# GENERAL LAB PROTOCOLS

## 1. Using the Balance (Denver Instrument Pinnacle Series)

1. Make sure the balance is level (the bubble should be in the center of the circle).
2. Use the brush to clean the balance (including underneath the plate).
3. Tare the balance before weighing (include your weighing paper/weighing boat if necessary).
4. Allow the balance to stabilize before taking a measurement.
5. Make sure both of the side doors and the top door are closed before you tare/weigh.
6. Weighing range for the balance is <210 g.

## 2. Pipetting

### Pipette Aid

1. To open a pipette (serological pipette):
   - Hold the middle of the pipette in your left hand, with the tip down, the paper side of the wrapper to the left, and the plastic side of the wrapper to the right.
   - With your right hand, grip the top of the wrapper and pull to the right, breaking the paper side of the wrapper with the top of the pipette.
   - Pull the plastic side of the wrapper down and grip with fingers from your left hand.
   - With your right hand, grip the paper side of the wrapper and pull down; grip with fingers in your left hand. Insert the top of the pipette into the pipette aid.
2. Using the pipette aid: To aspirate liquid, press the top button. To eject liquid, press the bottom button. Always release the liquid from the pipette slowly.
3. Things to remember: Never touch the pipette with your hand, only touch the wrapper. Never touch the pipette with the outside of the wrapper. If you touch anything that is not sterile with the pipette, remove the pipette and put a new sterile pipette onto the pipette aid. Never aspirate the liquid too high into the pipette, if the liquid reaches the top of the pipette the filter on the pipette aid must be changed.

### Micropipette

1. Set the pipette to the correct volume (Check the pippetting range on the pipette, and for improved accuracy, match the volume you need to the upper limit of the pipette).
2. Put a clean tip onto the pipette. (yellow for <200 µl, blue for >200 µl)
3. To aspirate liquid: Depress the button on the top of the micropipette until you feel resistance, then place the tip into the fluid you are pipetting. Release the button slowly while the tip is fully immersed in the fluid to draw up the fluid. Remove the pipette from the fluid when the depressed button has been returned to its original position.
4. To eject liquid: Depress the button on the top of the micropipette until you feel resistance, then press the button all the way down to release the last drop of liquid out of the tip.
5. To release the tip: Depress the front button on the micropipette.
6. Periodically check the calibration of the micropipettes.
7. Never spray the micropipette or pipette directly with alcohol or cleaning fluid, always spray a paper towel and then wipe the pipette.

# 3. Standardization and Measurement Using the pH Meter (Accumet AB15)

## How does a pH meter work?

pH is the measure of alkalinity or acidity of a solution and can be represented by the equation:

$$pH = -\log[H^+]$$

where $[H^+]$ represents the concentration of hydrogen ions in the solution. The values for this concentration are represented as a scale from 0 to 14 where 0 is the most acidic, 14 is the most basic, and 7 is neutral (pure water is an example of a 7).

The pH meter receives a signal in millivolts from a glass bulb electrode which is immersed in the solution to be measured. This signal (which is triggered by hydrogen ions) is translated into a pH reading. The glass bulb electrode is always used in conjunction with a reference electrode, which completes the circuit and serves as a reference point.

This pH meter can also use a field effect transistor (FET) electrode to measure pH. This electrode uses a solid state ion-sensitive membrane in a transistor to measure the concentration of hydrogen ions. This electrode is paired with a counter electrode and a reference electrode to maintain a constant potential while the FET responds to the sample.

