

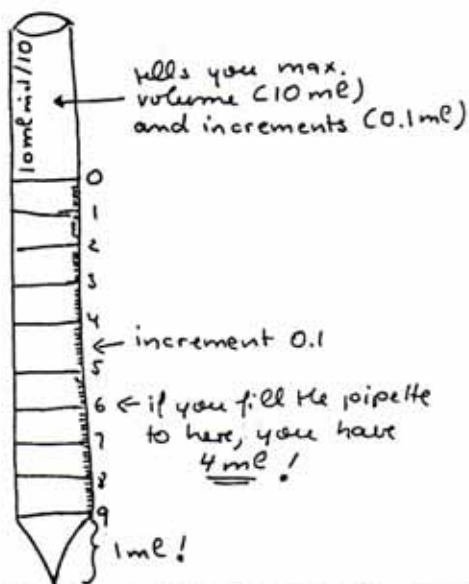
Biology 9 Pipette and Micropipette Use

Pipettes

Pipettes are plastic or glass tubes with one tapering end that are used to transfer specific amounts of liquid from one container to another. Common sizes are 1 milliliter (ml), 5 ml, 10 ml, and 25 ml. Glass pipettes frequently have a tuft of cotton located in the neck and require a dropper bulb or a pipette pump to draw up and dispense liquids.

Graduations on the sides of the pipettes allow you to measure the amount of material to be transferred. They are not as accurate as micropipettes (discussed on the next page) in part because any imperfection in their internal diameter will have a greater effect on the volume delivered as well as being more dependent on human observation and error.

- Note the volume scale before using a pipette. Both the largest and smallest volumes that a pipette can measure are marked on the upper end of the pipette (see figure below). For example, “10 ml in 1/10” means that the pipette can measure a maximum of 10 ml. The smallest increment it can measure accurately is 1/10 or 0.1 ml.
- Note also the numerical direction of the markings on the pipette. For example in the figure below, if you draw liquid into the pipette to the 6 ml mark, you have filled the pipette with 4 ml.



How to use a plastic pipette pump and glass pipette to draw up and dispense liquids

A pipette bulb or plastic pipette pump (see figure below) is used to draw liquid up into the pipette. We will use a plastic pipette pump.



- Insert the cotton-filled end of a sterile pipette into the end of a plastic pipette pump. Both pipettes and pumps come in different sizes. Be sure to use a pump that can "suck" the quantity of liquid you want (e.g. 10 ml pump with a 5 ml or 10 ml sterile pipette).
- Using the wheel at the top of the pump, raise the white top of the pump approximately 1 cm BEFORE inserting the pipette into the liquid. This step is necessary in order to create additional air pressure in the pipette as you expel the liquid.
- Next, place the tapered tip of the pipette into the liquid. The tip should be within the liquid during the pipetting to prevent air from entering.
- Hold the pipette pump with one hand. Your thumb should be placed on the wheel. Use your thumb to rotate the wheel downward. This will cause the liquid to rise into the pipette. Do this carefully and watch the meniscus* of the liquid rise to your desired level. While drawing up solution, make certain that the pipette tip remains below the surface of the liquid
 - Remember: Measure the level of the liquid at the BOTTOM of the meniscus.

- Next, take the tip of the pipette out of the liquid and move the entire apparatus to the place where you desire to put the measured liquid.
- Use your thumb to rotate the wheel upward. This will cause the liquid to be dispensed from the pipette. Lower the white top of the pump all the way to the pump shaft. Because you created the extra space before pipetting, the entire contents of the pipette should be now be dispensed!
- NEVER use your mouth to draw liquid into a pipette!

*Reading the volume at the bottom of the meniscus

Determine the volume of solution in a pipette by reading the bottom of the meniscus at eye level. The surface of a liquid confined in a cylinder curves to form what is known as a meniscus. The meniscus of most liquids curves up the sides of the container, making the center of the curve appear lower than the edges. Mercury is one of very few exceptions - it curves down at the edges. Since reading the meniscus at the top or at the bottom of the curve will make a difference in the volume measured, it is generally agreed to always read the bottom of the curve.

