

# General, Organic, and Biological Chemistry II Lab Manual

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## Drawing Organic Compounds

### Objective

To build organic structures

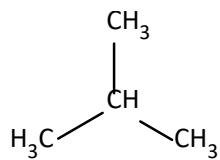
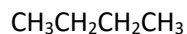
### Introduction

First, let's try to understand a bit more about structures before we draw some! Grab your model kits!

In most model kits, there are color designations for the atoms below. However, there is probably also a chart to help you with your kit:

| Atom     | Color          |
|----------|----------------|
| Carbon   | Black          |
| Hydrogen | White          |
| Oxygen   | Red            |
| Nitrogen | Blue           |
| Halogen  | Green          |
| Other    | Check your kit |

- 1) Let's start by building the molecules as presented below. When you are done, please show your instructor the models you built so they may record your completion for your grade.



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Faculty Initials that models are correct: \_\_\_\_

- 2) The models you built in example 1 are referred to as structural isomers. What do you think that could mean? Are the molecules identical or not?

Now that we have a bit more of an understanding of some basic chemical structures, let's practice! Make a model of each of the compounds below and draw the proper representations for each of the categories in the table below. They get harder as you go, and some involve applications of rules not explained above, but don't worry, your instructor is here to help when you get stuck! Structures 1-11. Each is worth **2 pts**, apart from Q9 (**1 pt**), and Q11 (**3 pts**)

| Entry | Condensed Structural Formula   | Expanded Structural Formula | Line-Angle Formula | Instructor Initials that Model Is Correct |
|-------|--|-----------------------------|--------------------|---|
| 1     | $\text{CH}_3(\text{CH}_2)_4\text{CH}_3$<br>The parentheses means this unit repeats. The 4 tells you how many times it repeats. |                             |                    |   |
| 2     | $\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)_2$   |                             |                    |   |

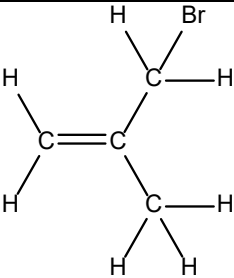
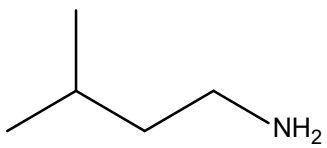
Name \_\_\_\_\_

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|   |  |  |  |   |
|---|--|--|--|---|
| 3 |  |  |  |   |
| 4 |  |  |  | <p>When you identify a bond to an atom that is not C or H, you must tell people what it is.</p> <p>(There are only 4 carbons here! Try to number them.)</p> |
| 5 |  |  |  | <p>Try to number the carbons again.</p>   |

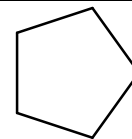
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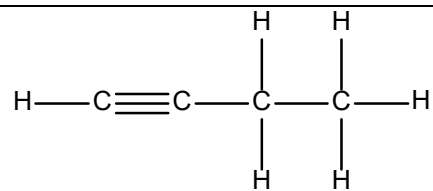
|   |  |   |  |  |
|---|--|---|--|--|
| 6 |  |  <p>(Yes, it is OK for a Carbon to not have Hydrogen associated with it!)</p> |  |  |
| 7 |  |   |  <p>When Hydrogen is connected to an atom that is not Carbon, we show that in the line-angle formula.</p> |  |
| 8 | $\text{CH}_3\text{CH}_2\text{NHCH}_2\text{CH}_3$ |   |  |  |
| 9 |  |   |  |  |

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10



11

Design a small molecule containing oxygen, carbon and hydrogen (only). Remember to follow the octet rule for all oxygen and carbon. Draw this structure as Entry 11 in the table.

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Lab Partner \_\_\_\_\_

## Nomenclature and Functional Groups

### Objective

- To learn how to name organic molecules
- To identify functional groups

### Introduction

To continue your understanding of organic molecules, you will learn to name compounds and identify them by the functional group that determines the physical and chemical properties of a molecule. In this lab you will identify the functional group in hydrocarbons, alcohols, thiols, ethers, amines, carboxylic acids, aldehydes, ketones, and amides.

Let's begin with the "root" names for different numbers of carbon in a chain:

- 1 Carbon = "meth"
- 2 Carbons = "eth"
- 3 Carbons = "prop"
- 4 Carbons = "but"
- 5 Carbons = "pent"
- 6 Carbons = "hex"
- 7 Carbons = "hept"
- 8 Carbons = "oct"
- 9 Carbons = "non"
- 10 Carbons = "dec"

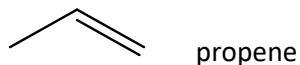
### Alkanes

If a hydrocarbon contains carbon-carbon single bonds, then these are referred to as alkanes. To name alkanes, simply put the root name of the longest chain and finish with the ending of "ane".

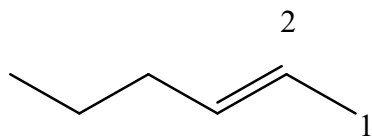
|                                |         |
|--------------------------------|---------|
| CH <sub>4</sub>                | methane |
| C <sub>2</sub> H <sub>6</sub>  | ethane  |
| C <sub>3</sub> H <sub>8</sub>  | propane |
| C <sub>4</sub> H <sub>10</sub> | butane  |

### Alkenes

If there is at least one double bond present between carbons, we refer to these compounds as alkenes. As above, we name these by noting the root of the word and ending the word in "ene".



We need to state WHERE the double bond begins, by "counting" the carbons. We can do this from any side of the molecule, but you want to number so that the first carbon you run into with the double bond has the **lowest number** possible.

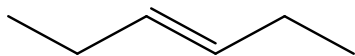


2-hexene or hex-2-ene  
(the numbers are here to help you in this case.)

Name \_\_\_\_\_

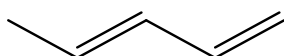
Lab Partner \_\_\_\_\_

In this case, we begin counting from the right side of the molecule and the name of the molecule becomes 2-hexene indicating that the double bond BEGINS on the second carbon and continues to the next highest carbon. Note the difference between this compound and one that would be named 3-hexene. When placing numbers before the name of a compound, put a dash between the number and the name.



3-hexene or hex-3-ene

Sometimes, there will be a more complicated molecule that has TWO double bonds between carbon atoms. These are referred to as dienes. In these cases, we need to use numbers to denote where both of the double bonds occur, giving the lowest numbers possible to each. Then, finish the root of the word with “diene”.

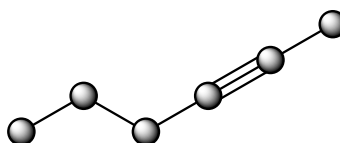
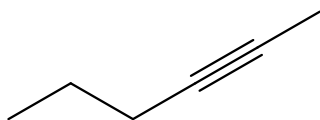


1,3-pentadiene or penta-1,3-diene

Note, now that we have 2 numbers, we need to put a comma between them to keep them distinct.

### Alkynes

When there is a triple bond in a molecule, it is called an alkyne. You name these just as you would an alkene, but with the end of the word being “yne”.

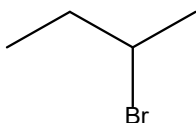


2-hexyne (both examples)

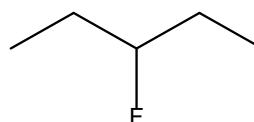
Note that in this case, the carbons on each side of the triple bond are aligned with the triple in a straight line. This is due to the linear geometry around these carbons. There are SIX carbons in this structure. Make sure you can note the location of each.

### Alkyl Halides

Recall that group 7A elements are referred to as Halogens or Halides. By having these halogens connected to a carbon chain, we refer to these compounds as alkyl halides. To name alkyl halides, keep the name of the carbon chain (methane, ethane, propane) and add a prefix of the halide with the following: fluoro, chloro, bromo, or iodo. The carbon that is attached to the halogen should have the lowest possible number.



2-bromobutane



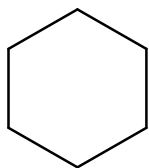
3-fluoropentane

### Cyclic Compounds:

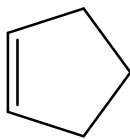
When carbon chains are connected to themselves, we call these cyclic compounds or rings. To name these compounds, count the number of carbons in a ring and place the prefix of “cyclo” before the rest of the name.

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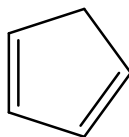


Cyclohexane



Cyclopentene

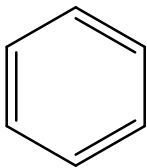
Again, if there is more than one double bond, number the double bonds so that the total location number will be as low as possible.



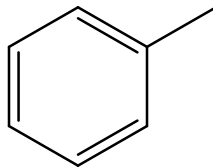
Should be named 1,3-cyclopentadiene and NOT 1,4-cyclopentadiene

### Benzene:

Benzene is a widely used structure both in and outside of the body and is incredibly stable. It features 6 carbons in a ring with alternating single and double bonds between the atoms. We can have substituents on these compounds as well.



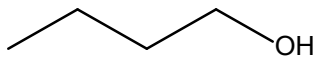
benzene



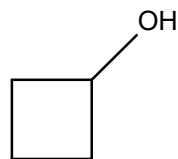
methylbenzene

### Alcohols:

Alcohols have an "OH" group attached to a carbon. To name alcohols, we name the rest of the molecule as you normally would (i.e. methane, ethane, butane), but replace the "e" at the end of the word with an "ol" indicating that the compound has an alcohol present.

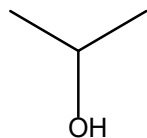


1-butanol or butan-1-ol

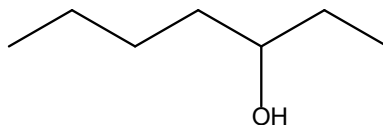


cyclobutanol

It is also possible to have alcohols that appear in the middle of the carbon chain. Number these as you have the alkenes and alkynes, where the lowest number goes where the alcohol group resides.



2-propanol or propan-2-ol



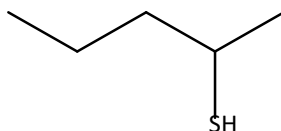
3-heptanol or heptan-3-ol

### Thiols

Thiols are very similar to alcohols, but instead contain an "SH" group. Name the carbon chain and then end that name with the term "thiol".

Name \_\_\_\_\_

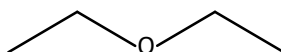
Lab Partner \_\_\_\_\_



2-pentanethiol

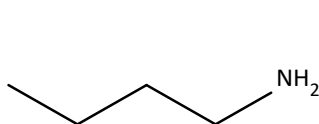
### Ethers

Ethers contain an oxygen connected between TWO carbons. Though we will not name these compounds in this lab it is important to recognize the functional group.

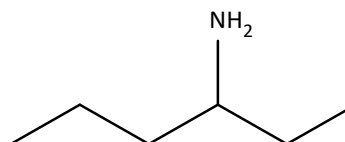


### Amines

Amines have a nitrogen attached to the carbon chain. The simplest version of these has "NH<sub>2</sub>" attached to a carbon chain or ring as seen below. Name amines with the same types of rules as with alcohols, but replace the "e" at the end of the word with "amine".



butanamine

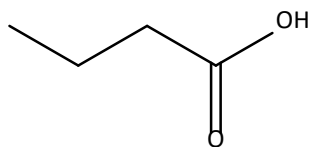


3-hexanamine

However, amines can get a bit more complex. At times, the nitrogen in amines may have more than one carbon attached to them! We will cover this later in the semester!

### Carboxylic Acids

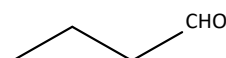
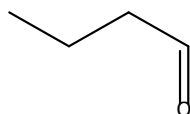
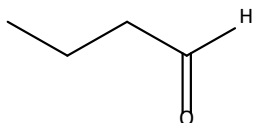
Carboxylic acids have a carbon at the end of a chain bonded to two oxygens. Take the name of the carbon chain INCLUDING the carbon in the carboxylic acid and remove the "e" and replace it with "oic acid" so that the name is now two words instead of one. Be careful, there are multiple ways to draw carboxylic acids, as seen below.



butanoic acid (all three examples!)

### Aldehydes

Aldehydes have a C=O bond at the end of the chain. Take the name of the carbon chain INCLUDING the carbon in the aldehyde, remove the "e" and replace it with "al". The aldehyde functional group can also be represented as "CHO". At times, structures of aldehydes show the hydrogen attached to the carbon with the C=O bond, and at other times, they do not. Do not let this confuse you – the carbon on the end in all cases has a C=O bond, and that is what makes it an aldehyde.



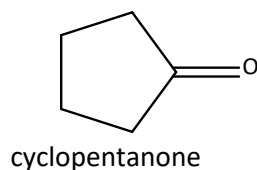
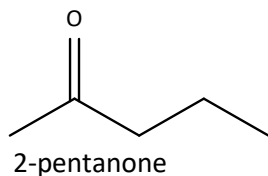
butanal (all examples!)

