Lab 2: Instruments and Equipment

Purpose:

An important part of working in any laboratory is the proper use and calibration of instruments and equipment. Students will become familiar with general information about the use of lab equipment, as well as more detailed information about the step-by-step procedures for the specific instruments that they use.

List of some equipment / apparatus used in biotech labs

1. Pipette	2. Balance	3. pH meter	4. Autoclave
5. Thermometer	6. Water bath	7. Centrifuge	8. Refrigerator
9. Gel Electrophoresis	10. UV Light	11.Thermal cycler	
12.Spectrophotometer	13. Nanodrop	14. ELISA	15. HPLC

1. Pipette: A pipette is used for measuring liquids and dispensing them from one vessel to the other.

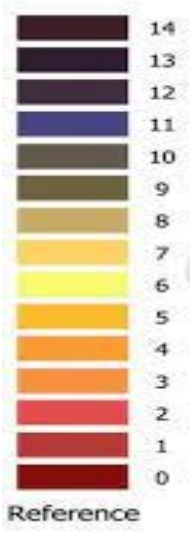
Types of Micropipette

Normally there are five piston positions in a pipette. They are listed and defined as follows:

1. Prepare: This is where the plunger is pressed down to the intermediate/ bottom stop at the beginning of the pipetting procedure in order to prepare liquid to enter the tip.
2. Aspirate: This is the point in the pipetting procedure where the plunger is slowly released and the liquid actually enters the tip.
3. Deliver: This is where the liquid is delivered to the receiving vessel by pressing the plunger down until the intermediate stop is reached
4. Blow out: This is the point at which the plunger is fully depressed until the bottom stop is reached in order to "blow out" any residual liquid that remains in the tip
5. Return: This is the final stage where the plunger is released and allowed to return to its starting position.

2. Analytical Balance: Balance is needed in biotechnology lab for weighing chemicals, powders, samples (e.g., tissue), etc. Digital balances are fast to work.

3.pH meter: pH meter is an electrical instrument used for measuring hydrogen ion concentration of solutions and mixtures. The pH meter must be standardized with buffer solutions before operation. It must not be dipped in hot or very cold solutions. The electrodes must always be kept immersed in suitable solutions.



4.Microcentrifuge: A micro-centrifuge holds micro-centrifuge tubes that can hold about 1.5 mL of liquid. These micro-centrifuges can also spin at high speeds and are sometimes refrigerated.

5. Autoclave: an autoclave is a robust, electrically heated steam vessel used for sterilizing glassware, culture media, and other materials that are not spoiled by moist heat. Microorganisms / cells are killed as a result of denaturation of cellular constituents (protein and nucleic acids). In routine process, sterilization can be achieved by operating the autoclave at 121°C for 15 min.

6.Thermometer: Thermometers are required to ensure the heating equipment is running at the correct temperature. The temperature of the water bath, incubator, etc., need to be frequently checked. (e.g., Mercury Thermometer).

7.UV (Ultraviolet): light Exposure to ultraviolet light can cause acute eye irritation. Since the retina cannot detect UV light, you can have serious eye damage and not realize it until 30 min to 24 hours after exposure. Therefore, always wear appropriate eye protection when using UV lamps.

8. NanoDrop spectrophotometer: a NanoDrop spectrophotometer is a brand of scanning UV/VIS spectrophotometer that allows the user to measure the absorbance of a very small sample of liquid (1-2 uL). This instrument makes it easy to quickly evaluate the quality and quantity of nucleic acids or proteins in a small sample prep.

9. Thermal cycler: thermal cycler is a machine that is used for amplification of a specific section of DNA by PCR (polymerase chain reaction). The machine cycles through several temperatures, which allows an enzyme called DNA polymerase to use chemicals in solution to build DNA molecules identical to a template provided.

Preparing Solutions

Introduction A common task for any technician or bio-technician is solution preparation. Solution is defined as a solute (smaller amount) dissolved in a solvent (larger amount). The concentration of a solution frequently must be known to a high degree of accuracy. All calculations are recorded in the lab notebook, even if a calculator is used. The exact mass and volume of reagents used is recorded in the notebook. This information, along with the date and the preparer name or initials, is recorded on a solution preparation form and on a label on the bottle itself.

There are several critical aspects to making solutions that should be followed at all times. Check and recheck each calculation.

1. Read each reagent bottle twice, once before using and once afterwards.
2. Complete a Solution Prep form for every solution you prepare. This includes the formula, as well as the concentration and the amount weighed out for each reagent.
3. Check pH by pH meter.
4. Label each bottle before filling. Write down the name of the solution, your initials and the date.
5. Record any changes observed. This record can be used to trace back a problem to its source quickly and easily or to confirm that a problem does not lie in the reagents or their preparation.

