

TITLE: Pipetting by Design Student Instructions

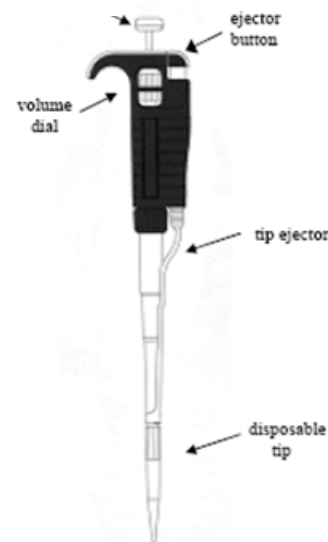
KEY QUESTION(S):

- What is a micropipette?
- How do you properly use a micropipette?
- Why is a micropipette necessary in biotechnology laboratories?

STUDENT LEARNING OBJECTIVES:

The student will be able to:

1. Properly operate a micropipette.
2. Determine the appropriate micropipette to use according to the volume of liquid being measured.
3. Correctly read the volume indicator on the micropipette.
4. Measure volume in microliters (μl) using a micropipette.
5. Convert volume into mass.
6. Use a scale to determine accuracy of pipetting.



MATERIALS:

- (1) Multicolor Food Coloring Package (Red, Blue, Yellow, Green)
- (1) 96 Well Plate
- (1) P20 Micropipette
- (1) P200 Micropipette
- (1) 2-200ul Tip Box

Directions on how to use a micropipette:

- Hold micropipette in one hand. With the other hand turn the black volume adjustment, dial 1/3 of a revolution above the desired setting then slowly down until the required volume shows on the digital indicator. *This prevents mechanical backlash from affecting accuracy.*
- Press disposable tips firmly onto the shaft to ensure an airtight seal. Do this by tapping the micropipette in the tip (tapping the tip on).

BACKGROUND INFORMATION:

Micropipettes are precise instruments used to accurately measure very small quantities of liquids in science laboratories. Image 1 shows a micropipette and the main components of the instrument. They are available in a variety of sizes to best match your measurement needs. The size of the micropipette is indicated directly on the instrument. The most commonly used micropipettes are the P10, P20, P200, and P1000. The number following the "P" refers to the maximum volume in microliters (μl) that can be measured using the instrument.

In this activity P20 and P200 micropipettes will be used. The proper method of reading the volume indicators and directions on how to use the P20 and P200 micropipettes is listed on the following page.

Reading the volume on the micropipette:

- **P20 Micropipettes:** The volume indicator consists of three number dials and is read from top to bottom. Black digits indicate tens of microliters and microliters; red digits indicate tenths of microliters. A P20 is used to measure volumes up to 20 μ l. **NOTE: Do not dial past 20 μ l.**

0
7
3

7.3 μ l

2
0
0

20.0 μ l

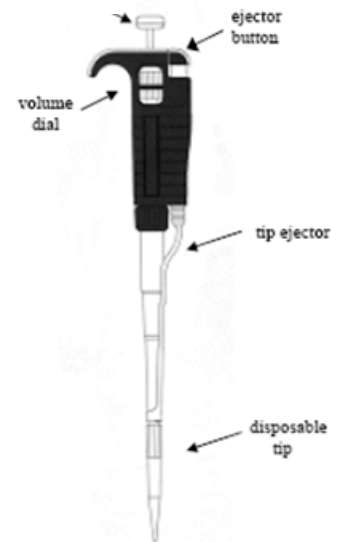
- **P200 Micropipettes:** The volume indicator consists of three number dials and is read from top to bottom. Black digits indicate hundreds and tens of microliters; red digits indicate microliters. A P200 is used to measure volumes between 20 μ l and 200 μ l. **NOTE: Do not dial past 200 μ l.**

0
7
3

73 μ l

2
0
0

200.0 μ l



Directions on how to use a micropipette:

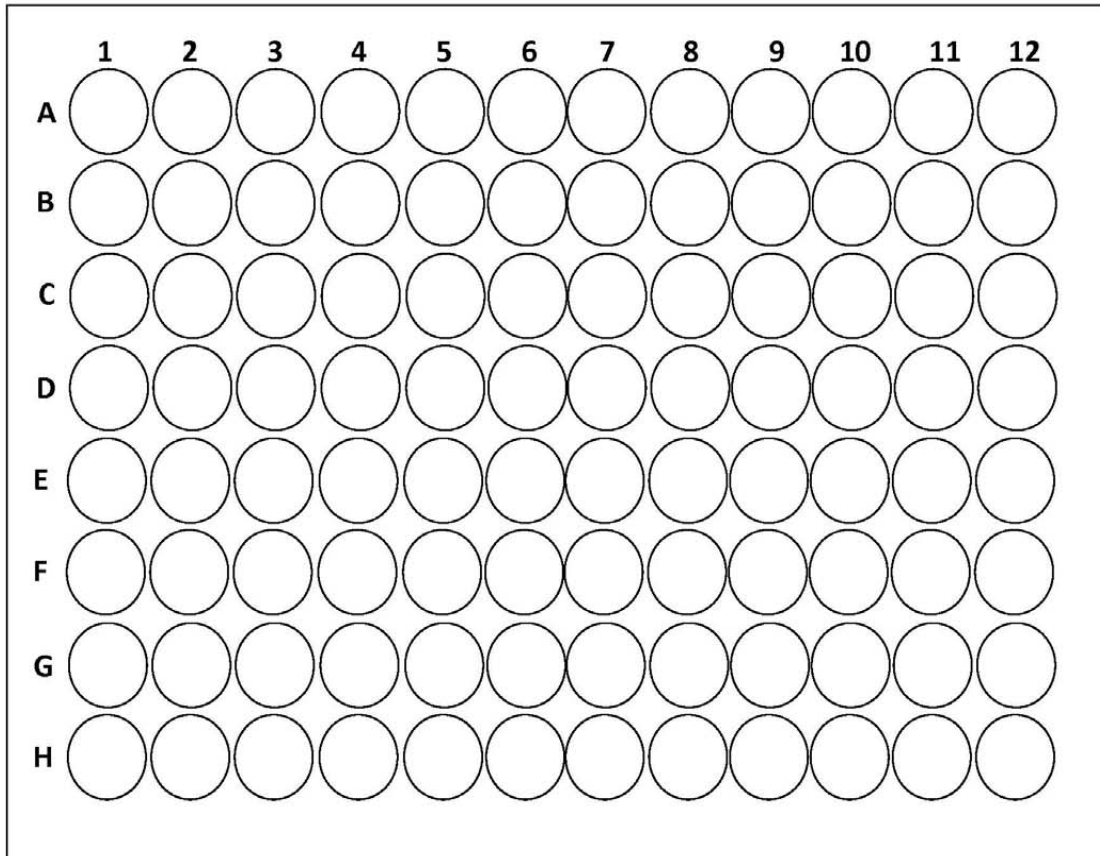
- Depress plunger to the **first stop**. Holding the micropipette vertically, immerse the tip approximately two mm into the sample liquid. **Allow the pushbutton to return slowly to the up position!**
- Withdraw the tip from the liquid. Touch the tip end against the side wall of the receiving vessel and depress the plunger slowly to the first stop.
- Wait one second then press the plunger to the second stop, expelling any residual liquid in the tip.
- With the plunger fully depressed, withdraw micropipette and allow the plunger to slowly return to the up position.
- Discard the tip by depressing the ejector button. **Use a fresh tip for the next sample to avoid contamination.**

Important information to note:

- Do not use the micropipette without a disposable tip in place. Moisture can damage the piston and reduce accuracy.
- Do not lay a liquid loaded micropipette down. Moisture can run back inside causing damage to the micropipette.
- Do not allow the button to snap back after pushing the plunger. Allow it to return gradually.

A. Micropipetting Activity

1. Work with your partner to complete the micropipette protocol activity.
2. You will need 10 mL of each color of water. The colors you need depend on your protocol.
3. When you get your protocol collect the water you need and follow the protocol directions, placing the appropriate color of water in the designated well of your plate.
4. A plate map is below.
5. When you complete the protocol check with your teacher to see if your design is correct, and then continue with part B to see how accurate you were.



B. Check for Accuracy

1. Check for accuracy by determining the mass of your design. To do so, you should do the following:
 - Determine the volume of colored water you added to your plate
 - Convert volume into mass ($1000\mu\text{l} = 1\text{ml}$; $1\text{ml} = 1\text{g}$)
 - Calculate the mass of your completed plate
 - Zero a scale with an empty 96-well plate
 - Weigh your complete plate
 - Determine your accuracy by comparing your calculated mass to the actual mass of your plate.