Name \_\_\_\_\_

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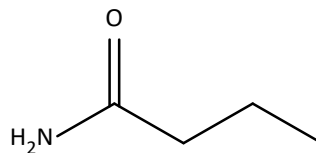
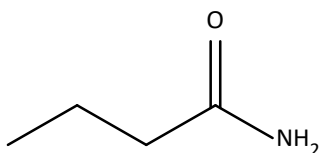
### Ketones

Ketones have a C=O bond that is NOT at the end of the chain. To name these, count as we have previously to give the C=O bond the lowest number possible in the chain and replace the ending of “e” with “one”.



### Amides

Amides are incredibly important in your body. They have a structure that ends with a single C that is attached to BOTH an O and a N. Similar to carboxylic acids, these compounds commonly have a CONH<sub>2</sub> motif. As with amines, N can also be attached to other carbons, but we will not name that type of compound at this time.



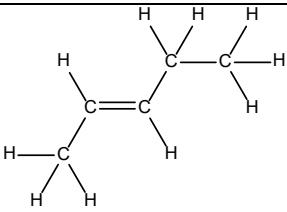
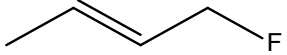
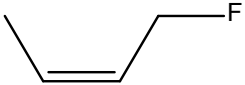
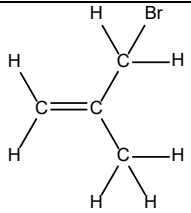
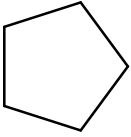
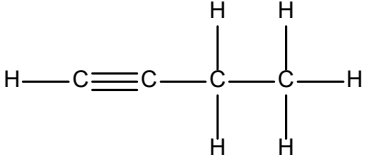
Butanamide (both examples)

Name \_\_\_\_\_

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**Lab 2 Laboratory Procedure**

Since you have already made some models in our last lab, try to name these compounds below. The last two entries are names, and you should instead draw the structure of the molecule. You will find some guides on naming at the beginning of this lab, using these items and what you have named in class, let's name what you identified in Lab 1.

| Compound  | Name                    |
|---|-------------------------|
| $\text{CH}_3(\text{CH}_2)_4\text{CH}_3$   |                         |
| $\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)_2$  |                         |
|    |                         |
|  <p>*This compound has a fluorine atom that is a substituent. We identify this as a "fluoro" group</p> |                         |
|    |                         |
|    |                         |
|    |                         |
|    |                         |
|   | 1,3-dimethylcyclohexane |
|   | propylcyclobutane       |

Name \_\_\_\_\_

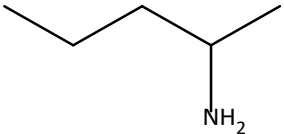
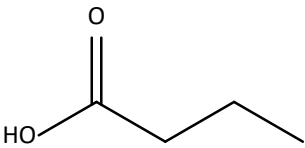
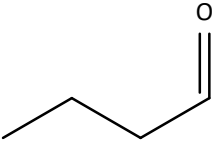
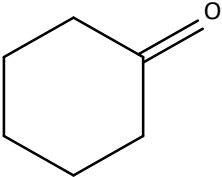
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**Lab 2 Functional Groups con't**

Name \_\_\_\_\_

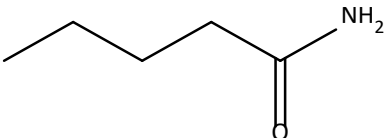
Functional groups are organic compounds containing different groups of atoms. Alkenes are an example of a functional group, since all alkenes have a double bond between two carbon atoms. Using the additional information in the naming guide at the end of this lab, you will construct models and name compounds with different functional groups in the table below. As with Lab 1, your instructor will need to verify that your models are correct.

| Entry | Condensed Structural Formula                        | Line-Angle Formula  | Name | Instructor Initials that Model Is Correct |
|-------|---|---|------|---|
| 1     | $\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$        |   |      |   |
| 2     | $\text{CH}_3\text{CHSHCH}_3$                        |   |      |   |
| 3     |   |    |      |   |
| 4     |   |   |      |   |
| 5     |   |  |      |   |
| 6     | We do not write condensed formulas cyclic compounds |  |      |   |
|       |   |   |      |   |



Name \_\_\_\_\_

Lab Partner \_\_\_\_\_

|   |   |   |         |  |
|---|---|---|---------|--|
| 7 | We do not write condensed formulas cyclic compounds |   | Benzene |  |
| 8 |   | <br><chem>CCCC(=O)N</chem> |         |  |

Name \_\_\_\_\_

Lab Partner \_\_\_\_\_

## Thin Layer Chromatography

### Objectives

- Prepare plates, and then run and analyze samples via Thin Layer Chromatography (TLC)
- Determine the identity of an unknown over-the-counter (OTC) medication through identification of its active ingredients via TLC

### Introduction

Have you ever seen a piece of fabric on which ink has been spilled, and the ink spreads out into its constituent colors? The colors spread in this way due to polarity differences in the dyes that account for these colors.

In this experiment, we will apply this concept of polarity difference, through a technique known as Thin Layer Chromatography (TLC), to identify the active ingredients in the over the counter pain relief medications Excedrin and Anacin. The active ingredients of interest are Aspirin, Caffeine and Acetaminophen (shown in Figure 1). Not all OTC medications contain all three of these active ingredients, therefore the goal of this experiment is to experimentally determine which active ingredients are present in Excedrin and Anacin.

Chromatography is the science of separation. Separation can be based on size, polarity or other factors. "Thin Layer" refers to a thin layer of silica gel, adhered to a plastic sheet (this is the TLC **plate**).

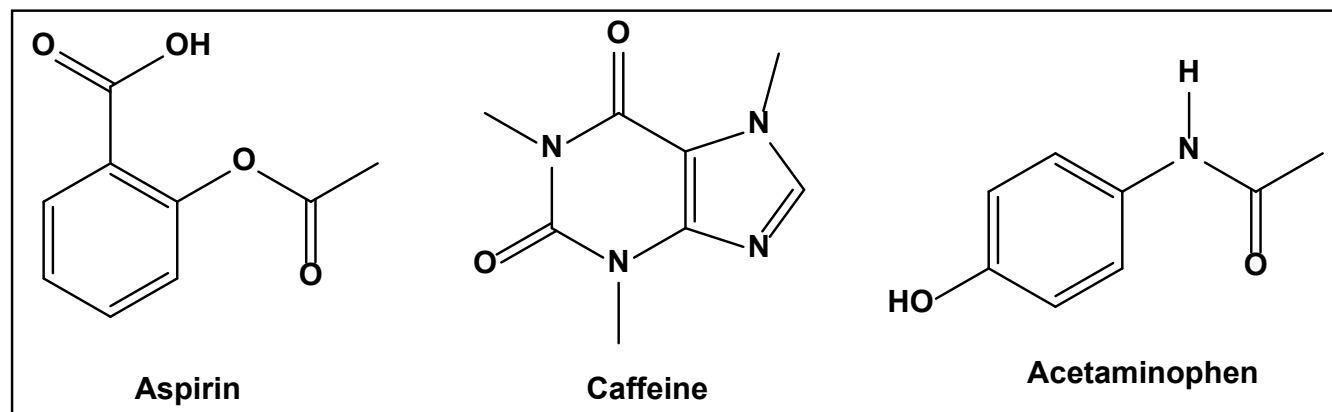


Figure 2. Bond-line structures of Aspirin, Caffeine and Acetaminophen

### Experimental

#### Working in pairs:

1. Two TLC plates in total will be needed for this experiment - one plate will be for the three knowns (aspirin, caffeine and acetaminophen), and the other plate will be used for our two unknowns, **1** and **2** (one unknown is Anacin, the other is Excedrin) and one of the known samples (you choose which one to use).

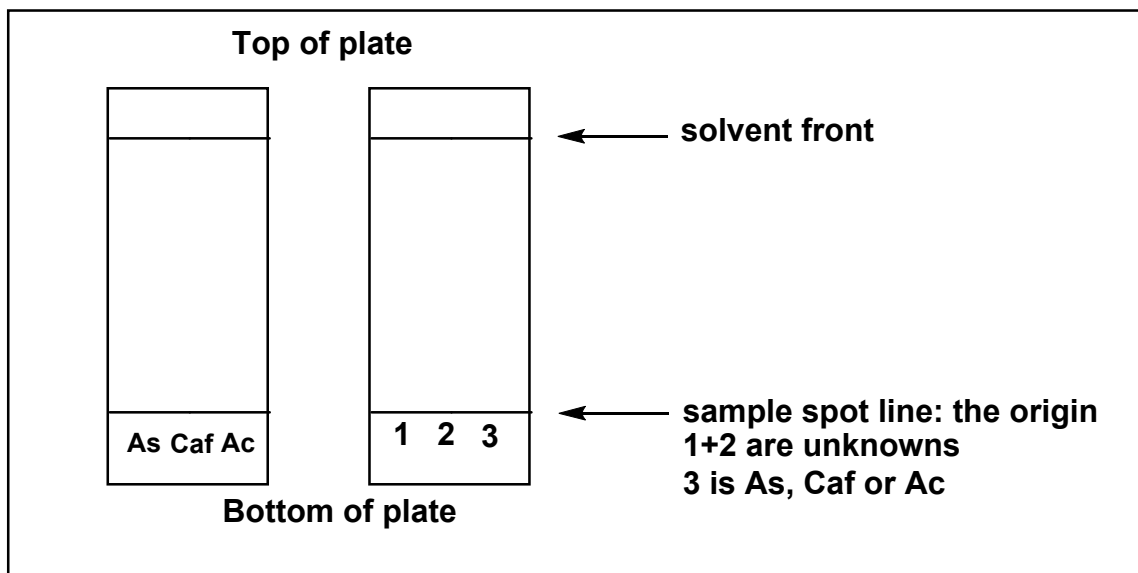
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2. Prepare each plate by (carefully and lightly) drawing two straight pencil lines (non-mechanical pencils work best for this, as they are less likely to break the thin layer of silica gel when drawing lines) – one line 1 cm from the bottom of the plate, and the other 0.5 cm from the top.

**Note - your instructor may give you different instructions for this step.**

3. Clearly label the two plates **in pencil**, on the rough white side of each plate. A labelling example is shown in **Figure 3**.



**Figure 3. Labelled TLC plates before sample spotting**

4. **Carefully** spot a **tiny amount** of each known solution (aspirin, caffeine, and acetaminophen) onto the sample spot line on the known's TLC plate using microcapillary tubes (your instructor will show you how to do this). **Use a fresh microcapillary tube for each solution.**
5. Place the TLC plate containing the three knowns spotted samples under the UV lamp. The presence of a dark spot on the sample spot line for each sample is an indication that enough of that compound is present for effective TLC analysis. If the spot cannot be seen or is very faint, that means too little compound is present. In this case, use a fresh microcapillary tube and spot more of the same sample over the original spot.
6. Prepare the elution chamber by adding enough elution solvent to cover the bottom of a 100 mL beaker. This is called the mobile phase (0.5% acetic acid in ethyl acetate). In order to obtain meaningful results, the sample spot line, the origin, on the TLC plate needs to be higher than the solvent level.
7. Place the TLC plate into the elution chamber. This needs to be done carefully: using a pair of tweezers will make it easier to perform. The bottom of the plate needs to be inserted parallel with the plane of the solvent (your instructor will demonstrate this technique). Place a watch glass over the top of the 100 mL beaker. Once the plate is in the chamber, it can be left until the solvent has reached the solvent front pencil line.
8. After the solvent has reached the solvent front pencil line, remove the TLC plate from the elution chamber, dry it by shaking it for a few seconds, and then place it under the UV lamp. Using a pencil, circle all spots that are present. An example of an eluted TLC plate under the UV lamp is shown in **Figure 4**.

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**Figure 4. Example of a TLC plate under UV light, showing the presence of eluted spots**

9. Repeat steps **4 - 8** on the second TLC plate for the unknown mixtures **1** and **2**, adding one of the knowns to this plate (your choice).
10. Dispose of any waste chemicals / glassware as indicated by your instructor.