Micropipettes

Most molecular biology experiments include procedures that require extremely small and precise volumes of solutions and reagents. The adjustable micropipette is the tool to measure these quantities accurately. The amounts of liquids transferred are usually less than 1 ml. The scales on micropipettes are in microliters (ul). Recall that 1000 ul equals 1 ml.

Rules to prevent damaging the micropipettes

Micropipettes are extremely expensive and delicate instruments. READ and FOLLOW these rules for their use.

- *Never use the micropipette or rotate its volume adjuster beyond its maximum or minimum range.*
- *Never use the micropipette without a clean tip in place.*
- *Always hold a filled micropipette vertically, and never lay a filled micropipette down - the fluid can flow back into the piston mechanism and ruin its ability to measure quantities accurately.*
- *Never allow any part of micropipette to be immersed in any fluid.*
- *Never allow the plunger to snap back after withdrawing or ejecting a sample - any extreme actions can damage the delicate piston inside.*

Our micropipettes come in three sizes that are capable of pipetting accurately three ranges of volumes:

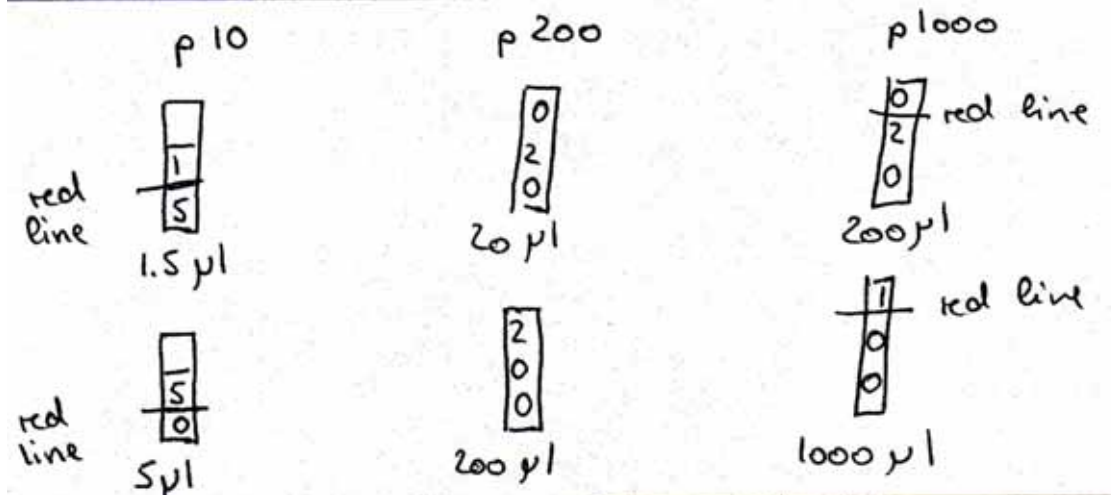
Pipette Ranges		
P20 (2 - 20 ul)	P200 (20 - 200 ul)	P1000 (200 - 1000 ul)

They are used in conjunction with disposable (often sterile) plastic tips. The smaller two micropipettes (P20 and P200) require the yellow tips and the P1000 uses the larger blue tips. The following is an illustration of a micropipette.

