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| **Lab1: Molecular laboratory tools and equipment** |
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| **YEAR 3-SEMSTER 1** |

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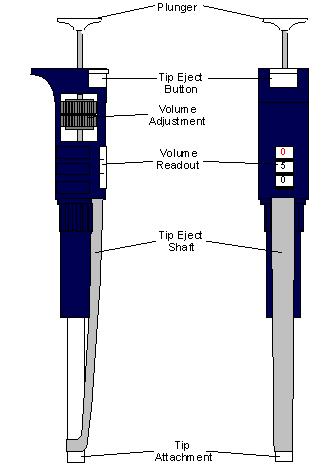
YEAR 3-SEMSTER 1

#### General Laboratory Procedures, Equipment Use, and Safety Considerations

**I.** **Equipment**

**A. Micropipettors**

Most of the experiments you will conduct in this laboratory will depend on your ability to accurately measure volumes of solutions using micropipettors. The accuracy of your pipetting can only be as accurate as your pipettor and several steps should be taken to insure that your pipettes are accurate and are maintained in good working order.Since the pipettors will use different pipet tips, make sure that the pipet tip you are using is designed for your pipettor. **DO NOT DROP IT ON THE FLOOR.** If you suspect that something is wrong with your pipettor, first check the calibration to see if your suspicions were correct, then notify the instructor.

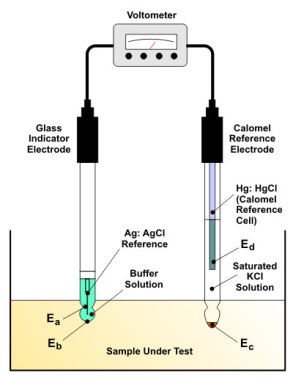


**B. Using a pH Meter**

Biological functions are very sensitive to changes in pH and hence, buffers are used to stabilize the pH. A pH meter is an instrument that measures the potential difference between a reference electrode and a glass electrode, often combined into one combination electrode. The reference electrode is often AgCl 2. An accurate pH reading depends on standardization, the degree of static charge, and the temperature of the solution.

**Operation of Orion PerpHecT pH Meter**

* Expose hole on side of electrode by sliding the collar down. Make sure there is sufficient electrode filling solution in the electrode (it should be up to the hole). If not, fill with ROSS filling solution only **(Do not use any filling solution containing silver (Ag**).
* Ensure that sample to be pHed is at room temperature and is stirring gently on the stir plate.
* Calibrate the pH meter with the two solutions that bracket the target pH - 4 and 7 or 7 and 10 as follows:
* Press the CAL key to initialize the calibration sequence. The last calibration range will be displayed (e.g. 7-4). Press YES to accept or use the scroll keys to select a different range. Press YES to accept.
* The number 7 will light up on the left hand side of the screen indicating that the meter is ready to accept the pH 7 standard buffer. Rinse off electrode and place in fresh pH 7 standard buffer solution. The READY light will come on when the value has stabilized. Press YES to accept the value.
* The number 4 (or 10) will light up next indicating that the meter is ready to accept the pH 4 (or 10) standard buffer solution. Rinse off electrode and place in fresh pH 4 standard buffer solution. The READY light will come on when the value has stabilized. Press YES to accept the value.
* SLP will be displayed. The meter will then go MEASURE mode.
* Rinse electrode and place into sample. The READY light is displayed when signal is stable.



**C. Autoclave Operating Procedures**

Place all material to be autoclaved in a autoclavable tray. All items should have indicator tape. Separate liquids from solids and autoclave separately. Make sure lids on all bottle are loose. **Do not crowd large number of items in tray- in order for all items to reach the appropriate temperature, one must allow sufficient air/steam circulation.**

1. Make sure chamber pressure is at 0 before opening the door.
2. Place items to be autoclaved in the autoclave and close the door. Some autoclaves require that you also lock the door after it's closed.
3. Set time - typically 20 minutes.
4. Temperature should be set at 121 deg C already, but double-check and change if necessary.
5. Set cycle: If liquid, set "liquid cycle" or "slow exhaust". If dry, set "dry cycle" or "fast exhaust" + dry time.
6. Start the cycle. On some autoclaves, the cycle starts automatically at step 5. On others, turn to "sterilize".
7. At the end of the cycle, check that: a. the chamber pressure is at 0; b. the temp is <100 deg C
8. Open door.
9. Remove contents using gloves and immediately tighten all caps.



**D. Operating Instructions for Spectrophotometer**

* To measure the absorbance of a solution in the short-wave range (<300 nM) use the quartz cuvettes. Disposable plastic cuvettes are available for reading in the visible range.
* Turn the spectrophotometer on - the switch is on the right in the back.
* Allow the instrument to calibrate. Do not open the chamber during this time. The deuterium lamp is OFF by default. To read absorbance in the UV range, turn the deuterium lamp on as follows after the machine has completed its calibration: Depress the function key until Fn5 is displayed. Press the mode key until d2on is displayed. Press enter. For best accuracy, the deuterium lamp should be warmed up for 20 minutes.
* Press the function key until Fn0 is displayed. Press enter. Using the up or down arrow keys, enter in the desired wavelength.
* Prepare a reference cuvette containing the same diluent as your sample.Prepare your sample.
* Place the reference cuvette in cell #1 and place your samples in cells #2-6.
* Press the cell key until cell #1 is in position. Press the Set Reference key to blank against the appropriate buffer. Press the cell key to advance to read the next sample.