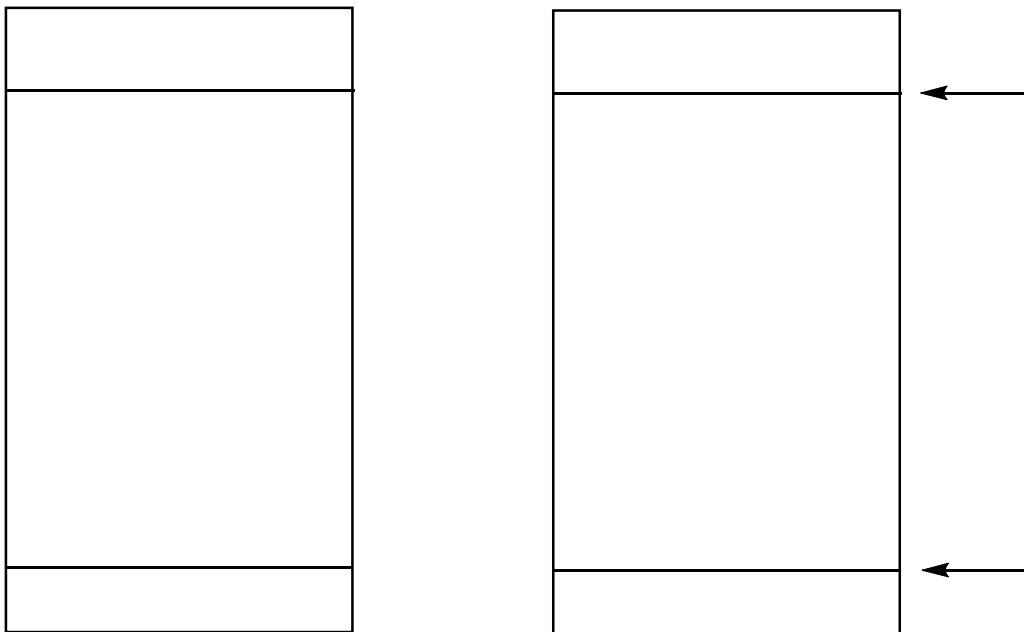
### **Analysis**

1. Show your experimental data in the representative TLC plates given below. Be sure to include, and, label all of the following:
  - a. Solvent front.
  - b. Origin.
  - c. The three knowns.
  - d. The two unknowns, and the stated known of your choice.
  - e. All of the measured distances obtained between:
    - (i) the center of each spot, and the origin (cm). This is the distance travelled by each component.
    - (ii) the origin and the solvent front (cm). This is the distance travelled by the solvent.

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**Top of plate**



**Bottom of plate**

2. Looking at the three knowns, please answer the following:

a. Which known component travels the smallest distance? \_\_\_\_\_

b. Which known component is more polar (interacts more with the TLC plate)? \_\_\_\_\_

c. Which known component is the least polar (interacts less with the TLC plate)? \_\_\_\_\_

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3. Using the measured distances obtained from the experimental TLC plates, now calculate the  $R_f$  values for **each** spot on **both** plates. Must show all numbers!

**Thought question:** How do you think the units for Retention Factor should be reported?

|  | Calculation of each retention factor | Retention Factor |
|--|--------------------------------------|------------------|
| Aspirin  |                                      |                  |
| Caffeine   |                                      |                  |
| Acetaminophen  |                                      |                  |
| Unknown 1 – spot 1                                   |                                      |                  |
| Unknown 1 – spot 2                                   |                                      |                  |
| Unknown 1 – spot 3*                                  |                                      |                  |
| Unknown 2 – spot 1                                   |                                      |                  |
| Unknown 2 – spot 2                                   |                                      |                  |
| Unknown 2 – spot 3*                                  |                                      |                  |
| Known developed on the<br>unknown plate was<br>_____ |                                      |                  |

\*If applicable

4. Which of Aspirin, Caffeine and Acetaminophen is/are present in **unknown 1**, and, is this unknown Anacin or Excedrin? Explain how this can be determined.

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5. Which of Aspirin, Caffeine and Acetaminophen is/are present in **unknown 2**, and, is this unknown Anacin or Excedrin? Explain how can this be determined?

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## Synthesis of Aspirin

### Week One

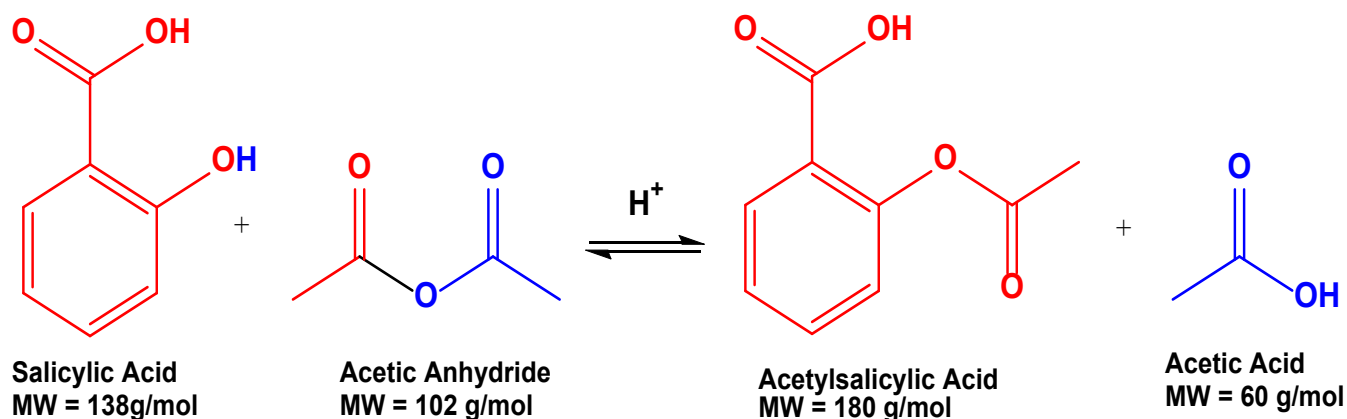
This lab will take two weeks to complete. During the first week, you will synthesize crude aspirin and determine the % yield. During the second week, you will recrystallize the impure aspirin, calculate the % recovery and then perform TLC analysis of both the crude and pure aspirin products.

### Objectives

Prepare a sample of crude aspirin.

### Introduction

The industrial scale synthesis of aspirin involves several steps, the last of which is shown in Figure 1. Salicylic acid is reacted with acetic anhydride (in the presence of an acid catalyst), to form aspirin and acetic acid. This industrial synthesis is the reaction that we will be using in this experiment.



**Figure 1. Synthesis of aspirin from salicylic acid and acetic anhydride.**

The equation shown in **Figure 1** is balanced as written; thus, one mole of salicylic acid reacts with one mole of acetic anhydride to give one mole of acetylsalicylic acid (aspirin). The  $H^+$  on top of the arrow is an acid catalyst; without this, the reaction would occur too slowly to be practical.

The use of color in **Figure 1** shows how the reaction product is formed during the reaction. The **red atoms from the salicylic acid** combine with the **red atoms from acetic anhydride** to form the **aspirin** product, and the **blue atoms from each molecule combine to form the acetic acid side product**.

This reaction goes only partially to completion (it's an example of an *equilibrium* process); the acid catalyst greatly accelerates the reaction, but, like all catalysts, it cannot make a reaction go all the way to completion. Thus, the reaction will be stopped as soon as it has reached equilibrium, even though less than 100% of the products are formed at that point. The aspirin product must then be isolated and purified, to remove unreacted salicylic acid and acetic anhydride, as well as the acetic acid side product.



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## Experimental Procedure

### A. Preparation of Acetylsalicylic acid

1. Accurately weigh 1g of salicylic acid to the nearest 0.01g. Transfer this to a clean, dry 50 mL Erlenmeyer flask.
2. Measure out 4 mL of acetic anhydride in a graduated cylinder and add it to the flask. (**WARNING! Acetic anhydride will burn, so handle it carefully. Keep it off your skin, clothes and lab bench.**) If you come into contact with acetic anhydride, notify your instructor and immediately wash your hands with soap and water until they no longer smell like vinegar.
3. Swirl the flask gently to mix all the salicylic acid in the acetic anhydride. Add two drops of phosphoric acid ( $\text{H}_3\text{PO}_4$ ). The salicylic acid may not completely dissolve at room temperature. (**WARNING! Although Phosphoric Acid is not a particularly strong acid, it still needs to be handled with care. Keep it off your skin, clothes, and lab bench.**) If you come into contact with phosphoric acid, notify your instructor and immediately wash your hands with soap and water.
4. Heat this mixture in a water bath at 60 - 70 °C, made using a 250 mL beaker on a hot plate. Be sure to add a single boiling stone to the water bath. Make sure that the temperature of the water bath is 60 -70 °C before inserting the Erlenmeyer flask. Heat this solution with an occasional swirling for 20 minutes. Record the appearance of the reaction mixture (compare before, during and after 20 minutes). Also record the temperature of the bath.
5. During this 20-minute heating period, calculate the theoretical yield of aspirin (in g), based on the mass of salicylic acid weighed out in step 1.
6. At the conclusion of the heating period, slowly add around 2 mL of distilled water into the flask, to destroy any excess acetic anhydride. Remove the flask from the bath and allow it to cool for a few minutes at room temperature. Add around 10 -20 mL of ice chips (more can be added as needed) and swirl the flask to facilitate mixing. **It is the addition of ice that will cause precipitation of the product.** Place the flask in ice to cool it further. White crystals should appear as the mixture cools. If no crystals appear, try scratching the inside of the flask with a stirring rod or spatula – this may promote crystal growth.
7. The acetylsalicylic acid will be collected by vacuum filtration (your instructor will direct you as to how to perform this filtration). After pouring your product mixture into the filter funnel, rinse out the remaining solid product in the reaction flask with a small amount of ice-cold distilled water, and add to the filter funnel. Wash the crystals with ice-cold water to melt any remaining ice chips; this will also help to remove any water-soluble impurities.
8. Carefully remove the crystals (with the filter paper) from the funnel and transfer them to a pre-weighed watch glass. Dry the crystals further by placing them on a watchglass in the oven. Weigh this crude product to the nearest 0.01 g after it is cool and dry (product is more than likely dry if it does not stick to a spatula). Use this mass of dried aspirin, to calculate the % yield of crude aspirin.

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9. Save a small amount of crude product (about half the size of a grain of rice) for TLC analysis. Save the crude acetylsalicylic acid. Your instructor will setup vials for storage.

### Data Sheet

-Use full sentence to write down your entire observations from this experiment. Must include the following words in your description: dissolve; crystals; wet; dry; white; acrid smell; cold and hot.

-Theoretical Yield of aspirin product (in grams). **Show all of your work below:** \_\_\_\_\_

-Actual Yield of Product (crude, in grams): \_\_\_\_\_

$$\text{Percentage yield} = \left( \frac{\text{Actual yield}}{\text{Theoretical yield}} \right) \cdot 100$$

-Percent Yield of Product (crude). **Show your full working below:** \_\_\_\_\_

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## Purification and Analysis of Aspirin

### Week Two

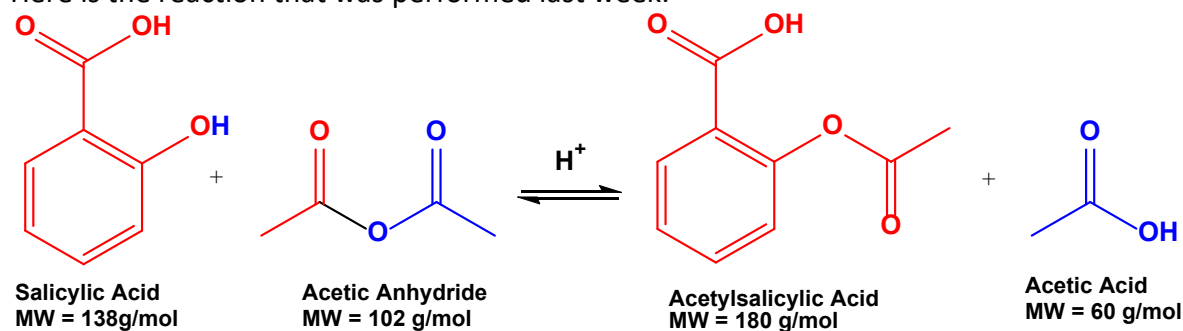
This is the second week of the two-week aspirin lab. You will recrystallize the impure aspirin that was saved from last week, calculate the % recovery and then perform TLC analysis of both the crude and recrystallized aspirin products, and the pure aspirin standard.

### Objectives

- Purify the crude aspirin via recrystallization.
- Analyze the purity of the crude and recrystallized samples of aspirin via thin-layer chromatography.

### Introduction

Here is the reaction that was performed last week:

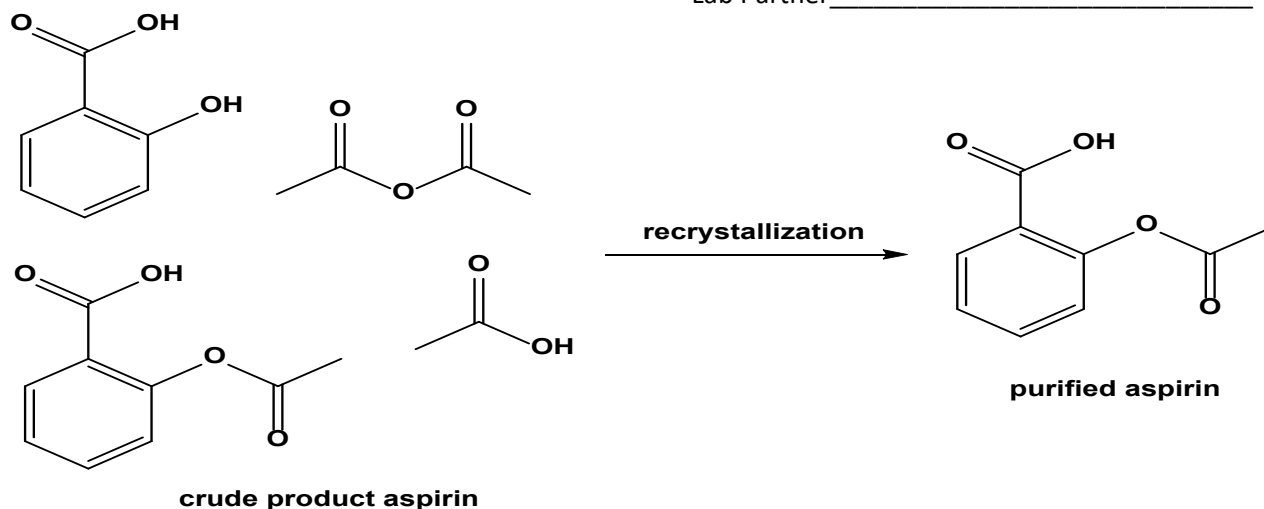


**Figure 1. Synthesis of aspirin from salicylic acid and acetic anhydride.**

An impure solid sample which is primarily one substance but that contains some impurities (as illustrated below in **Figure 2**) can be purified by a process known as **recrystallization**. *The desired substance must completely dissolve in a selected solvent at a high temperature, but it must be only slightly soluble in this solvent at a low temperature.* The solvent is added in small volumes to the impure solid, and heated until the impure solid is completely dissolved. This solution is then left to cool, and as it does so, the desired product will precipitate out. Since the impurities are present in relatively small amounts, they will remain in solution. The substance thus obtained will be purer than before recrystallization.

