT GO PAST THE FIRST STOP OR YOU WILL GET AN INCORRECT VOLUME), then...

7. ... dip the tip into the solution to be pipetted.

8. Draw fluid into the tip by slowly releasing the plunger. (Cap and put down the tube with the liquid or hand it to your partner.)

C. How to Expel a Sample From the Micropipette

9. With your other hand (or, have your lab partner do this), open the cap or lid of the tube into which you are ejecting the fluid.

10. Hold the micropipette in one hand, almost vertical; hold the tube in your other hand. Both should be about at eye-level.

11. Touch the micropipette tip to the inside wall of the reaction tube into which you want to expel the sample. This creates a tiny surface-tension effect, which helps bring fluid out of the tip.

12. Slowly push down the plunger of the micropipette to the first stop. Then, continue to the second stop to expel the last bit of fluid, and hold the plunger in this position.

13. Slowly remove the pipette out of the tube, keeping the plunger pushed down to avoid sucking any liquid back into the tip.

14. Always change tips for each new reagent you need to pipette. To eject a tip, depress the large blue button on the top of the micropipette.

Upon completion of this lab

Dispose of all tips and used reaction tubes in waste container.

Dispose of designated materials as outlined by your instructor.

Leave equipment as you found it.

Check that your workstation is in order.

Wash your hands.

Exercise: Measure the volumes given below using appropriate micropipette/s and weigh it. Then plot volume vs weight graph. Volumes (microliters) give are:

Gp1/10: 2, 9, 33, 87, 541

Gp2/11: 3, 6, 21, 91, 640

Gp3/12: 5, 8, 32, 107, 710

Gp4/13: 4, 9, 34, 98, 650

Gp5/14: 9, 15, 42, 108, 800

Gp6/15: 6, 11, 34, 118, 870

Gp7/16: 4, 16, 44, 126, 560

Gp8/17: 3, 15, 67, 110, 680

Gp9/18: 6, 15, 56, 130, 944