

Biol/Chem 405 Biochemistry Laboratory I

Experiment 1 Introduction to the course, and pipetting exercise.

INTRODUCTION: Welcome to Biochem lab. This semester is designed to give you a chance to experience an integrated research-like laboratory. The whole semester is one long experiment. In place of individual week(s) of separate experiments, you will be working on a research style problem where critical skills and techniques are introduced throughout the semester embedded into the work you will be doing. The initial handouts will be more detailed and as the semester progresses, you will receive less specific instructions and instead, you will be involved in deciding what to do and how. Your handouts will give your guidance; there will be protocols on the web as well as streaming video (Tegrity files) to help you along. The intent is that your instructor will become a mentor/advisor rather than the instructor to tell you what to do step by step. The outcomes for the laboratory are that each student will by the end of the semester be able to think through a bench-based problem, become familiar and proficient with a number of critical skills and techniques, gain confidence in their own abilities to conduct "wet" biochemistry and learn to think more independently.

On the course website, there are a series of links to streaming videos called Tegrity files. These are a special set of short video/audio/presentations designed to help you through using some of the equipment in the laboratory as well as topics that typically are challenging for new biochemists. There will also be links to protocols for you to use. These protocols are reference materials designed to be more general in using equipment or methods. Specific instructions for your experiments will be in the handout. Listed on each handout will be a set of required reading. These readings will be supporting information and background help from the required textbooks. The handouts and class time will not be spent going over this material so it will be best if you take the time to read the assignment, it will make your time much easier and fun. Finally many of the laboratory experiments will have quizzes assigned. These are prelab quizzes and must be completed prior to coming to lab. The best way to get the most from this lab and avoid frustrations is to read the handout and assignments. The quizzes are designed to have straightforward questions that should help you focus on the key concepts or events of the experiments.

The first two weeks of the laboratory are more traditional and will introduce you to several important skills so that you can successfully navigate the experience planned for this semester. The rest of your time will focus on working with a fusion protein created just for this lab, Malate Dehydrogenase-GFP-6XHis (MDH-GFP). You will be working in a team of two students to first purify, quantitate and transform the plasmid DNA for MDH-GFP. Then each group will use the fluorescent properties of this protein to quantitatively detect the protein as you decide which of several chromatographies to use to purify the protein. You will learn to analyze the protein using SDS-PAGE and Western blot and finally you will create your own protocol to examine the kinetic properties of your purified protein. At the end of the semester, you will be required to write a professional publication-style paper on your results. One day of the semester is devoted to learning how to write scientifically. Remember, the goal for this semester is that this style of laboratory will help ensure that students explore and discover concepts through inquiry rather than simply achieve expected outcomes. Good luck and have fun.

Required Reading: At the Bench; pages 89-99, 165-184.

LABORATORY I – PIPETING AND USING THE SPECTROPHOTOMETER:

Pipetting:

Several different brands of micropipettors will be used in the laboratory, and it is important to familiarize yourself with the instrument you are using. Typically, these devices use disposable tips to hold the liquid. The tips should fit snugly over the ends of the pipettor. Different instruments may require different sizes of tips. A simple rule of thumb is that if it fits tight it should be OK, except for pipettes of 10 μ l and smaller. These pipettes use

the smaller tips, and only these pipettes. If the tip does not fit snugly to the instrument, then precise measurements cannot be made, because air will leak past the poor seal allowing solution to leak from the pipette.

To pick up a desired volume of liquid into the tip, adjust the volume adjustment knob just past the desired mark, then reverse to the correct volume. Then press down on the plunger until the first "stop" is encountered, and place the tip into the liquid to

be picked up. The tip should be just into the liquid. Too far and you are likely to leave additional liquid on the outside of the tip and this can lead to a significant error. Slowly release the plunger. Never snap the plunger up. Pause for a second or two. Then place the tip in the receiving vessel, and depress the plunger all of the way down past the first stop to the blow out region of the plunger

Simple pipetting tips

1. Always keep an eye on the tip to see if all of the liquid was drawn into the tip.
2. If you have picked up a significant amount of liquid with the tip touch it against a tube or a tissue, but do not wipe the tip. Capillary action will draw out some of the liquid.
3. Always add appropriate amounts of a single reagent first to reduce contamination
4. Release the liquid onto a new location in the tube or just into the liquid. DO not just shoot small volumes into the tube. This will lead to a very inaccurate pipetting.
5. Use a fresh tip when switching to a new reagent
6. If the tip becomes contaminated, switch to a new one.
7. Do not contaminate the stock reagent by using a used tip from one of your tubes
8. Pipettes can be used to mix samples but be very careful in that the solution does not get into the barrel of the pipettor
9. Do not lay the pipettor down or place the tip higher than the barrel while liquid is in the tip

Spectrophotometry:

Spectrophotometry refers to using the absorbance characteristic of some compound. Many (but not all) compounds will absorb in either the UV or visible light range. UV/Vis spectroscopy is often used to measure the presence of a substance and then quantify the amount of substance. Many transition metal ions and compounds with conjugated double bonds lend themselves well to this type of analysis. The basis of UV/Vis spectrophotometry is the Beer-Lambert law, which states that the absorbance of a solution is due to the solution's concentration.