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**Figure 2: Recrystallization to purify crude aspirin**

To determine if the recrystallization was successful, the impure and pure samples of aspirin will both be analyzed by thin-layer chromatography (TLC). The procedure for performing this TLC analysis will be like the procedure used in the “TLC Analysis of Over-the-Counter Medications” experiment.

## Experimental Procedure

### B. Purification

1. Remove a very small amount of crude aspirin (no more than the tip of a small spatula), and place in the dimple of a spot plate to use later.
2. On a hot plate, heat ~ 10 mL of ethanol in a small beaker containing a boiling stone. Transfer the rest of the crude acetylsalicylic acid into a 50 mL Erlenmeyer flask and dissolve the crystals in a minimum amount of hot ethanol, warming and swirling the solution on the hot plate. Start with 2mL of ethanol, and if needed, add 1 mL portions of ethanol until all the sample has just completely dissolved. Keep track of the total volume of ethanol used. Swirl the flask occasionally during this process but keep it on the hot plate until all the solids are dissolved.
3. When all the crystals have dissolved, remove the flask from the hot plate, allow it cool for about 5 minutes, and then slowly add about twice the volume of distilled water as added for ethanol, to cause recrystallization. This is an example of a mixed-solvent recrystallization. Since water is more polar, it will decrease the solubility of acetylsalicylic acid in ethanol. Cool in an ice bath until recrystallization is complete. Filter, wash with ice-cold water, and dry the crystals as before.
4. Determine the weight (in g) of the recrystallized, dry acetylsalicylic acid and determine the % yield based upon your theoretical yield. Also determine the % recovery after recrystallization, based upon the yield of crude acetylsalicylic acid. Label it as such in your notebook.
4. Give the dry, recrystallized sample of acetylsalicylic acid to your instructor, and dispose of all the waste chemicals as directed.

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### C. TLC Analysis

1. Using the methodology learned in the "TLC of over-the-counter medications" experiment, prepare a TLC plate with a pencil line origin about 1cm up from the bottom of the plate (remember to wear gloves when handling the TLC plate).
2. Place a small amount of both recrystallized aspirin and the pure aspirin standard, into two separate wells of a spot plate. *The crude aspirin should already be in a separate dimple.* Add a few drops of acetone to each dimple and stir. Carefully spot these samples (crude aspirin, recrystallized aspirin and pure aspirin) onto the bottom pencil line. Check the plates under a UV lamp to make sure that they are visible.
3. Using tweezers, place the TLC plate into a TLC chamber. The TLC mobile phase is 1:1 hexane: ethyl acetate. Remove the plate when solvent elution is complete, and mark off the solvent front with a pencil. Dry the plate and mark the location of the spots when placed under the UV lamp.
4. Calculate an  $R_f$  value for each spot observed on the plate.

### Data Sheet

-Using your own words and full sentences, write down your observations for the purification procedure. Must include the following words in your description, but not limited to: hot, ice cold, dry, dissolve, °C, crystals, ethanol, water, and ml.

-Actual yield of product (**purified**, in grams): \_\_\_\_\_

-Theoretical yield of aspirin product from last week (in grams): \_\_\_\_\_

$$\text{Percentage yield} = \left( \frac{\text{Actual yield}}{\text{Theoretical yield}} \right) \cdot 100$$

-Percent yield of product (**purified**): \_\_\_\_\_ (*Show all of your work below*)

-Actual yield of product (**crude**, in grams): \_\_\_\_\_

- Percent recovery after recrystallization, based upon the yield of crude acetylsalicylic acid.

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***(Show all of your work below)***

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-Draw your developed TLC plate below. Include the following:

-title e.g., this is a developed TLC plate of ... (*complete the rest*)

-all relevant labels of lines and spots for both the crude and purified aspirin, and aspirin control

-all measurements and  $R_f$  calculations

-Give a reasoned explanation regarding the purity of your purified product.



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# Serial Dilution of Food Dye

## Lesson Objectives

- Perform serial dilutions to prepare solutions of specified concentrations from a stock solution
- Be able to accurately use a micropipette.

## Introduction

In this exercise, you will systematically dilute solutions of food dye with water. You will observe how the color intensity, or saturation, of each subsequent solution changes as it becomes more dilute. You will calculate the concentration of each of the diluted solutions. You will also check the accuracy of your pipetting skills by developing a “secret code” in a microtiter culture plate.

## Experimental

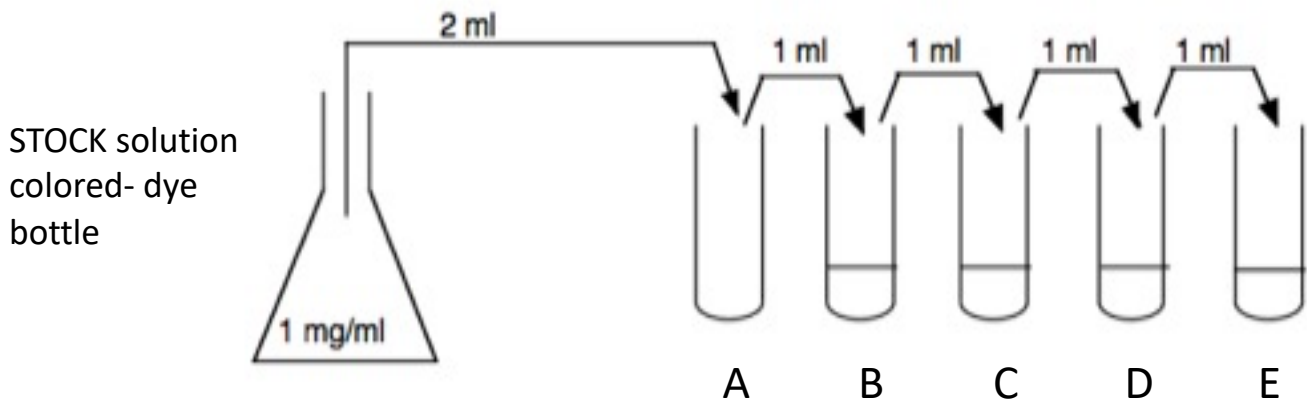
### Steps to prepare a 1:10 serial dilution

**Part I: Each pair prepares their own blue stock solution** (Use a graduated cylinder to measure out 10 ml of distilled water and pour into a beaker. Add two drops of blue food dye. Mix). Keep this stock solution for part II.

- 1) Refer to figure below. Set up 5 test tubes in a rack. Label them A-E with marker.
- 2) Pipette 9 ml of water into tubes B-E.
- 3) Transfer 2 ml of blue stock solution to tube A.
- 4) Using a P1000 micropipette, transfer 1 ml (1000ul) from tube A to B. Mix well. Dispose tip.
- 5) Using a new tip, transfer 1ml from B to C, and so on as in the figure, each time mixing well and changing the tip.
- 6) Fill in the table for part I (refer to data sheet).
- 7) Serial dilutions are multiplicative, so to find the overall (total) dilution in a tube, simply multiply the dilution ratios for each step.

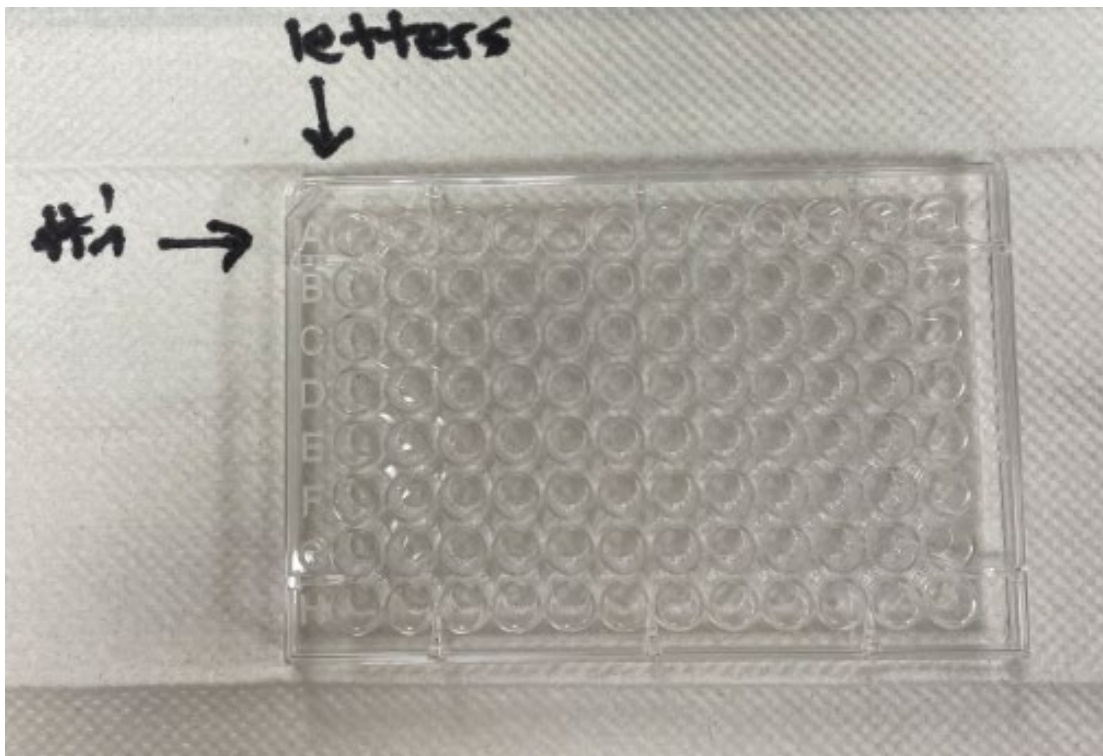
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### *Steps to Prepare a Secret Code*

**Part II: Each pair prepares their own stock solutions for the red, yellow and green food dyes** (follow the same instructions as for the blue stock solution). Use a graduated cylinder to measure out 10 ml of distilled water and pour into a beaker. Add two drops of food dye. Mix.



1) Here is a microtiter plate. Use an appropriate micropipette to dispense the required aliquots of food dye solutions into the wells of a 96-well microtiter plate.

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- 2) If there is no call for any color food dye to be placed in a well, then leave it empty (i.e. A1). Some wells will have multiple colors of food dye to be dispensed into them (i.e. E6)
- 3) In some cases, the volume to dispense is given in  $\mu\text{l}$  and in other instances the volume to pipet is given in ml. You will have to convert to the appropriate units for the micropipette in order to complete the exercise properly.
- 4) Once you've dispensed all of the dyes into the wells, show your instructor the plate, and then describe and draw what you see, in the data sheet.
- 5) Wash and dry the microtiter plate, and return to instructor.