## Standardization of pH Meter – This should be done every time before use

1. Check to see that the fluid level within the electrode casing is ~1/4" below the top of the casing. Fill the casing with KCl electrode solution if the level is low.
2. Place a beaker under the electrode to catch the distilled water used for rinsing the electrode.
3. Before taking the meter off STANDBY, remove the electrode storage bottle from the electrode.
4. Obtain a wash bottle filled with distilled water. Rinse the electrode with the distilled water. Blot dry, never wipe, the electrode with a paper towel.
5. Place the electrode into the buffer solution that will be tested.
6. Take the pH meter off STANDBY, by pressing the "**stdby**" button once, after the electrode is immersed in a buffer solution of known pH.
7. On the top of the display screen, check to see if the system is on pH mode. If it is not, press and release the "**mode**" key until the digital display indicates pH mode. The key can switch between **pH**, **mV**, and **Rel mV** modes.
8. Clear the existing standardization of the meter by pressing the "**setup**" key twice and then the "**enter**" key.
9. Press the "**std**" key to access the standardization mode. Press the "std" key again when the pH reading is stable, which is noted by an icon that reads "STABLE" under the measurement.
10. The meter will automatically recognize the buffer and display the value on the screen.
11. Remove the electrode from the buffer solution. Rinse the electrode with distilled water, allowing the beaker to catch the rinse. Between every measurement, you should ALWAYS rinse the electrode with distilled water.
12. Blot-dry the electrode with a paper towel.
13. Place the electrode in another buffer solution of known pH. Repeat steps 9-11 for each buffer solution used to standardize the meter. The selected buffers should be at least two pH units apart.

## 3. Standardization and Measurement Using the pH Meter (Accumet AB15) (continued)

### Measurement of pH

1. Check to see that the fluid level within the electrode casing is ~1/4" below the top of the casing, and standardize the pH meter as described above.
2. Before immersing the electrode into the sample, always remember to rinse the electrode with distilled water. Between every measurement, you should ALWAYS rinse the electrode with distilled water and if possible, the fluid you will be measuring in.
3. After immersing the electrode into the sample solution, the display screen will adjust to display the pH of the sample. When the meter senses that the reading has stabilized, the STABLE icon will appear under the reading. The reading may be recorded at this time.
4. Repeat steps 2 and 3 for each sample that you are measuring pH for.
5. After finishing measurements, rinse the electrode with distilled water, blot dry the electrode, and place the electrode storage bottle back onto the electrode. Make sure the electrode is immersed in the storage solution in the storage bottle (if necessary, fill the storage bottle with storage solution).
6. Press "**stdby**" to put the meter back in standby mode.

NOTE: If you will not be using the electrode for consecutive measurements, but want to keep your calibration, place the electrode storage bottle back onto the electrode until you take another measurement.

# 4. Making Solutions

Objective: Precise production of chemical solutions will help to make experimental results reproducible. Thus, please follow the pertaining steps as closely as possible.

## Glassware preparation

1. Wash container with soapy water
2. Then rinse it twice in deionized water
3. Rinse once with the solvent of your desired solution (i.e. rinse with DMEM if you are making a solution containing DMEM and other additives)
4. Remove as much volume of solvent as possible

## Making solution

1. Calculate the mass/volume of solutes and mass/volume of solvents
    a. Note: The balance can measure masses above 10 mg accurately. If you need less than that, make a dilution of solution where you use at least 10 mg of solute.
    b. v/v% = (solute volume)/(total volume including solvent) x100%.
    c. w/v%= (grams solute)/(total volume of solution) x100%
    d. **Molarity** = moles solute per 1L of total solution
    e. **Molality** = moles solute per kilogram of solvent
2. Ask someone else in lab to double check your calculations
3. Add your solute and then bring the volume up to the desired calculated amount with the solvent. (i.e. 5M of salt is not 5 moles salt added to 1L of water)
4. Mix well – vortex if applicable

Note:

- Check storage directions – some solutions should be refrigerated!!!!
- Check if solution is light sensitive – if so, make solution in the dark
- Always wear gloves – especially if there is a toxicity warning on the MSDS sheet

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# 5. Using the Cell Culture Hood

Cell culture hoods maintain a sterile environment for cell culturing. The basis of operation is a mono-directional flow of filtered air that ensures no contamination to the cultures inside the hood. Air can be blown in two ways. The first is from the back of the hood towards the user. This method ensures that the cells do not get contaminated but does not provide protection for the user. The second (our hood) blows air from a small section in the ceiling directly behind the glass panel to a grid located at the bottom of the hood. This creates a wall of sterile air that separates the interior and exterior of the hood. It is therefore important to remember not to obstruct this air flow and to sterilize any body that enters the hood from the exterior (see below for exceptions).

