

WORKING WITH MICROPIPETS

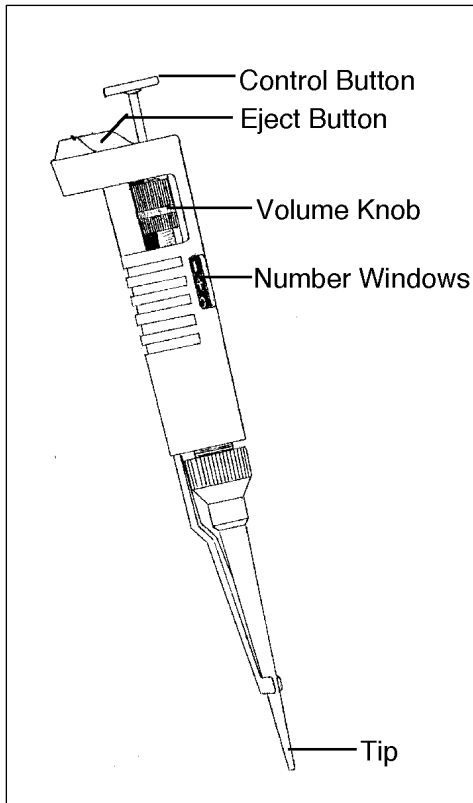
INTRODUCTION

Laboratory work in molecular biology and biotechnology is usually done in minute quantities. The unit of measure used for setting up reactions is the **microliter (μl)**. One microliter is one millionth (10^{-6}) of a liter.

So: 1 L = 1,000,000 μl , and 1 ml = 1,000 μl

Practice these conversions:

1. Convert the following to ml:
100 μl
500 μl
3,000 μl
10 μl
2. Convert the following to μl :
5 ml
0.5 ml
0.004 ml
0.000001 ml



The **micropipet** is an instrument that allows us to accurately measure μl volumes of reagents. Micropipets are delicate, very expensive, and the cornerstone of our work with DNA. In this lab, you will learn to properly use and care for micropipets. A micropipet uses suction to draw up specific amounts of liquid. Its parts allow you to control how much liquid to suck up and dispense. It is essentially a hollow barrel with an adjustable plunger through it. On the left is a diagram of a micropipet and its specific parts.

The *control button*, or *plunger*, allows the user to suck up and dispense liquid.

The *eject button* allows ejection of micropipet tips after use.

The *volume knob* allows the user to dial the amount of liquid to be measured.

The *number window* shows the amount dialed.

The *tip* of the micropipet is where the micropipet tips are placed. The entire white part is called the *barrel*.

Damaging these instruments can be avoided by following a few simple rules:

- **Never rotate the volume knob beyond the upper or lower range of the micropipet.**
- **Never use a micropipet without a tip in place.**
- **Never lay down a micropipet with a filled tip.**
- **Never allow plunger to snap back after ejecting fluid.**
- **Never immerse barrel of micropipet in fluid.**
- **Never flame micropipet tips.**

Micropipets are designed to deliver a specified volume within a certain range, with the appropriate tip in place. You have micropipets for the following ranges:

Name of micropipet	Range of Volumes Delivered	Tip To Use
P1000	200-1,000 μ l	Blue
P200	20-200 μ l	Yellow
P20	2-20 μ l*	Yellow

*We use this micropipet to measure down to 1 μ l

Perhaps the most difficult part of using micropipets is setting them properly. On each of the micropipets, you will find 3 numbers places in the number windows. However, the numbers represent different volumes for P1000, P200, and P20:



Number	P1000	P200	P20
1 st	X,000 μ l	X00 μ l	X0 μ l
2 nd	X00 μ l	X0 μ l	X μ l
3 rd	X0 μ l	X μ l	0.X μ l

You will notice a red line on the P1000. This represents a decimal point in ml. The red line on the P20 is the decimal point for μ l.