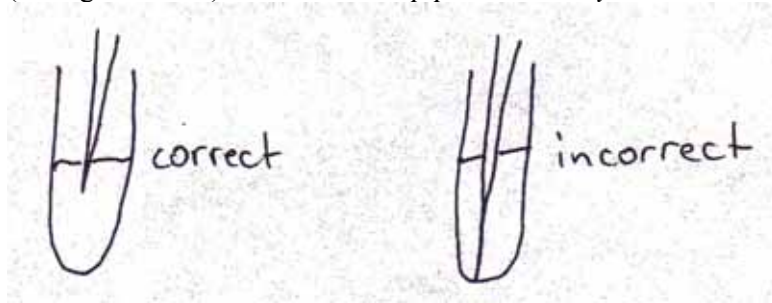


Directions for use of the micropipette

- Select a micropipette that has a volume range that includes the volume you need. **DO NOT USE MICROPIPETTES FOR VOLUMES OUTSIDE OF THEIR INTENDED RANGE!**
- Adjust the micropipette to the desired volume. Be sure to locate the decimal point (a red line) properly when reading the volume setting! Some models are adjusted using the plunger button. Others are adjusted with a separate knob or wheel. Turn the wheel clockwise to increase volume or counterclockwise to decrease volume. Ask your instructor if you need help with a particular micropipette. Some examples of settings are provided in the figure below.



- Firmly press a new tip on the discharge end of the micropipette. Note if sterile conditions are necessary do not allow the tip to touch any object including your hands. Replace the lid on the pipette tip box to keep them sterile.
- To draw liquid into the micropipette tip:
 - Depress the control button to the FIRST STOP and hold it in that position.
 - The plunger will stop at two different positions when it is depressed. The first of these stopping points is the point of initial resistance and is the level of depression that will result in the desired volume of solution being transferred. Because this first stopping point is dependent on the volume that is being transferred, the distance you have to push to reach the point of initial resistance will change depending on the volume being pipetted.
 - The SECOND stopping point can be found when the plunger is depressed beyond the initial resistance until it is in contact with the body of the micropipette. The second stopping point is used for the complete discharging of solutions from the plastic tip. You should not reach this second stop when drawing liquid into the micropipette, only when expelling the last drop.
 - Before continuing, practice depressing the plunger to each of these stopping points until you can easily distinguish between these points.
 - Insert the tip into the solution, just barely below the surface of the liquid and not as deep as possible (see figure below). *Hold the micropipette vertically and the tube close to eye level.*



- Release your thumb slowly and carefully from the plunger to draw the fluid into the pipette tip - *if you release the plunger too quickly, it will suck liquid up into the micropipette and damage it.* Check that you are not aspirating any air bubbles.

- To avoid pipetting errors, learn to recognize the approximate level to which particular volumes fill the tip.
 - If air is noted in the tip during intake, dispense the sample back into the reagent vessel, and repeat more slowly and evenly. If air is noted a second time, discard the tip and use a new one.
 - Now lift the micropipette out from the tube by gently *dragging* the filled tip against the inside wall of the tube.
- To dispense the liquid:
 - While still holding the micropipette in an upright position, use your free hand to hold your destination tube at eye level.
 - Touch the filled micropipette tip lightly against the inside wall of the tube. The resulting capillary action will later help to draw all of the fluid out of the pipette tip.
 - Steadily depress the plunger beyond the FIRST STOP and down to the SECOND STOP (to push out every last bit of the sample) but *do not let go of the plunger!* Keep your thumb pressed on the plunger.
 - Now, with your thumb still pressing on the plunger at the SECOND STOP, slowly lift the micropipette out while dragging its tip against the inside wall of the tube with a rotational motion of your wrist. All of the fluid within the tip should now be expelled and left within the receptor tube.
- Dispose of the used micropipette tip by pressing the eject button on the micropipette.
- *Always* use a fresh pipette tip when changing and dealing with a different solution. You may reuse a tip for the same solution, provided you have not contaminated the tip by touching any solution that may have already been in the receptor tube. *Be sure not to contaminate solutions with used tips!*
- When combining reagents together in the single tube, it is a good idea to position each one at a different location midway along the inside wall of the tube. This will help you keep track of which solutions have already been dispensed and will prevent contaminating the pipette tips.
- See the figure below for an overview of the steps in micropipetting.