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### Secret Code Instructions

| Pipet the following amounts in the designated assay plate cells: |             |             |             |             |  |      |            |             |             |             |
|--|-------------|-------------|-------------|-------------|--|------|------------|-------------|-------------|-------------|
| Cell   | Yellow      | Red         | Blue        | Green       |  | Cell | Yellow     | Red         | Blue        | Green       |
| D4   | 0           | 0           | 350 $\mu$ L | 0           |  | C7   | 50 $\mu$ L | 50 $\mu$ L  | 50 $\mu$ L  | 200 $\mu$ L |
| E4   | 0           | 0           | 350 $\mu$ L | 0           |  | D7   | 50 $\mu$ L | 50 $\mu$ L  | 50 $\mu$ L  | 200 $\mu$ L |
| F4   | 0           | 0           | 350 $\mu$ L | 0           |  | F7   | 50 $\mu$ L | 50 $\mu$ L  | 50 $\mu$ L  | 200 $\mu$ L |
| C8   | 0.2 mL      | 0           | 50 $\mu$ L  | 100 $\mu$ L |  | C6   | 0.1 mL     | 50 $\mu$ L  | 50 $\mu$ L  | 150 $\mu$ L |
| F8   | 0.2 mL      | 0           | 50 $\mu$ L  | 100 $\mu$ L |  | E6   | 0.1 mL     | 50 $\mu$ L  | 50 $\mu$ L  | 150 $\mu$ L |
| A12  | 325 $\mu$ L | 25 $\mu$ L  | 0           | 0           |  | F6   | 0.1 mL     | 50 $\mu$ L  | 50 $\mu$ L  | 150 $\mu$ L |
| G12  | 325 $\mu$ L | 25 $\mu$ L  | 0           | 0           |  | C5   | 25 $\mu$ L | 100 $\mu$ L | 225 $\mu$ L | 0           |
| B11  | 225 $\mu$ L | 100 $\mu$ L | 0           | 25 $\mu$ L  |  | F5   | 25 $\mu$ L | 100 $\mu$ L | 225 $\mu$ L | 0           |
| C11  | 225 $\mu$ L | 100 $\mu$ L | 0           | 25 $\mu$ L  |  | C3   | 0          | 0           | 300 $\mu$ L | 50 $\mu$ L  |
| D11  | 225 $\mu$ L | 100 $\mu$ L | 0           | 25 $\mu$ L  |  | F3   | 0          | 0           | 300 $\mu$ L | 50 $\mu$ L  |
| E11  | 225 $\mu$ L | 100 $\mu$ L | 0           | 25 $\mu$ L  |  | C2   | 0          | 75 $\mu$ L  | 250 $\mu$ L | 25 $\mu$ L  |
| F11  | 225 $\mu$ L | 100 $\mu$ L | 0           | 25 $\mu$ L  |  | F2   | 0          | 75 $\mu$ L  | 250 $\mu$ L | 25 $\mu$ L  |
| C10  | 75 $\mu$ L  | 0.25 mL     | 0           | 25 $\mu$ L  |  | D1   | 0          | 100 $\mu$ L | 250 $\mu$ L | 0           |
| E10  | 75 $\mu$ L  | 0.25 mL     | 0           | 25 $\mu$ L  |  | E1   | 0          | 100 $\mu$ L | 250 $\mu$ L | 0           |
| D9   | 0           | 350 $\mu$ L | 0           | 0           |  | F1   | 0          | 100 $\mu$ L | 250 $\mu$ L | 0           |

### References:

- 1) Mathbench Biology Modules at University of Maryland. <http://mathbench.umd.edu/index.html>
- 2) DrexelUniversityGK-12program, *Engineering as a Contextual Vehicle for Science and Mathematics Education*, supported in part by National Science Foundation Award No. DGE-0538476
- 3) Pipetting Secret Code, Ellyn Daugherty [www.BiotechED.com](http://www.BiotechED.com)

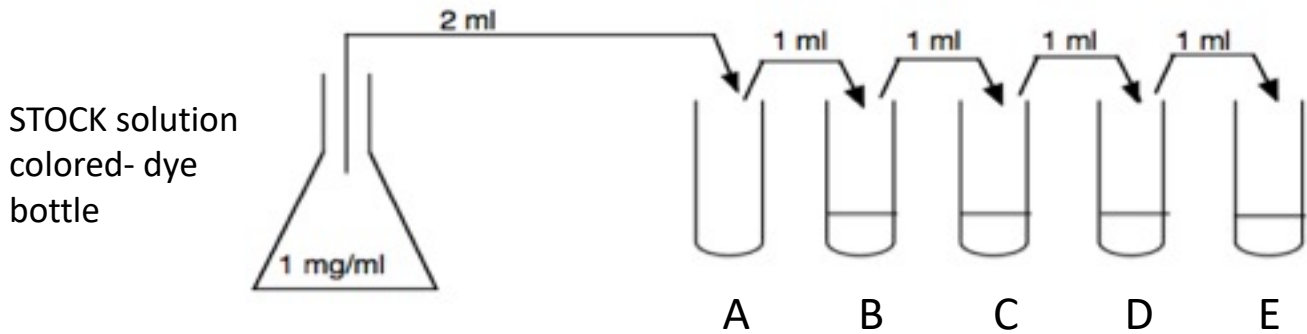
Name \_\_\_\_\_

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## Data Sheet

### Part I

Complete the table below **and** show all of your work:



|                          |   |     |     |     |     |
|--------------------------|---|-----|-----|-----|-----|
| Volume of water          | 0 | 9ml | 9ml | 9ml | 9ml |
| Dilution ratio           |   |     |     |     |     |
| Dilution factor          |   |     |     |     |     |
| Final Concentration      |   |     |     |     |     |
| Describe color intensity |   |     |     |     |     |

-Explain your work for this table: (**10 pts**)

-If tube E was diluted the same way one more time to make tube F, what is the final dilution factor for tube F?  
Show your full working. (**5 pts**)

- How do you construct a 1:100 dilution series? Explain **and** diagram. (**5 pts**)

### Part II

Describe and draw what you see on the microtiter plate. (**10 pts**)

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## Got Protein? How much is in that sample? (Week One)

### Objectives

- Prepare standard samples and compare color visually
- Graph a standard curve
- Use a spectrophotometer
- Perform serial dilutions
- Interpret a standard curve
- Prepare duplicate samples
- Calculate concentration in sample

### Introduction

#### Week One: Protein Standard Curve and Graphing (30 pts)

This is a two-week lab series. In the first week, we will learn about standards, and preparing a high-quality graph. In the second week, we will dilute samples of unknown protein concentration and conduct a protein assay to determine the protein concentration.

#### Lab objectives:

#### Lab Skills:

- Pipet accurately
- Label and keep track of test tubes
- Examine colors by eye
- Use a spectrophotometer correctly
- Transfer samples efficiently between tubes

### Week 1. Protein Standard Curve and Graphing

#### Colorimetric Assays

There are three colorimetric assays used for protein concentration determination. The **Bradford assay** is the most robust assay and it is the one that we will use today. The dye, Coomassie Blue G-250, binds to a number of the side chains of amino acids in a protein. When the protein binds the dye, the dye converts to a stable unprotonated, blue form. The intensity of the blue is proportional to the concentration of the protein. The pH of the solution must be slightly alkaline.

The absorbance of each of the samples will be measured using a **spectrophotometer**. The spectrophotometer measures the amount of light that is absorbed by a sample. Colored solutions have color because they absorb certain wavelengths of light. One can measure the amount of light that is absorbed at a particular wavelength of light and determine how much of the colored substance is present.

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Samples of the standards will be treated in the protein assay and the absorbances measured. These absorbances are graphed to develop the **standard curve**, which is actually a straight line, graphing protein concentrations versus the absorbance. One can take the absorbance of a protein solution, compare it to the standard curve and determine the concentration of the unknown. We will do this in week two of this lab, when you will determine the concentration of protein in various milk beverages.

## ***Experimental Procedure***

### **Part 1. Prepare the Standards**

The “**standards**” are solutions of known protein concentration. The protein is called BSA (bovine serum albumen) and the concentration is in mg/ml. You will take a small volume of each standard protein concentration solution and add the Bradford reagent to make the standards to measure the absorbance. The proteins of standard concentrations are provided.

Procedure:

1. Keep the standards on ice at all times. Do not throw them away. Must return all of the standards to your instructor at the end of the assay.
2. Label 7 small test tubes: 0 mg/ml (tube #1), 0.125 mg/ml (tube #2), 0.25 mg/ml (tube #3), 0.5 mg/ml (tube #4), 0.75 mg/ml (tube #5), 1 mg/ml (tube #6), 1.5 mg/ml (tube #7).
3. Pipet 20  $\mu$ l of water into 0 mg/ml (tube #1). This tube has no protein and is the control.
4. Pipet 20  $\mu$ l of 0.125 mg/ml BSA into the corresponding tube.
5. Pipet 20  $\mu$ l of 0.25 mg/ml BSA into the corresponding tube.
6. Repeat for the rest of the tubes.
7. Add 1 ml Bradford reagent to each of the 7 tubes.
8. Vortex (swirl) to mix.
9. Visually examine your tubes to check your work.
  - a. Do they all have the same volume? 1 ml Bradford + 20  $\mu$ l sample? The solution should be the same height in every tube.
  - b. Does the color vary in the protein standards tubes? Which tube has the darkest color? Which has the most protein?

*Pro tip: When placing small volumes in tubes, place the droplet at the bottom of the tube by touching the side of the tube with the tip. Don't just squirt as the sample might cling to the tip. If you put the sample at the bottom of the tube, you know where it is even if you can't see it.*



Name \_\_\_\_\_

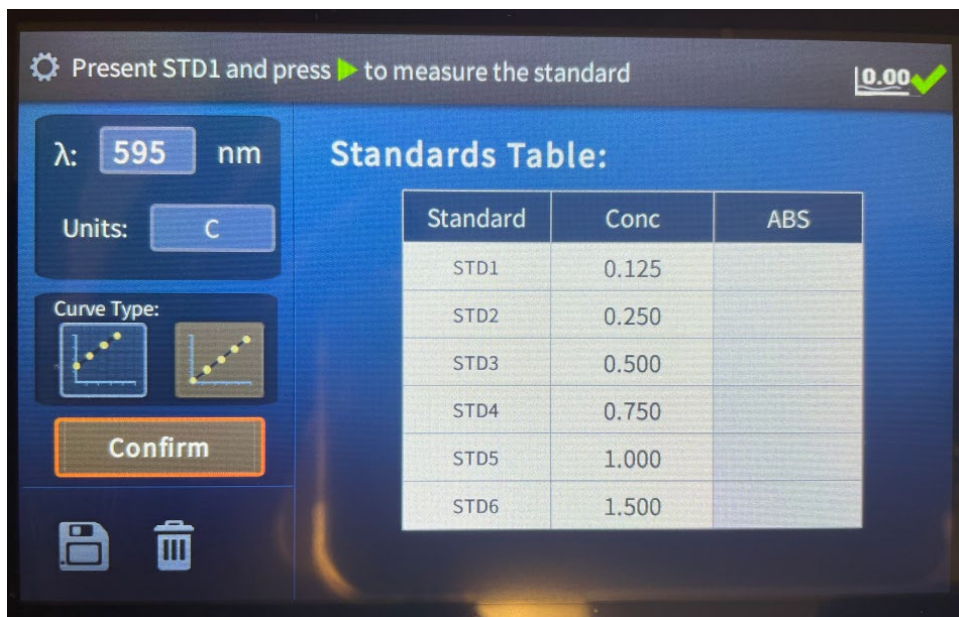
Lab Partner \_\_\_\_\_



Your assay might look like this

## Part 2. Use the spectrophotometer

Turn on the spectrophotometer and allow the machine to warm up for at least 5 minutes before taking any measurements. Your instructor will show you how to use the spectrophotometer. For example, the arrows are used to navigate around the screen. The data for this experiment can be collected in “Quant” mode. All of the instructions are given on the screen.



1. **Set the wavelength:** Set the wavelength for this lab to 595 nm.
2. **Blank the machine:** Pour the contents of tube #1 into the cuvette. Place the cuvette in the sample holder with the arrow facing you. Close the sample holder lid. Press the 0.00% Abs/T yellow button.
  - a. *By blanking the machine, you subtract the absorbance of the colored Bradford reagent from your results since the colored reagent is added to each tube.*
  - b. *When you measure each sample, you will only measure the color produced by the Bradford reagent reacting with the protein.*
  - c. *Pour the contents of the cuvette back into tube #1.*

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- d. *Use a Kim wipe to catch the remaining drips from the cuvette, and continue to use this SAME cuvette throughout the exercise.*
3. **Set the standard concentrations (mg/ml):** These are the BSA concentrations from each tube #2-#7.
  4. **Record the absorbance:** Fill your cuvette with each solution to be measured, close the lid, and record the absorbance of all samples at 595 nm in Table 1. POUR back each sample into original tube so that you can reuse the sample if you need to read it again for any reason. If you start with tube #1 and proceed, you need not rinse the cuvette between samples. *Use the same cuvette for all samples.*

### Part 3. Graph the data

Using the data from Table 1, you will prepare the Standard Curve, or graph of absorbance of the protein standard solutions.

#### Graphing by Hand: General Notes

1. **The x-axis is the independent variable.** The independent variable is the one you control e.g. temperature, pH, number of days, concentration of substrate, time.
  - a. Note: minor divisions on each axis are equal e.g. 10, 20, 30...not 10, 20, 50, 75, 100. If a square is worth 2 or 5 or 10, it is always worth 2 or 5 or 10 along that axis.
  - b. Choose your divisions wisely. Make it easy for you to USE your graph. If a point is  $\frac{1}{2}$  way between 2 lines, it is easier to note that it is 1 or 2.5 or 5 rather than 0.33, 1.8 or some other number. Best to select 1, 2, 5 or 10 for ease of use.
2. **The y-axis is the dependent variable.** Some change in x causes a change in y e.g. higher temperature (x-axis) causes an increase in reaction rate (y-axis), longer time (x axis) produces more product absorbance (y-axis).
3. **Label each axis** with name and units.
4. **Every good graph deserves a title.** The title should be informative; consider the informational difference between "The enzymes in the tubes" versus "The Dependence of Reaction Rate on pH".
5. **The origin does not have to be 0,0.**
6. Know when to use a **line graph** or a bar graph. When in doubt, a line graph is the best choice. Line graphs demonstrate how one aspect changes in response to another. Bar graphs demonstrate composite parts.
7. **Put each data point on the graph paper** and circle it. Then draw the best fit line. **DO NOT play connect the dots.** Yes, sometimes one data point doesn't fit in well, by drawing the best fit line you average out that data point.
8. **Graphs should be large.** If using graph paper\*, use most of the page, not a tiny corner. If using a program, print the graph to be at least half the page.
9. **Use a ruler to draw a straight line.**
10. If you have more than one line, **use a legend to identify each line.**

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\*Note on graph paper: Real graph paper has dark lines at 1 cm divided by 10 smaller lines, so that one can read 3 significant figures from it.