**E.VORTEX**

A **vortex mixer** is a simple device used commonly in laboratories to mix small vials of liquid. It consists of an [electric motor](https://en.wikipedia.org/wiki/Electric_motor) with the drive shaft oriented vertically and attached to a cupped rubber piece mounted slightly off-center. As the motor runs the rubber piece oscillates rapidly in a circular motion. When a test tube or other appropriate container is pressed into the rubber cup (or touched to its edge) the motion is transmitted to the liquid inside and a [vortex](https://en.wikipedia.org/wiki/Vortex) is created. Most vortex mixers have variable speed settings and can be set to run continuously, or to run only when downward pressure is applied to the rubber piece.



**II.** **Preparation of Solutions**

**A. Calculation of Molar, % and "X" Solutions .**

1. A molar solution is one in which 1 liter of solution contains the number of grams equal to its molecular weight. Ex. To make up 100 ml of a 5M NaCl solution = 58.456 (mw of NaCl) g/mol x 5 moles/liter x 0.1 liter = 29.29 g in 100 ml of solution

**2. Percent solutions.**Percentage (w/v) = weight (g) in 100 ml of solution; Percentage (v/v) = volume (ml) in 100 ml of solution. Ex. To make a 0.7% solution of agarose in TBE buffer, weight 0.7 of agarose and bring up volume to 100 ml with TBE buffer.

**3. "X" Solutions.** Many enzyme buffers are prepared as concentrated solutions, e.g. 5X or 10X (five or ten times the concentration of the working solution) and are then diluted such that the final concentration of the buffer in the reaction is 1X. Ex. To set up a restriction digestion in 25 μ l, one would add 2.5 μ l of a 10X buffer, the other reaction components, and water to a final volume of 25 μ l.

**B. Preparation of Working Solutions from Concentrated Stock Solutions .**

Many buffers in molecular biology require the same components but often in varying concentrations. To avoid having to make every buffer from scratch, it is useful to prepare several concentrated stock solutions and dilute as needed. Ex. To make 100 ml of TE buffer (10 mM Tris, 1 mM EDTA), combine 1 ml of a 1 M Tris solution and 0.2 ml of 0.5 M EDTA and 98.8 ml sterile water. The following is useful for calculating amounts of stock solution needed: C i x V i = C f x V f , where C i = initial concentration, or conc of stock solution; V i = initial vol, or amount of stock solution needed C f = final concentration, or conc of desired solution; V f = final vol, or volume of desired solution

**C. Steps in Solution Preparation:**

1. Refer to a laboratory reference manual for any specific instructions on preparation of the particular solution and the bottle label for any specific precautions in handling the chemical. Weigh out the desired amount of chemical(s). Use an analytical balance if the amount is less than 0.1 g. Place chemical(s) into appropriate size beaker with a stir bar. Add less than the required amount of water. Prepare all solutions with double distilled water When the chemical is dissolved, transfer to a graduated cylinder and add the required amount of distilled water to achieve the final volume. An exception is in preparing solutions containing agar or agarose. Weigh the agar or agarose directly into the final vessel. If the solution needs to be at a specific pH, check the pH meter with fresh buffer solutions and follow instructions for using a pH meter. Autoclave, if possible, at 121 deg C for 20 min. Some solutions cannot be autoclaved, for example, SDS. These should be filter sterilized through a 0.22 μ m or 0.45 μ m filter. Media for bacterial cultures must be autoclaved the same day it is prepared, preferably within an hour or two. Store at room temperature and check for contamination prior to use by holding the bottle at eye level and gently swirling it Solid media for bacterial plates can be prepared in advance, autoclaved, and stored in a bottle. When needed, the agar can be melted in a microwave, any additional components, e.g. antibiotics, can be added and the plates can then be poured.
2. Concentrated solutions, e.g. 1M Tris-HCl pH=8.0, 5M NaCl, can be used to make working stocks by adding autoclaved double-distilled water in a sterile vessel to the appropriate amount of the concentrated solution.

**D. Glassware and Plastic Ware .**

Glass and plastic ware used for molecular biology must be scrupulously clean. Dirty test tubes, bacterial contamination and traces of detergent can inhibit reactions or degrade nucleic acid.

Glassware should be rinsed with distilled water and autoclaved or baked at 150 degrees C for 1 hour. For experiments with RNA, glassware and solutions are treated with diethyl-pyrocarbonate to inhibit RNases which can be resistant to autoclaving. Plastic ware such as pipets and culture tubes are often supplied sterile. Tubes made of polypropylene are turbid and are resistant to many chemicals, like phenol and chloroform; polycarbonate or polystyrene tubes are clear and not resistant to many chemicals. Make sure that the tubes you are using are resistant to the chemicals used in your experiment. Micro pipet tips and microfuge tubes should be autoclaved before use.