## Sterilizing the hood

1. Ensure the hood is devoid of living material, solutions and any other body that may be altered by UV light.
2. Fully lower the glass panel until shut, turn off the blower and switch to UV light for 15 minutes minimum.
3. Switch the blower on, return light to ON and slowly raise the glass panel ensuring it never rises past the set threshold mark.
4. Wipe the hood workspace with 70% ethanol solution. Remember to wear latex gloves while performing this operation.

## Aspirator Setup

1. Clean the aspirator flask and tubes with the appropriate agent.
2. Place glass flask on the floor beside the hood.
3. Wipe down both tubes externally with 70% ethanol.
4. Connect the lower rubber tube (the one that connects just below the neck of the flask) to the yellow vacuum (VAC) tap in the hood.
5. Turn the vacuum tap on by aligning the handle with the nozzle (handle should be orthogonal to the wall).
6. Spray the second tube (the one with the rubber stopper that attaches to the top of the flask) with 70% ethanol both internally and externally and place the tube in the hood. Ensure it is safely inside the hood and won't fall out spontaneously.

## General Guidelines

1. Sign the log sheet.
2. Use sterile techniques while working in the hood.
   a. Use latex gloves at all times.
   b. Wear a lab coat.
   c. Use 70% ethanol to sterilize all equipment that will be used within the hood prior to usage (including your gloves each time you transition from outside to within the hood). The only exceptions are cell culture dishes.
   d. Wipe your work area with 70% ethanol before and after use of the hood.
   e. Do not raise the glass panel beyond the mark to ensure sterility within the hood.
   f. Do not obstruct air flow on the grid with excessive equipment.
   g. Refer to a senior member of the lab for further details and instructions.

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## Aspirator Dismantling

1. Turn off the vacuum tap by turning the handle parallel to the wall (make sure you are using gloves and they are sprayed with 70% ethanol).
2. Remove tubes from hood.
3. Dispose of the liquid in the flask according to protocol (may vary depending upon what is in the flask). Clean tubes and flask with soap solution and/or other necessary agents.

## Hood shutoff

1. Restock all solutions in fridge/shelves according to their specifications.
2. Note any items that need refilling/replacement such as Pasteur pipettes, pipette tips and centrifuge tubes.
3. Wipe the hood with 70% ethanol solution.
4. Close all pipette/tube containers.
5. Fully lower the glass panel until shut. Turn off the blower and lights. Good night!

# 6. Using the Incubator

Incubators provide an adequate homogeneous environment for cells to proliferate. Incubators actively control the temperature and atmosphere composition within them and 100% humidity can easily be achieved by adding distilled water in a container at the bottom. For virtually all cell cultures in the lab the operating parameters for the incubator resemble human physiologic conditions: 37°C, 4% $CO_2$ atmosphere (provided by a $CO_2$ tank) and 100% humidity. There are two doors to the incubator. The outer door is the main heat insulator and contains the controls panel and display. The inner glass door is used to further seal the incubator interior to preserve the atmosphere and to allow the user to visualize the samples without having to disrupt the internal conditions. It is important to work in a time-conscious manner when dealing with the incubator i.e. do not leave the doors open too long in order to maintain a relative constant environment for the cells to survive in.

## Placing samples in the incubator

1. Using latex gloves open the incubator's outer door and identify an appropriate spot for your samples through the glass door (do not open the glass door yet). If there is no space ask a senior lab member for advice in re-organizing samples. Always ensure an even distribution of samples in the incubator.
2. Check your samples for internal and external contamination. If samples seem contaminated or you are unsure ask a senior lab member for advice.
3. Open the glass door and gently place your samples in the incubator. Do not tip dishes to avoid spilling solutions and try to avoid sliding the samples along the metal shelves.
4. Promptly close both doors of the incubator. Remember to lock the glass door by turning the black knob.