## Data Sheet

*Be sure to explain your answers for full credit.*

1. What is a “standard” in this experiment?
2. Which tube had the darkest color?
3. Which tube had the most protein?

**Record the absorbance of the standards in Table 1 and to the right of this, place a labelled sketch of your protein standard curve.**

Table 1 Absorbance of standards

| Tube | $\mu\text{l}$    | Standard<br>mg/ml | Absorbance |
|------|------------------|-------------------|------------|
| 1    | 20 $\mu\text{l}$ | water             |            |
| 2    | 20 $\mu\text{l}$ | 0.125             |            |
| 3    | 20 $\mu\text{l}$ | 0.250             |            |
| 4    | 20 $\mu\text{l}$ | 0.500             |            |
| 5    | 20 $\mu\text{l}$ | 0.750             |            |
| 6    | 20 $\mu\text{l}$ | 1.00              |            |
| 7    | 20 $\mu\text{l}$ | 1.50              |            |

**Record the equation of the line and the  $R^2$  value.**

**Prepare a graph of the data from Table 1 using the graph paper provided.**

Be sure to follow the graphing notes, including:

- S.W.E.A.R: state what each axis represents
- include all units
- must give your graph a descriptive title

4. What is the independent variable?

Name \_\_\_\_\_

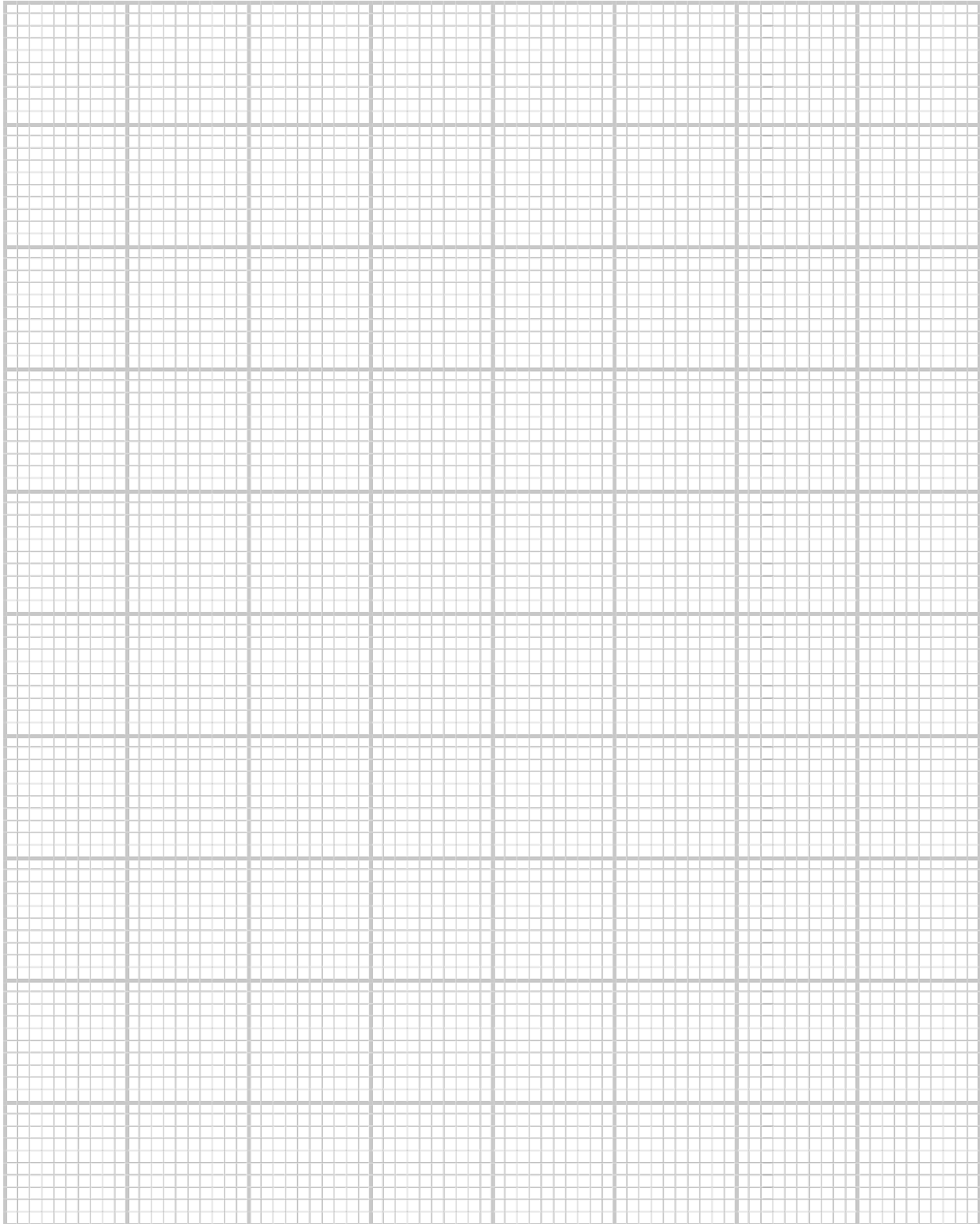
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5. What is the dependent variable?

6. As the amount of protein increases, what happens to the absorbance?

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## **Dilutions and Protein Assay**

### **Week Two**

### **Objective**

To determine the concentration of protein in unknown samples.

### **Introduction**

This is the second week of the two-week lab series. Last week, we learned about standards, and how to prepare a high-quality graph. In this second week, we will dilute samples of unknown protein concentration and conduct a protein assay to determine the protein concentration.

### ***Experimental Procedure***

#### **Part 1. Prepare the Standards (*repeat of week one*)**

1. Always keep the standards on ice. Do not throw them away. Must return all the standards to your instructor at the end of the assay.
2. Label 7 small test tubes: 0 mg/ml (tube #1), 0.125 mg/ml (tube #2), 0.25 mg/ml (tube #3), 0.5 mg/ml (tube #4), 0.75 mg/ml (tube #5), 1 mg/ml (tube #6), 1.5 mg/ml (tube #7).
3. Pipet 20  $\mu$ l of water into 0 mg/ml (tube #1). This tube has no protein and is the control.
4. Pipet 20  $\mu$ l of 0.125 mg/ml BSA (Bovine Serum Albumen) into the corresponding tube.
5. Pipet 20  $\mu$ l of 0.25 mg/ml BSA into the corresponding tube.
6. Repeat for the rest of the tubes.
7. Add 1 ml Bradford reagent to each of the 7 tubes.
8. Vortex (swirl) to mix.

#### **Part 2. Use the spectrophotometer for the standards**

Turn on the spectrophotometer and allow the machine to warm up for at least 5 minutes before taking any measurements and recording your data in Table 1. The data for this experiment will be collected in "Live Display" from the home screen. Follow the instructions given on the screen. E.g., adjust the wavelength to the same wavelength used last week, select "absorbance" as the measurement mode, blank the spec and take the readings. POUR back each sample into original tube so that you can reuse the sample if you need to read it again for any reason. If you start with tube #1 and proceed, you need not rinse the cuvette between samples. *Use the same cuvette for all samples.*

#### **Part 3. Graph the data in Excel**

Using the data from Table 1, you will prepare the Standard Curve, or graph of absorbance of the protein standard solutions in Excel. Your instructor will show you how to use this program. Record a sketch of your labelled Excel graph next to Table 1 and include the equation of the line and the  $R^2$  value.

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#### **Part 4. Prepare the samples using serial dilutions.**

You may choose samples from three different milk beverages such as soy milk, oat milk, Muscle Milk or other. You know the names of these samples, but you do not know the protein concentration. These beverage samples probably have too much protein and are outside of the range of Beer's Law for the provided protein standards. These milk samples must be diluted, but you do not know by how much. Therefore, you will prepare a range of concentrations of the milk beverages to be sure that one is dilute enough to read on the standard curve. *Only one dilution of each sample has to be in the range of the standard curve for each sample.*

1. Place 1 ml of each milk beverage in a tube labeled with the name of the beverage e.g., soymilk, oat milk, goat milk.
2. Prepare 3 tubes for each beverage with 900  $\mu$ l of water and label each tube 1:10, 1:100, 1:1000 and the name of the beverage. E.g., Soy 1:10, Soy 1:100, Soy 1:1000
3. Dilute one milk beverage as follows:
  - a. Place 100  $\mu$ l of one beverage into the tube labelled 1:10.
  - b. Mix.
  - c. Place 100  $\mu$ l from the 1:10 tube into the corresponding 1:100 tube.
  - d. Mix.
  - e. Place 100  $\mu$ l from the 1:100 tube into the corresponding 1:1000 tube.
  - f. Mix.
4. Repeat for each of the three beverages.
5. Arrange these diluted samples in your rack, in order, for Part 5 below.

*Pro tip: move each tube back one row, or over one space in your rack as you add the sample. That makes it easier to keep your place.*

#### **Part 5. Prepare milk samples to read absorbance**

1. Place 20  $\mu$ l of sample from each of the diluted tubes from part 4 into a fresh and labelled tube. E.g., Soy 1:10, Soy 1:100, Soy 1:1000
2. Place 20  $\mu$ l of water in a tube labeled "Blank"
3. Add 1 ml of Bradford reagent to the tubes from step 1 containing 20  $\mu$ l of each sample and the blank tube.  
\*\*\*\*DO NOT ADD BRADFORD TO THE 1 ML DILUTION TUBES\*\*\*\*
4. Mix each tube.
5. Visually examine your tubes to check your work.
  - a. Do they all have the same volume? 1 ml Bradford + 20  $\mu$ l sample? The solution should be the same height in every tube.
  - b. Does the color vary in the tubes? Check to verify that the lightest color tube has the least amount of protein and the darkest tube, the most protein.

*The colorimetric reaction should be complete after about 5 minutes, and the color should remain stable for about 1 hour.*

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*Pro tip: Always check your work visually. Are the samples the same volume? Are the colors and order what you expect?*

## **Part 6. Use the spectrophotometer for the milk samples**

The spectrophotometer is already warmed up and is ready for taking measurements.

1. **Return to the home screen:** Select "Live Display."
2. **Adjust the wavelength:** Set to 595 nm.
3. **Select the mode:** Choose "absorbance"
4. **Blank the machine:** Pour the contents of the blank tube into the cuvette. Place the cuvette in the sample holder with the arrow facing the direction of light.

**Measure samples:** Once the machine is blanked, fill your cuvette with each solution to be measured, close the lid, and read the absorbance. Record values in Table 2 in the worksheet. POUR back into original tubes. Use the SAME cuvette throughout the exercise.

## **Part 7. Calculate the concentration of each milk beverage**

**Determine the concentration of your milk samples.**

**Use the equation of the line**

- a. Determine the equation of your standard curve line from your Excel graph.
- b. Choose the absorbance of your sample that falls within the values of your protein standard absorbances.
- c. Y is absorbance in the equation of the line. Substitute the absorbance of your sample and solve for X. This x-value is mg/ml.
- d. Record the value.

**Calculate the concentration of your original milk beverage:**

Remember that you used a diluted sample for the protein assay? Now you need to calculate the protein concentration of the original sample, undiluted. Since dilution is division (dividing the concentration of the sample), now you must multiply.

Multiply the x value (in mg/ml) from above by the dilution factor. If this value came from the 1:100 dilution, multiply by 100.

If your sample was diluted 1:100, for example, and the concentration in the diluted tube was 0.25 mg/ml, then the milk stock container would be  $0.25 \text{ mg/ml} * 100 = 25 \text{ mg/ml}$ .

## **Part 8. Finally, how close to the nutrition label is your sample?**

Compare the protein concentration measured in the lab with that of the label on the beverage. Examine the nutrition label on the beverage container. The label will give the grams of protein per serving. Most servings are 1 cup which is 250 ml and is noted at the top of the nutrition label.

For example, goat's milk has 9 grams of protein for 250 ml. The protein concentration would be  $9 \text{ grams} / 250 \text{ ml} = 0.036 \text{ g/ml}$ .

The protein concentration from above is in g/ml, so convert to mg/ml.

$0.036 \text{ g/ml} * 1000 \text{ mg/g} = 36 \text{ mg/ml}$ .