Thus as long as one knows the molar absorptivity (you can look them up in tables) or use calibration curves (also known as standard curves) the actual concentration of a substance can be determined by measuring the

absorbance at its maximum absorbing wavelength.

$$A = \alpha lc$$

A is absorbance

α is the molar absorptivity

l is the pathlength of the cell

c is the concentration of the absorbing species

Fig 1. Beer-Lambert Law

The basic components of a spectrophotometer are a light source, a series of mirrors and gratings to select wavelengths and move the beam of light through our sample, and a detector to measure the light that has gone through the sample (transmittance). See the link on Beckman DU specs for a more complete background.

Spectrophotometers typically have absorbance maximums at 2.5 to 3.0 absorbance units (not absorbency – that is for paper towels). It is dangerous when using readings at the extremes of a machine. To best understand this, you need to remember that the instrument is reading transmitted light and then converting that to absorbance for you. Thus a high absorbance is the result of a very low transmittance. In this case, there is very little light to be detected and thus the noise of the machine may have just as much significance as your sample has. Ultimately, what this means is that your sample is absorbing nearly all of the light. Under these conditions you cannot tell if one sample is different from another.

Important points when using spectrophotometers:

- Make certain that your cell or cuvette is in the proper place in the holder and that the sample is in the right spot in the holder. DON'T assume it is already there.
- If using the sipper, look to see that your sample is going through the tubing – if not, look for the problem.
- When using UV (320 nm and lower) you must use a quartz cuvette. Glass and plastic will absorb most of the light at these wavelengths.
- Prepare a blank that has the buffer/solution your absorbing species is dissolved in.
- Shut off the light source when done, these lamps cost over \$300 each!

Most of the problems students' encounters are from the first two points!

Micropipetting Exercise

Exercise 1:

1. Place 10 ml of distilled water in 18 test tubes. Use the serological pipets.
2. Place the following volumes of 0.5 % bromophenol blue in the tubes. Each volume will be done in triplicate (three of each) 5µl, 10µl, 25µl, 50µl, 75µl, 100µl. Split the pipetting duties between partners.
3. Read the samples from least to most concentrated using the sipper and record the absorbance for each
4. Calculate the average and standard deviation for each point.
5. Graph the results. (NEXT WEEK) Average the readings and include the error bars (standard deviation) on the graph. Do the math and bring your results to class next week.

Exercise 2:

1. Acquire one of the pipettors with the correct sized tips and set the pipettor at an appropriate range. Record the size and style of the pipettor as well as the volume you've set. (Use a range between 20 µl and 200 µl)
2. Place a weigh boat on a balance and tare the weight to zero.
3. Draw up the designated volume of deionized water and dispense it onto the weighing boat by "shooting" it out with out touching the tip to anything. Record the weight of the water. Repeat for a total of four times.
4. Repeat step three but this time allow the tip of the pipet to touch the side while dispensing the water. Repeat for a total of four times.
5. Repeat step three again but place a small amount of water into the boat prior to taring. Now dispense the same volume of water four times, but now immerse the tip into the water. Repeat for a total of four times.

I. Record the weight you've measured for the three trials for step three.

Weight 1 (x₁) _____

Weight 2 (x₂) _____

Weight 3 (x₃) _____

Weight 4 (x₄) _____

II. Average the three trials: _____

III. Calculate the % error between the average of the four trials and the true value

$$\% \text{ error} = \frac{\text{Avg weight} - \text{expected mass}}{\text{expected mass}} \times 100 = \underline{\hspace{2cm}}$$

IV. Calculate the mean deviation for the three trials:

$$\text{Mean deviation} = \frac{\sum |x_i - X_{\text{avg}}|}{n} = \underline{\hspace{2cm}}$$

Repeat steps I through IV. For each of the methods of pipetting the water.

Discuss the error and % error mean deviation for each of the three methods.

What is the take home message for this exercise?