

Basic Laboratory Techniques

Pipetting

In molecular biology the ability to accurately and reproducibly measure and transfer small volumes of liquids is critical for obtaining useful results.

For volumes less than 1 ml, the most common method for measuring liquid volumes involves the use of a device known as a pipette.

Make sure that you are using the correct pipette for the volume you need. Also, make sure that the pipette is actually set for the volume you need by looking in the “volume window”, and, if necessary, turning the “volume control knob” until the pipette displays the correct volume

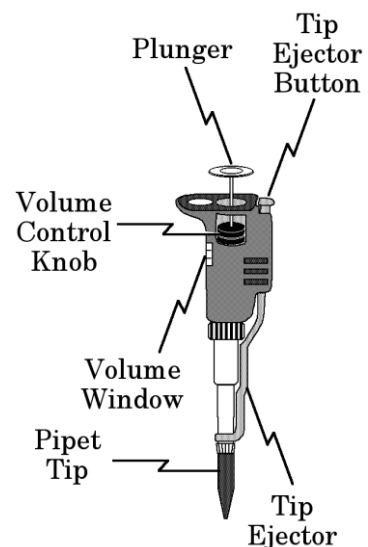
All pipettes use disposable tips (**do not pipet liquids without using the appropriate tip**, because this will contaminate the pipette and may damage it). When attaching the tip, make certain that the tip is the correct type for the pipette you are using, and that the tip is properly seated on the end of the pipette.

Try depressing the plunger. As the plunger depresses, you will feel a sudden increase in resistance. This is the first “stop”. If you continue pushing, you will find a point where the plunger no longer moves downward (the second “stop”). When using the pipette, depress the plunger to the first “stop”, place the tip into the liquid, and **in a slow, controlled manner**, allow the plunger to move upwards. (Do not simply let the plunger go; doing so will cause the liquid to splatter within the tip, resulting in inaccurate volumes and in contamination of the pipet.)

Now, take the pipette (carrying the pipetted liquid in the tip) to the container to which you wish to add liquid. Depress the plunger to the first, and then to the second stop. If you watch carefully, you will note that depressing to the second stop expels all of the liquid from the tip. (Actually, this is true for most aqueous solutions. In some cases, however, such as for organic solvents, or for solutions containing large amounts of protein, it is often difficult to get all of the liquid out of the tip. In these cases, it is best to “wet” the tip, by pipetting the original solution once, expelling it, and then taking up the liquid a second time.)

Although pipettes are tremendously useful, they have a potential drawback. If used improperly, pipette will transfer inaccurate volumes. In addition, pipette may lose calibration. If used incautiously, therefore, pipette may yield misleading or even totally useless results. Checking the calibration of pipette is a simple procedure that can save considerable time, energy, and reagents.

Fig. 1: Pipette



Performing Dilutions

Many solutions used in molecular biology are prepared by the dilution of a more concentrated stock solution. In preparing to make a dilution (or series of dilutions), you need to consider the goal of the procedure. This means that you need to consider both the desired final concentration and required volume of the diluted material. A simple equation allows the dilution to be calculated readily:

$$C_1V_1 = C_2V_2$$

where **C₁** is the concentration of the initial solution; **V₁** is the volume of the initial solution available to be used for dilution (this may not be the total volume of the initial solution, and instead may be a small fraction of the initial solution), **C₂** is the desired final concentration, and **V₂** is the desired final volume.

In most cases, the initial concentration and the final concentration are either known or are chosen in order to work correctly in the experiment being planned. The final volume is usually an amount that is chosen based on the amount required for a given experiment. This means that at least three of the required terms are either known or can be chosen by the experimenter.

Let us consider an example. You are setting up a standard curve. You have a stock solution of 1000 µg/ml PBS, and for one of the points on the curve, you want 200 µl of 20 µg/ml. In this case, $C_1 = 1000 \text{ µg/ml}$; $C_2 = 20 \text{ µg/ml}$, and $V_2 = 200 \text{ µl}$. This leaves V_1 as the unknown value (*i.e.* how much of the stock solution must be diluted to 200 µl final volume to yield the desired concentration). Rearranging the dilution equation gives:

$$V_1 = V_2 \frac{C_2}{C_1} = 200 \text{ µl} \left(\frac{20 \text{ µg/ml}}{1000 \text{ µg/ml}} \right) = 4 \text{ µl}$$

Thus, you need to dilute 4 µl of the stock solution to a final volume of 200 µl (*i.e.* by adding 196 µl).

In some cases, you may not know the actual starting concentration. If, for example, you need to measure the enzyme activity in a sample, and you find that the activity is too high to measure accurately, you will need to dilute the starting material. Since you don't know the actual starting concentration, all you know is the concentration **ratio** between starting and final solutions. As long as you keep track of the concentration ratio in all of your dilutions, you can easily determine the enzyme activity in the initial solution, even though you cannot measure it directly.

Concentration ratios are frequently of considerable value. For example, you have a stock solution of buffer that contains 450 mM Tris-HCl, 10 mM EDTA, and 500 mM NaCl. You actually wish to use a final concentration of 45 mM Tris-HCl, 1 mM EDTA, and 50 mM NaCl. In each case the concentration of the final buffer is one tenth that of the original. Simply performing a 1:10 dilution of the stock solution then gives the appropriate final concentration of each component. The stock solution of buffer is typically called a 10x stock, because it is ten-times more concentrated than the final, useful buffer.

Buffers

A buffer is a solution that is used to control the properties of a process occurring in an experimental aqueous medium. The term "buffer" is related to the ability of these solutions to resist changes in the hydrogen ion concentration, but buffers also contain other molecules, and are used to attempt to influence the ionic strength, the activity of

proteases, and other parameters of the experiment in addition to the hydrogen ion concentration.

in order to produce most biochemically useful buffers, several components must be added together. This frequently requires careful consideration of the necessary dilutions for each of the components.

The Phosphate Buffered Saline is designed to allow preparation of Phosphate Buffered Saline (PBS) at various pH and ionic strength, its includes the following reagents:

- Per Liter of Distilled Water
 - a. Dipotassium Hydrogen Phosphate 1.1 g
 - b. Potassium Dihydrogen Phosphate 0.32 g
 - c. Sodium Chloride 8.5 g
 - d. pH 7.2 +/- 0.1

Table 1: Phosphate Buffer Formulation Guide

1 Liter of 0.05 M Phosphate Buffer (25 °C)			1 Liter of 0.05 M Phosphate Buffer/0.15 M NaCl (25 °C)		
pH	1 M KH ₂ PO ₄ (ml)	1 M K ₂ HPO ₄ (ml)	1 M KH ₂ PO ₄ (ml)	1 M K ₂ HPO ₄ (ml)	5 M NaCl (ml)
6.6	32.0	18.0	26.6	23.4	30
6.7	29.8	20.2	23.7	26.3	30
6.8	26.5	23.5	20.9	29.1	30
6.9	24.0	26.0	18.1	31.9	30
7	21.1	28.9	15.6	34.4	30
7.1	18.4	31.6	13.2	36.8	30
7.2	16.8	34.2	11.1	38.9	30
7.3	13.4	36.6	9.2	40.8	30
7.4	11.2	38.8	7.6	42.4	30
7.5	9.4	40.6	6.3	43.7	30
7.6	7.8	42.2	5.1	44.9	30

$$M = (\text{wt}/M_{\text{wt}}) * (V/1000)$$