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From the example above, we measured 25 mg/ml for the goat milk which is less than indicated on the label.  $25 \text{ mg/ml} / 36 \text{ mg/ml} * 100 = 69.5\%$  of the reported value.

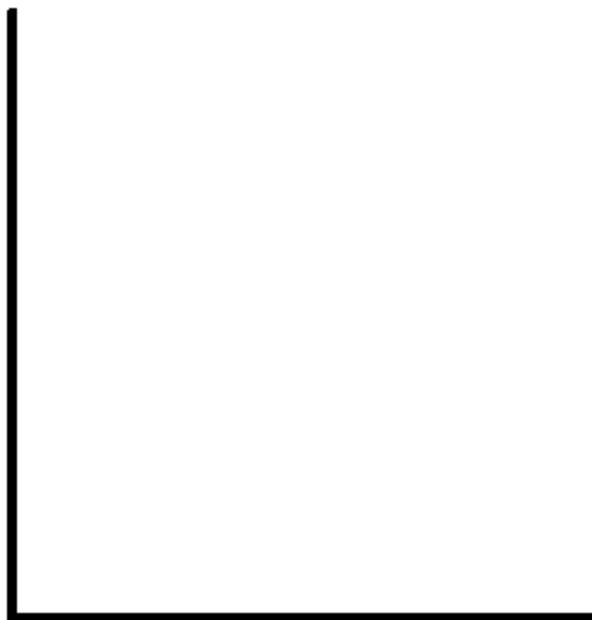
## Week 2 Protein Assay Worksheet

Record the absorbance of the standards in Table 1, and *to the right of this*, sketch the Excel Protein Standard Curve.

Table 1. Absorbance of standards

| Tube | $\mu\text{l}$    | Standard<br>mg/ml | Absorbance |
|------|------------------|-------------------|------------|
| 1    | 20 $\mu\text{l}$ | water             |            |
| 2    | 20 $\mu\text{l}$ | 0.125             |            |
| 3    | 20 $\mu\text{l}$ | 0.250             |            |
| 4    | 20 $\mu\text{l}$ | 0.500             |            |
| 5    | 20 $\mu\text{l}$ | 0.750             |            |
| 6    | 20 $\mu\text{l}$ | 1.00              |            |
| 7    | 20 $\mu\text{l}$ | 1.50              |            |

Sketch the protein standard curve.



Record the equation of the line and the  $R^2$  value.

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**Record the absorbance of your milk samples at 595 nm.** Use Table 2 to fill in the identity of each of your milk samples. Using the Excel protein standard curve, you developed this week, determine the concentration of protein in each \*dilution.

\*For each milk sample, choose an absorbance value that lies within the range of your standard curve. You must not extend the line beyond the last standard data point. For example, if the absorbance of the 1 mg/ml protein is 0.880 and your 1:10 diluted sample is 0.913 (which is higher than your curve goes), you cannot just extend the line and determine the concentration. BUT since you were smart and prepared other dilutions of your sample, you may use the 1:100 dilution instead. Since ALL three dilutions were from the same protein sample, ONLY ONE of these samples needs to fit the curve to determine the concentration in the original carton.

1. Select ONE absorbance value that lies on the standard curve for EACH set of dilutions.
2. Use the equation of the line (absorbance is Y) to determine the concentration in mg/ml.
3. Correct the concentration for dilution. Since dilution is division, you need to multiply by the dilution factor, the number on the other side of the colon. Example: The 1:100 dilution gave an absorbance of 0.397 which corresponds to 0.28 mg/ml. That is the concentration in the diluted sample. Correct for the dilution by multiplying by 100, so  $0.28 \text{ mg/ml} \times 100 = 28 \text{ mg/ml}$ . That should be the concentration of the original milk beverage in the carton.

Show all your work in Table 2.

Table 2 Absorbance of milk samples

| Tube:<br>Identity | dilution | absorbance | Protein conc. from curve | Corrected for<br>dilution |
|-------------------|----------|------------|--------------------------|---------------------------|
| Milk 1            | 1:10     |            |                          |                           |
| Milk 1            | 1:100    |            |                          |                           |
| Milk 1            | 1:1000   |            |                          |                           |
| Milk 2            | 1:10     |            |                          |                           |
|                   |          |            |                          |                           |

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|        |        |  |  |  |
|--------|--------|--|--|--|
| Milk 2 | 1:100  |  |  |  |
| Milk 2 | 1:1000 |  |  |  |
| Milk 3 | 1:10   |  |  |  |
| Milk 3 | 1:100  |  |  |  |
| Milk 3 | 1:1000 |  |  |  |

**Complete below for each milk sample:**

(Identity) \_\_\_\_\_ milk gave a protein concentration from curve of \_\_\_\_\_ mg/ml,  
which when corrected for \_\_\_\_\_ dilution, gave a final protein concentration of  
\_\_\_\_\_ mg/ml.

(Identity) \_\_\_\_\_ milk gave a protein concentration from curve of \_\_\_\_\_ mg/ml,  
which when corrected for \_\_\_\_\_ dilution, gave a final protein concentration of  
\_\_\_\_\_ mg/ml.

(Identity) \_\_\_\_\_ milk gave a protein concentration from curve of \_\_\_\_\_ mg/ml,  
which when corrected for \_\_\_\_\_ dilution, gave a final protein concentration of  
\_\_\_\_\_ mg/ml.

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**Record each nutrition label concentration for your milk samples.** Convert from g/ml into mg/ml (show your full working). (6 pts)

(Identity) \_\_\_\_\_ milk has a nutrition label protein concentration of \_\_\_\_\_ g/ml. Below is the conversion of g/ml into mg/ml to obtain \_\_\_\_\_ mg/ml.

(Identity) \_\_\_\_\_ milk has a nutrition label protein concentration of \_\_\_\_\_ g/ml. Below is the conversion of g/ml into mg/ml to obtain \_\_\_\_\_ mg/ml.

(Identity) \_\_\_\_\_ milk has a nutrition label protein concentration of \_\_\_\_\_ g/ml. Below is the conversion of g/ml into mg/ml to obtain \_\_\_\_\_ mg/ml.

**Compare each milk sample nutrition label protein concentration, with your calculated experimental final corrected protein concentration.** Comment on your results. What percentage of the reported nutrition value was obtained?

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## Enzyme Activity Assay

### Week One: How fast is the enzyme?

#### Objectives

- Perform enzyme assays
- Measure enzyme product using the spectrophotometer;
- Calculate the moles of product produced from the absorbance value
- Graph the enzyme progress and determine the speed of the enzyme.

#### Introduction

This is a two-week lab series. In the first week, you will determine the activity of the enzyme, acid phosphatase. In the second week, you will observe what affects the activity of an enzyme.

**In this procedure, you will overlap your reaction times to maximize your efficiency.** Each reaction will be started in turn, incubated, and then stopped in turn so that each tube incubates for the correct length of time. Read the procedure below and walk it through in your head to understand how the procedure works. Copy Table 1 and Table 2 into your lab notebook before lab begins. **Only begin adding enzyme once you have every tube labeled and all your reagents and pipettes set up and ready to go.**

Prepare 4 ml of the **Reaction Cocktail**, which contains all the reagents required for the assay. Preparing a cocktail ensures uniformity across samples and reduces opportunities for pipetting errors. **Record the enzyme concentration in mg/ml in your lab notebook.**

In this experiment, the amount of enzyme is varied. To reduce any effects resulting from having protein in the tube, an equivalent amount of an inactive protein called BSA will be added. This will ensure that each tube receives the same mass of protein and only the proportion of active enzyme is changed.

#### *Experimental Procedure*

##### **Part 1. Prepare the Reaction Cocktail**

**Table 1: Reaction Cocktail**

|                           |              |
|---------------------------|--------------|
| 1 M acetate buffer pH 5.7 | 500 $\mu$ l  |
| 0.1 M $MgCl_2$            | 500 $\mu$ l  |
| 50 mM pNPP                | 500 $\mu$ l  |
| Water                     | 2500 $\mu$ l |
| Total volume              | 4000 $\mu$ l |

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**NOTE: The reaction starts as soon as you add the enzyme! Keep enzyme on ice and add it when you are ready to initiate each reaction.**

1. Prepare Reaction Cocktail from Table 1 in a large tube, mix and set aside.
2. Label six tubes 1-6.
3. Add 400  $\mu$ l Reaction Cocktail to each tube, 1-6.

## Part 2. Run the Reaction Cocktail with Enzyme

**Table 2: Acid Phosphatase Assay Protocol**

Record enzyme concentration here: \_\_\_\_\_ mg/ml (from your instructor)

| Tube # | Reaction Cocktail ( $\mu$ l) | 0.1% BSA ( $\mu$ l) | Enzyme ( $\mu$ l) | Mass enzyme (mg)<br>(calculate this) | Time   | A <sub>410</sub> |
|--------|------------------------------|---------------------|-------------------|--------------------------------------|--------|------------------|
| 1      | 400                          | 100 $\mu$ l         | 0                 | 0                                    | 15 min | 0.00             |
| 2      | 400                          | 80                  | 20                |                                      |        |                  |
| 3      | 400                          | 60                  | 40                |                                      |        |                  |
| 4      | 400                          | 40                  | 60                |                                      |        |                  |
| 5      | 400                          | 20                  | 80                |                                      |        |                  |
| 6      | 400                          | 0                   | 100               |                                      |        |                  |

1. Add the amount of BSA to each tube as indicated in Table 2 below. **Do NOT add enzyme yet.**
2. *Tube #1 is the control, you will not add enzyme; instead, you will determine if product can form in the absence of enzyme.*
3. Initiate the assay by placing tube 1 in the 30°C water (or bead) bath and record the time. (NO Enzyme).
4. 30 seconds later, add 20  $\mu$ l enzyme to tube 2, mix and place in water (or bead) bath.
5. 30 seconds later, add 40  $\mu$ l enzyme to tube 3, mix, and place in water (or bead) bath. Repeat the pattern for the remainder of the tubes.
6. After 15 min, stop the reaction in tube 1 by adding 4.5 ml Stopping Mix from the Repipet. (This Stopping Mix is an alkaline solution to halt the reaction and cause the product to turn yellow).
7. 30 seconds later, stop tube 2 by adding 4.5 ml Stopping Mix, then stop each reaction at 30 second intervals by adding 4.5 ml Stopping Mix, so that each tube incubates for 15 min.
8. Hold the samples at room temperature.
9. Record the concentration of the enzyme from your instructor.

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*An important part of being a scientist, or a health care provider, is paying attention to even simple details. LOOK at your tubes. Do they all have the same volume, or level of solution in the tube? If so, great, you did good work pipetting. If not, your pipetting skills need some attention.*

*Examine the tubes, do you see some yellow color?*

*Which tubes had the most enzyme?*

*Which tubes had the most color?*

### **Part 3. Determine Absorbance of the Reaction Cocktail with Enzyme**

*(Complete this after all reactions have been stopped)*

Turn on the spectrophotometer and allow the machine to warm up for at least 5 minutes before taking any measurements and recording your data in Table 2. The data for this experiment will be collected in “Live Display” from the home screen. Follow the instructions given on the screen. E.g., adjust the wavelength to 410 nm. Select “absorbance” as the measurement mode, blank the spectrophotometer with tube #1 (which has zero enzyme, and serves as a control for spontaneous appearance of product during the incubation), take the readings, and record in Table 2. POUR back each sample into original tube so that you can reuse the sample if you need to read it again for any reason. If you start with tube #1 and proceed, you need not rinse the cuvette between samples. *Use the same cuvette for all samples.*

*Pro tip: if you measure from the lightest yellow to the darkest, you do not have to rinse the cuvette each time!*

### **Part 4. Graph the Data in Excel**

Using the data from Table 2, you will prepare a graph in Excel using the absorbance and mass of the acid phosphatase solutions. Your instructor will show you how to use this program. Record a sketch of your labelled Excel graph and include the equation of the line and the  $R^2$  value.

### **Data Analysis**

1. Between the mg of acid phosphatase, and, the absorbance of product, decide which is the dependent variable, and which is the independent variable
2. Data from tube #1, zero absorbance, zero enzyme, is a real data point and should be included in the graph.
3. Determine the best-fit line, either using a computer program or by hand drawing.
4. **Pick a point** on your graph to use in the calculation of velocity. A calculated point is more accurate than any single data point. The coordinates of this point (X,Y) will be used in the following calculation.

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## Part 5. Enzyme Kinetics Results

### Calculations and Questions (Part I): *must show all working*

1. Calculate the concentration of substrate in the Reaction Cocktail (from Table 1).
2. Calculate the concentration of the substrate in the reaction tube (from Table 2).
3. Calculate the number of moles of substrate that are present in the reaction tube.
4. If you add more enzyme, what should happen to the velocity? Explain your answer.



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## Calculation of Enzyme Velocity (Part II)

Recall that Beer's law ( $A = \epsilon cl$ ) describes the relationship between the absorbance of a compound and the concentration. The molar extinction coefficient ( $\epsilon$ ) is a constant and is characteristic of each compound. Thus, if we know the absorbance of a solution ( $A$ ) and the extinction coefficient ( $\epsilon$ ), as well as the path length of the light ( $l$ , usually 1 cm) we can calculate the concentration ( $c$ ) of that substance. Using this information, we can determine the quantity of product produced by the enzyme, which is needed for calculation of velocity.