Concentration Expression:

Percent concentrations may be expressed as:
1. Weight per volume (wt/vol or w/v), which indicates the weight (in grams) of solute per 100 mL of solution (used to indicate the concentration of a solid in a liquid) Eg. 0.7% solution of agarose in TBE buffer, weight 0.7g of agarose and bring up volume to 100 ml with TBE buffer.
2. Volume per volume (v/v), which indicates the volume (in mL) of solute per 100 mL of solution (used to indicate the concentration of a liquid dissolved in liquid)
3. Weight per weight (w/w), which indicates the weight (in grams) of solute per 100 g solution (used to indicate the concentration of a solid mixed in another solid)

Molarity:

Molarity is the most common unit of concentration in the biotechnology lab. The molarity of a solution is defined as the number of moles of solute per liter of solution. The symbol for molarity is M, but it can also be written as moles/Liter, or mol/L. A mole of any element always contains 6.02 X 1023 (Avogadro's number) atoms. Because some atoms are heavier than others, a mole of one element weighs a different amount than a mole of another element. The weight of a mole of a given element is equal to its atomic weight in grams. For example, one mole of the element carbon weighs 12.0 g.
Molarity= weight gram_ x 1000 Molecular weight
Request volume Weight gram= molecular weight x request volume x molarity 1000.

Molality:

Molality is the number of moles of solute per kilogram of solvent. The density of water at 25°C is about 1 kilogram per liter, molality is approximately equal to molarity for dilute aqueous solutions at this temperature.
Molality (m) = Moles of solute (n)/ kilograms of solvent

Normality:

Normality is equal to the gram equivalent weight of a solute per liter of solution. A gram equivalent weight or equivalent is a measure of the reactive capacity of a given molecule. Normality (N) = Gram Equivalent Weight of solute/1Liter of solution The

Equivalent Weight is the amount of solute needed to be the equivalent of one mole of hydrogen ions. Therefore, the equivalent weight is dependent on the valence of the solute.

E.g., Hydrochloric acid (HCl) has one replaceable hydrogen ion (H+). E.g., Sulfuric acid (H2SO4) has two replaceable hydrogen ions (2H+). So, for pure HCl, its MW is 36.46, its EW is 36.46 and therefore a 1N solution would be 36.46 grams of the pure chemical per liter.
To make a 1N H2SO4 solution from pure H2SO4, its MW is 98.08, and its EW is: 98.08/2 Therefore, EW = 49.04 grams per liter (98.08/2) (or 49.04 grams per 1000 milliliters). So, a 1 N solution would be 49.04 grams of the pure chemical per liter.

Buffers:

Buffers are solutions help maintain a biological system at its proper pH. It is essential when working with protein, nucleic acid (and other biomolecular) solutions that you pay attention to pH. When a solution is buffered, it resists a change in pH, even when H+ ions are added or lost from the system.

TBE Buffer:

Function: TBE Buffer is a containing a mixture of Tris base, acetic acid and EDTA and used for polyacrylamide and agarose gel electrophoresis. It is ideally suited for DNA and RNA separation on longer run, higher voltage or ampere gels. A 1X solution is obtained by adding 1 part of the10X TBE buffer to 9 parts of deionized water.
Composition (10X): 890mM Tris, 890mM Boric acid, 20mM EDTA, pH:8 at 25 °C
5X Stock solution Component:
54 g of Tris base
27.5 g of boric acid
20 ml of 0.5 M EDTA (pH 8.0)
For one liter
Working solution Component:
Adding1 part of the 5X TBE buffer to 4 parts of deionized water

Dilutions:

(Preparation of Working Solutions from Concentrated Stock Solutions)

Dilution consists of adding additional solvent (usually water) to a solution to reduce its concentration. Stock solutions in biotechnology labs are concentrated and must be diluted before using. A buffer that is ten-fold more concentrated than the usable concentration is referred to as a 10X solution. One must dilute a 10X solution by a factor of 10 (by adding 1 part of the 10X stock to 9 parts of solvent) before using. To dilute a more concentrated stock solution to a less concentrated solution, the following formula is used:

C1V1 = C2V2 C1= original concentration (of stock solution) C2 = final concentration (of diluted solution) V2 = original volume (to be taken from stock solution) V1 = final volume (of diluted solution) Example: Calculate how many mL of a 1.0 M stock solution of NaCl are needed to prepare, 100 mL of a 0.050 M solution (also referred to as 50 mM). C1V1 = C2V2 (1.0 M)*(V1) = (0.050 M) * (100 mL) V1 = (0.050 M) * (100mL)/1.0 M V1 = 5.0 ml of stock

The original volume (V1) is almost always the quantity you must calculate, since you usually know the stock concentration and the final volume and concentration desired (if the final volume is not given, you should estimate how much you will need for the task at hand). It is not necessary for the volume to be measured in Liters in this formula, but the units for V1 and V2 must be the consistent.

Steps in Solution Preparation

1. Weigh out the desired amount of chemical(s). Use an analytical balance if the amount is less than 0.1 g.
2. Place chemical(s) into appropriate size beaker with a stir bar.
3. Add less than the required amount of double distilled water.
4. When the chemical is dissolved, transfer to a graduated cylinder and add the required amount of distilled water to achieve the final volume.
5. If the solution needs to be at a specific pH, check the pH meter using a pH meter.
6. Autoclave, if possible, at 121 °C for 20 minutes. Some solutions cannot be autoclaved, for example, SDS. These should be filter sterilized through a 0.22 μ m or 0.45 μ m filters.
7. Store at room temperature and check for contamination prior to use by holding the bottle at eye level and gently swirling it.