## Removing samples from the incubator

1. Using latex gloves open the incubator's outer door and identify your samples through the glass door (do not open the glass door yet).
2. Open the glass door and carefully remove your samples from their spot. Do not tip dishes to avoid spilling solutions and try to avoid sliding the samples along the metal shelves.
3. Promptly close both doors of the incubator. Remember to lock the glass door by turning the black knob.

## General guidelines

1. Should any liquid spill in the incubator, wipe the area with 70% ethanol and report the spill.
2. Report any fungal growth and contamination of the incubator. Also alert a senior lab member if you notice that the water containing pan at the bottom is dry/almost dry.
3. Do not use the incubator to warm up/defrost solutions. Use the water bath instead.

# 7. Tecan Usage

Tecan's SPECTRAFluor Plus is a multifunctional microplate designed to replace several instruments. It also integrates into robotic systems for full automation. Suggested applications include cell and metabolic assays and protein or DNA quantification, using any plate format up to 1,536 wells. The SPECTRAFluor Plus measures fluorescence or absorbance from UV to near-IR wavelengths, as well as glow luminescence, and can be quickly switched between the different modes of measurement.

## Warming up

1. Turn SPECTRAFluor Plus on (switch on the back) and allow it to warm up for approximately 5 minutes.

## Loading Samples

1. Open "F:\Program Files\Tecan\XFluor4\XFluor4.xls." You can also access this by clicking on the Start button → All Programs → Tecan → XFluor4.
2. Wait for program to load and click on XFluor4 → Connect from the dropdown menu. An interface will appear.
3. Under Setup Port choose SPECTRAFluor Plus (default) under instrument and click OK.
4. A plate holder will move out of the machine. Load your plate and click on XFluor4 → Move Plate and Filter…
5. A small interface will appear with the options of moving the plate and both the excitation/absorbance and emission filters in/out.
6. Click on "Plate → In" and observe the plate move in.
7. Make sure the correct filters are loaded. You may make changes by moving filters in/out and replacing them with the correct filter. If you are unsure which filter you should use ask a senior member of the lab. Filters are located in the drawer beneath the SPECTRAFluor Plus.
8. When you are satisfied click OK.

## Analyzing Samples

1. To select from a range of readings click on XFluor4 → Edit Measurement Parameter…
2. An interface will appear with 5 labels: General, Plate, Meas. Params, Kinetics, and Shaking.
3. Select the appropriate settings for your assay. If you are unsure or if this is your first time using the machine please ask for supervision by a senior member of the lab.
4. Under "General" select the type of analysis you wish to perform. You may also select to have the machine automatically eject the plate at the end of the analysis.
5. For most assays you will be selecting a "Costar 96ft .pdf" under the "Plate" label.
6. The remaining labels will change according to the option you chose under "General." Fill the options in accordingly making sure you have inserted the correct filters. If not, cancel the assay and return to "Loading Samples, 4." above.
7. For Fluorescence studies set the "Gain" under "Meas. Params" to optimal for the first plate (containing the standards). See below for multiple plates.
8. When all the parameters have been established click OK.
9. Click on XFluor4 → Start Measurement to begin the reading.

9. At the end of the reading a new Excel spreadsheet will be generated containing the data.

## General guidelines

1. You should avoid leaving the plate holder in the "Out" position for too long.
2. When conducting assays make sure there are no air bubbles in your samples.
3. When conducting fluorescence (DNA-picogreen for example) on multiple plates using only one set of standards you must adjust the "Gain" under XFluor4 → Edit Measurement Parameter… → Meas. Params to "manual" and report the gain used for the plate containing the standards.
4. Always remember to sign in/out when using the machine.