Practice setting the following volumes:

1. P1000: 324, 1000, 546 μ l
2. P200: 24, 156, 87 μ l
3. P20: 2.4, 18.9, 6.0 μ l

USE OF MICROPIPETS



1. Observe the instructor's demonstration on the proper use of the micropipet before beginning this exercise.
2. Obtain two 1.5 ml microfuge tubes and fill one with distilled water. You will practice transferring liquid from one tube to the other.
3. Choose a micropipet and set the dial to a desired volume. To operate, your thumb should be at the top of the plunger, and your fingers wrapped around the body. You may have the ejector positioned under your thumb (see picture above) or facing out (I prefer it facing out).
4. Place a tip onto the micropipet by pressing the tip of the micropipet barrel firmly into a tip of the appropriate type (blue or yellow)
5. Depress the plunger to the first stop.
6. While holding the plunger down, place the tip into microfuge tube and into the liquid.
7. **Slowly** withdraw your thumb to suck liquid into tip. Watch that it goes up without air bubbles. Do not snap back plunger!
8. Place the tip into the bottom of the receiving microfuge tube.
9. Press plunger to first stop to dispense liquid. Continue to press beyond to first stop to get out all of the remaining liquid in the tip.
10. Pull tip out of liquid before relaxing the plunger back to original position.
11. Eject tip into waste container by pressing the ejector button.

USE OF MICROFUGES

1. Observe the instructor demonstrate the proper use of the microfuge and how to insert tubes in a balanced configuration. This is extremely important, because **spinning tubes in an unbalanced position will damage the microfuge!**
2. Be sure tubes you are spinning are in pairs and have approximately the same weight/volume in them.
3. Open lid and remove rotor cover. Place tubes in pairs arrange so that they are at opposite ends in the rotor.
4. Replace rotor cover and close lid.
5. Select appropriate time and push start. For short pulses, hold the pulse button for the desired time.
6. Wait for rotor to stop completely before opening lid and removing your tubes.

SMALL VOLUME PIPETTING EXERCISE

1. Obtain a P20 micropipet, yellow tips and 2 microfuge tubes.
 2. Label the tubes A and B. Use the matrix below as a guide for adding appropriate volumes of the colored solutions to each tube.
- | Tube | Red | Blue | Yellow | Water | Color Observed |
|------|-----------|-----------|-----------|------------|----------------|
| A | - | 2 μ l | 2 μ l | 11 μ l | |
| B | 3 μ l | 2 μ l | - | 10 μ l | |
3. First add the appropriate amount of water to the bottom of each tube. Be sure all of the liquid comes out and forms a small bubble of liquid at the bottom of the tube.
 4. Next add the colored solutions one at a time. Dispense the solution directly into the small bubble of liquid at the bottom of the tube.
 5. After you have added all of your solutions into each tube, practice mixing the contents with a micropipet. Set your micropipet to 15 μ l and slowly pipet the mixture up and down until well mixed.
 6. Place all the tubes in the microfuge and apply a short (1-2 seconds) pulse. **Make sure the tubes are placed in a balanced configuration!**
 7. Record the final color of the solution in each tube in the table above.
 8. A total of 15 μ l was pipetted into each tube. Check that your measurements were accurate by setting the P20 to 15 μ l and withdrawing the contents of each tube.

LARGE VOLUME PIPETTING EXERCISE

1. Obtain a P200 and P1000 micropipet, yellow tips and blue tips and 4 microfuge tubes.
 2. Label the tubes C, D, E, & F
 3. Use the matrix below as a guide for adding appropriate volumes of the colored solutions to each tube.
- | Tube | Red | Blue | Yellow | Water | Color Observed |
|------|------------|-------------|-------------|-------------|----------------|
| C | 40 μ l | - | 40 μ l | 920 μ l | |
| D | - | 20 μ l | 260 μ l | 720 μ l | |
| E | | 120 μ l | 20 μ l | 860 μ l | |
| F | 60 μ l | 30 μ l | 30 μ l | 880 μ l | |
4. Mix well (by finger vortexing or micropipetting) and place all the tubes in the microfuge and apply a short (1-2 seconds) pulse. **Make sure the tubes are placed in a balanced configuration!**
 5. Record the final color of the solution in each tube in the table above.
 6. A total of 1,000 μ l (1ml) was pipetted into each tube. Check that your measurements were accurate by setting the P1000 to 1,000 μ l and withdrawing the contents of each tube.

ANALYSIS

Today, you will take a quiz and demonstrate your micropipetting skills. Be sure you know how to:

1. Convert from liters to milliliters to microliters.
2. Identify micropipet parts and their purposes.
3. Choose and set the correct micropipet for the job.
4. Properly use the micropipet.
5. Properly use a microfuge.