

Pipetting 101

How to Use a Pipetman:

A Pipetman is very useful for measuring very small volumes of liquid. The Pipetmans we use at CITYLAB measure volumes in the **microliter** (μl) range. A microliter is 1/1000 of a milliliter and a milliliter is 1/1000 of a liter. At CITYLAB, we use 2 different types of Pipetmans depending on the volume of liquid needed.

Pipetman	Volume range	Tip to use
P200	20-200 μl	Yellow tip
P1000	200-1000 μl	Blue tip

NOTE: There are only 3 number boxes on the P1000 Pipetman. When using this Pipetman, you must multiply the number seen in the window by 10 to read the volume. For example, if the number in the window is 100, then the volume is $100 \times 10 = 1000 \mu\text{l}$.

NOTE: Be careful not to drop the Pipetman, it can be damaged very easily

How to Use a Pipetman

1. Select the correct Pipetman using the table above.
2. To set a volume, hold the Pipetman in one hand and turn the volume adjustment knob with the other hand until the correct volume shows on the digital indicator.
3. Attach a new disposable tip to the pipette shaft. Press firmly.
4. Depress the plunger to the **FIRST STOP**.
5. Holding the Pipetman vertically, immerse (dip) the disposable tip into the liquid. The tip should be immersed only 1-2 millimeters into the liquid.
6. Allow the push-button to return slowly to the UP position. **Never Let It Snap Up!!**
7. Wait a few seconds to ensure that the full volume of sample is drawn into the tip.
8. Withdraw the tip from the sample liquid.
9. To dispense the sample, touch the tip against the side wall of the receiving tube and depress the plunger slowly to the **FIRST STOP**.
10. Then press the plunger to the **SECOND STOP**, expelling any liquid left in the tip.
11. With the plunger fully depressed, withdraw Pipetman from vessel carefully.

12. Allow the plunger to return to the UP position.
13. Discard the tip by depressing the tip ejector button. A fresh tip should be used for each sample.

Pipetting Exercise

This exercise is to help all students to become comfortable with the Pipetman and pipetting.

Please follow the protocol and fill in the blanks in the table. Make sure that all members of your group take turns completing the exercises. The Pipetmans we will be using in the lab are designed to accurately measure **microliter** volumes of liquid.

A microliter is 0.000001 of a liter and is abbreviated with the symbol μl .

1. Create your **reference tubes**:

STANDARD #	COMBINE		RESULTING COLOR
#1	100 μl blue dye	100 μl yellow dye	
#2	100 μl red dye	100 μl yellow dye	
#3	100 μl red dye	100 μl blue dye	

2. Use the table to create the following dye combinations:

TUBE	COMBINE		RESULTING COLOR	COMPARE TO STANDARD For example: lighter than, darker than, same as...
A	50 μl blue dye	50 μl yellow dye		#1
B	37 μl red dye	37 μl yellow dye		#2
C	658 μl red dye	658 μl blue dye		#3
D	100 μl yellow dye	25 μl red dye (4 times)		#2
E	25 μl red dye (4 times)	25 μl yellow dye (4 times)		#2

3. Using your newly acquired pipetting skills, solve the mystery of the unknown tubes. You will be given 2 known & 4 unknown tubes. One known tube will contain iodine, and the other will contain starch. Starch and iodine will give a color change when mixed. Your first task will be to make a standard tube of an iodine/starch mix. This will give you a reference for the reaction between iodine and starch. Using the 4 unknown tubes (W,X,Y,Z) we have given you, perform micropipeting procedures to mix samples from **two** tubes at a time (into a clean tube) to determine which tube contains starch, which tube contains iodine and which two tubes contain water. You should compare each tube to the standard that you have made. Please make sure that you are labeling all of your tubes!

TUBE LABEL	COMBINE		RESULTING COLOR
Standard	50 μ l Starch	50 μ l iodine	
1	50 μ l tube W	50 μ l tube X	

Conclusions:

Which tube do you think is:

Iodine _____ Starch _____ Water _____