1. **Pick a point** from your line with the coordinates (X,Y).
  - This point from the best fit line or equation is more accurate than any of your data points.
  - X= mg of enzyme, Y= absorbance
2. Determine the **concentration of product produced** by the enzyme.
  - The molar extinction coefficient,  $\epsilon$  in Beer's Law, for pNPP is  $1.88 \times 10^4$  L/mole cm. Note the units.
  - Absorbance has no units.
  - Divide **Absorbance (the Y of your point)** by  $1.88 \times 10^4$  L/mole cm
    - Units are now **# moles cm/L**
    - The length of the cuvette path is 1 cm, so divide by 1 cm.
  - Units are now **#moles/L**
  - Convert to  $\mu\text{mol}$  per liter by multiplying by  $10^6$   $\mu\text{moles/mole}$ .
3. Calculate **how many moles of product were made**.
  - The final absorbance was measured in 5 ml. (0.5 ml reaction + 4.5 ml stop buffer)
  - Multiply the concentration from step 2 by 0.005 L
  - Units are now  **$\mu\text{mol}$  of product**
4. This product was made in a quantity of time. Divide by 15 min to reflect this.
  - Units should now be  $\mu\text{mole/min}$

This quantity is known as the **velocity, rate, or activity of the enzyme: the amount of product formed in a certain amount of time.**

### Sample Calculation

- |  |   |
|--|---|
| 1. Selected data point is                      | (0.132 mg, 0.478)   |
| 2. Divide absorbance by $\epsilon$             | $0.478 / 1.88 \times 10^4 \text{ L/mole cm} = 2.5 \times 10^{-5} \text{ mol/L}$       |
| 3. Multiply by $10^6$ $\mu\text{moles/mole}$ . | $2.5 \times 10^{-5} \text{ mol/L} * 10^6 \mu\text{moles/mole} = 25 \mu\text{moles/L}$ |
| 4. Multiply by volume                          | $25 \mu\text{moles/L} * 0.005\text{L} = 0.125 \mu\text{moles of product}$             |
| 5. Divide by time                              | $0.125 \mu\text{moles}/15 \text{ min} = 0.00834 \mu\text{mole/min}$                   |

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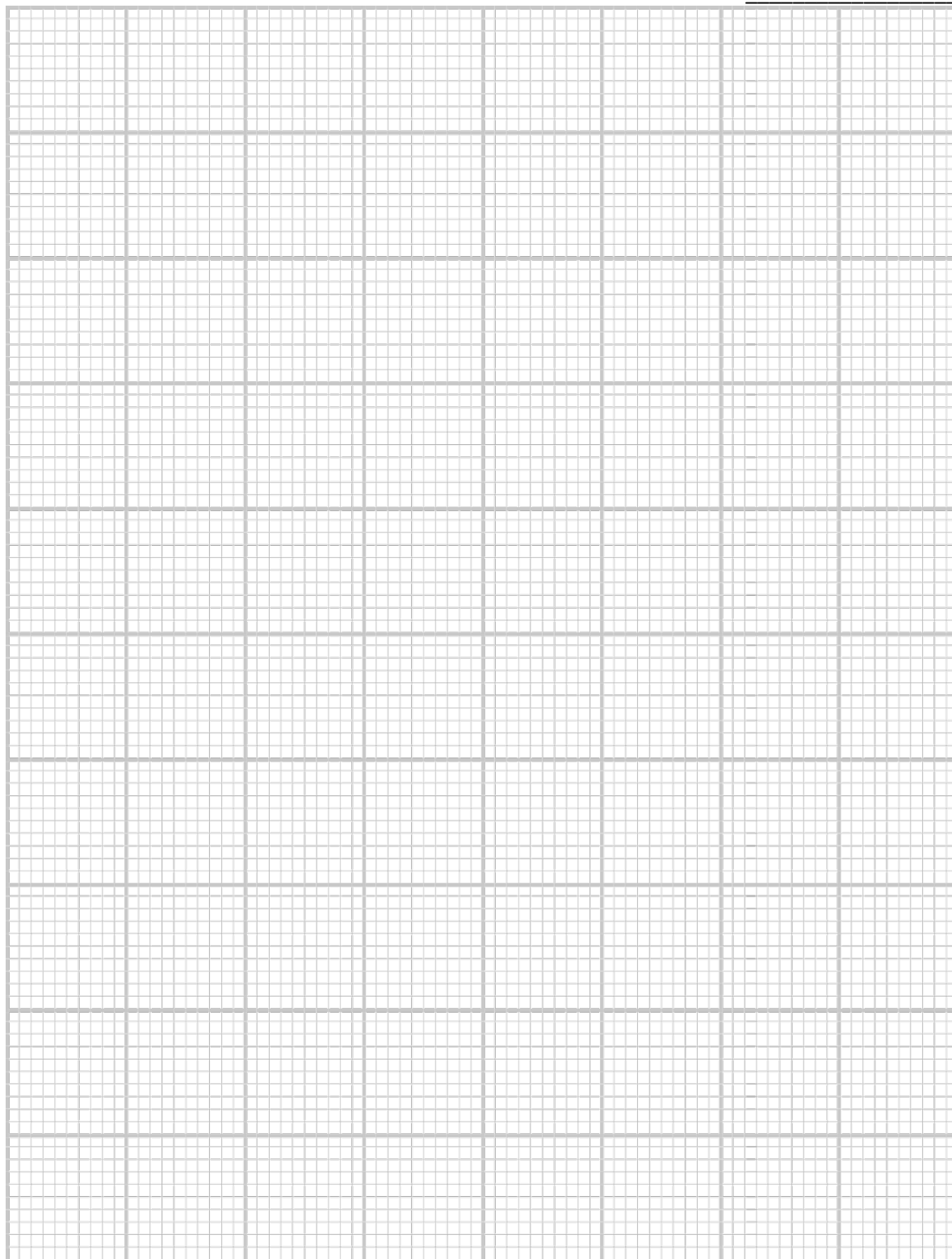
**The velocity of the enzyme in this example is 0.00834  $\mu\text{mole}/\text{min}$**

### Calculations

1. Calculate the velocity of your enzyme. Show all your work, explain your working, and show all units for each step.
2. Graph of data in Excel. Be sure to label x and y axes. Include the equation of the line and  $R^2$ .

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## Enzyme Activity Assay

### Week Two: How does the concentration of substrate affect product appearance?

#### Objectives

- Perform enzyme assays
- Measure enzyme product using the spectrophotometer;
- Calculate the moles of product produced from the absorbance value
- Graph the enzyme progress and determine the speed of the enzyme.

#### Introduction

This is the second week of the two-week enzymology lab series. In this week's experiment, you will keep the amount of enzyme the same and vary the amount of substrate.

Prepare 3 ml of the **Reaction Cocktail**, which contains all the reagents required for the assay. Preparing a cocktail ensures uniformity across samples and reduces opportunities for pipetting errors. **Record the enzyme concentration in mg/ml in your lab notebook.**

The reaction cocktail for this experiment must be different from last week; it should omit substrate so that you can manipulate the amount.

#### Experimental Procedure

##### Part 1. Prepare the Reaction Cocktail

Table 1: Reaction Cocktail

|                           |        |
|---------------------------|--------|
| 1 M acetate buffer pH 5.7 | 500µl  |
| 0.1 M MgCl <sub>2</sub>   | 500µl  |
| Water                     | 2000µl |
| Total volume              | 3000µl |

**NOTE: The reaction starts as soon as you add the enzyme! Keep enzyme on ice and add it when you are ready to initiate each reaction.**

1. Prepare Reaction Cocktail from Table 1 in a large tube, mix and set aside.
2. Label six test tubes 1-6.
3. Add 300 µl Reaction Cocktail to each tube, 1-6.
4. Prepare substrate for addition to each tube by dilution. The stock pNPP is 50 mM. Prepare a 1:2 dilution series of the stock in water by adding 150 µl of 50 mM pNPP to 150 µl water.

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Dilution series:

| Dilution | pNPP                         | Water in tube | Conc of substrate |
|----------|------------------------------|---------------|-------------------|
| 1:2      | 150 $\mu$ l                  | 150 $\mu$ l   | 25 mM             |
| 1:4      | 150 $\mu$ l of 1:2 dilution  | 150 $\mu$ l   |                   |
| 1:8      | 150 $\mu$ l of 1:4 dilution  | 150 $\mu$ l   |                   |
| 1:16     | 150 $\mu$ l of 1:8 dilution  | 150 $\mu$ l   |                   |
| 1:32     | 150 $\mu$ l of 1:16 dilution | 150 $\mu$ l   |                   |

5. Prepare reaction tubes according to Table 2. **Remember to keep your enzyme on ice** until you are ready to start the entire reaction series.

**Table 2: Acid Phosphatase Assay Protocol.**

Record enzyme concentration here: \_\_\_\_\_ mg/ml (from your instructor)

| Tube # | Reaction Cocktail ( $\mu$ l) | Substrate (dilution) | Enzyme ( $\mu$ l) | Time   | A <sub>410</sub> |
|--------|------------------------------|----------------------|-------------------|--------|------------------|
| 1      | 300                          | 100 $\mu$ l water    | 100 $\mu$ l       | 15 min | 0.00             |
| 2      | 300                          | 100 $\mu$ l 1:2      | 100 $\mu$ l       |        |                  |
| 3      | 300                          | 100 $\mu$ l 1:4      | 100 $\mu$ l       |        |                  |
| 4      | 300                          | 100 $\mu$ l 1:8      | 100 $\mu$ l       |        |                  |
| 5      | 300                          | 100 $\mu$ l 1:16     | 100 $\mu$ l       |        |                  |
| 6      | 300                          | 100 $\mu$ l 1:32     | 100 $\mu$ l       |        |                  |

**DO NOT ADD ENZYME** until you are ready with each time point. Add enzyme to tube 1, start timer, place in 30 °C incubator. At 30 sec, add enzyme to tube 2, at 30 sec add enzyme to tube 3, repeat until each tube has enzyme. At 15 min, stop tube 1 by adding 4.5 ml stopping mix as before, at 15:30 stop tube 2 and so on. Read absorbance as before at 410 nm.

Complete all the substrate concentration calculations in part 3.

Prepare a graph of final substrate concentration vs absorbance.

Calculate enzyme velocity as before and report in micromoles of product.

## Part 2. Graph the Data in Excel

Using the data from Table 2, you will prepare a graph in Excel using the absorbance and substrate concentration. Your instructor will show you how to use this program. Record a sketch of your labelled Excel graph and include the equation of the line and the R<sup>2</sup> value.

## Data Analysis

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1. Between the concentration of substrate, and, the absorbance of product, decide which is the dependent variable, and which is the independent variable
2. Data from tube #1, zero absorbance, zero substrate, is a real data point and should be included in the graph.
3. Determine the best-fit line, either using a computer program or by hand drawing.
4. **Pick a point** on your graph to use in the calculation of velocity. A calculated point is more accurate than any single data point. The coordinates of this point (X, Y) will be used in the following calculation.

### Part 3. Enzyme Kinetics Results

#### Calculations and Questions: *must show all working*

1. Give the concentration of substrate in the 1:2 dilution series with water. Show your working for all dilutions.

| Dilution | pNPP                         | Water in tube | Conc of substrate |
|----------|------------------------------|---------------|-------------------|
| 1:2      | 150 $\mu$ l                  | 150 $\mu$ l   | 25 mM             |
| 1:4      | 150 $\mu$ l of 1:2 dilution  | 150 $\mu$ l   |                   |
| 1:8      | 150 $\mu$ l of 1:4 dilution  | 150 $\mu$ l   |                   |
| 1:16     | 150 $\mu$ l of 1:8 dilution  | 150 $\mu$ l   |                   |
| 1:32     | 150 $\mu$ l of 1:16 dilution | 150 $\mu$ l   |                   |

2. Give the concentration of substrate in each tube from **Table 2**. *Hint: remember to include the volume of stopping mix in the final assay volume.* Show your work.

| Tube # | Reaction Cocktail ( $\mu$ l) | Substrate (dilution) | Enzyme ( $\mu$ l) | Conc of substrate mM |
|--------|------------------------------|----------------------|-------------------|----------------------|
| 1      | 300                          | 100 $\mu$ l water    | 100 $\mu$ l       |                      |
| 2      | 300                          | 100 $\mu$ l 1:2      | 100 $\mu$ l       |                      |
| 3      | 300                          | 100 $\mu$ l 1:4      | 100 $\mu$ l       |                      |
| 4      | 300                          | 100 $\mu$ l 1:8      | 100 $\mu$ l       |                      |
| 5      | 300                          | 100 $\mu$ l 1:16     | 100 $\mu$ l       |                      |
| 6      | 300                          | 100 $\mu$ l 1:32     | 100 $\mu$ l       |                      |

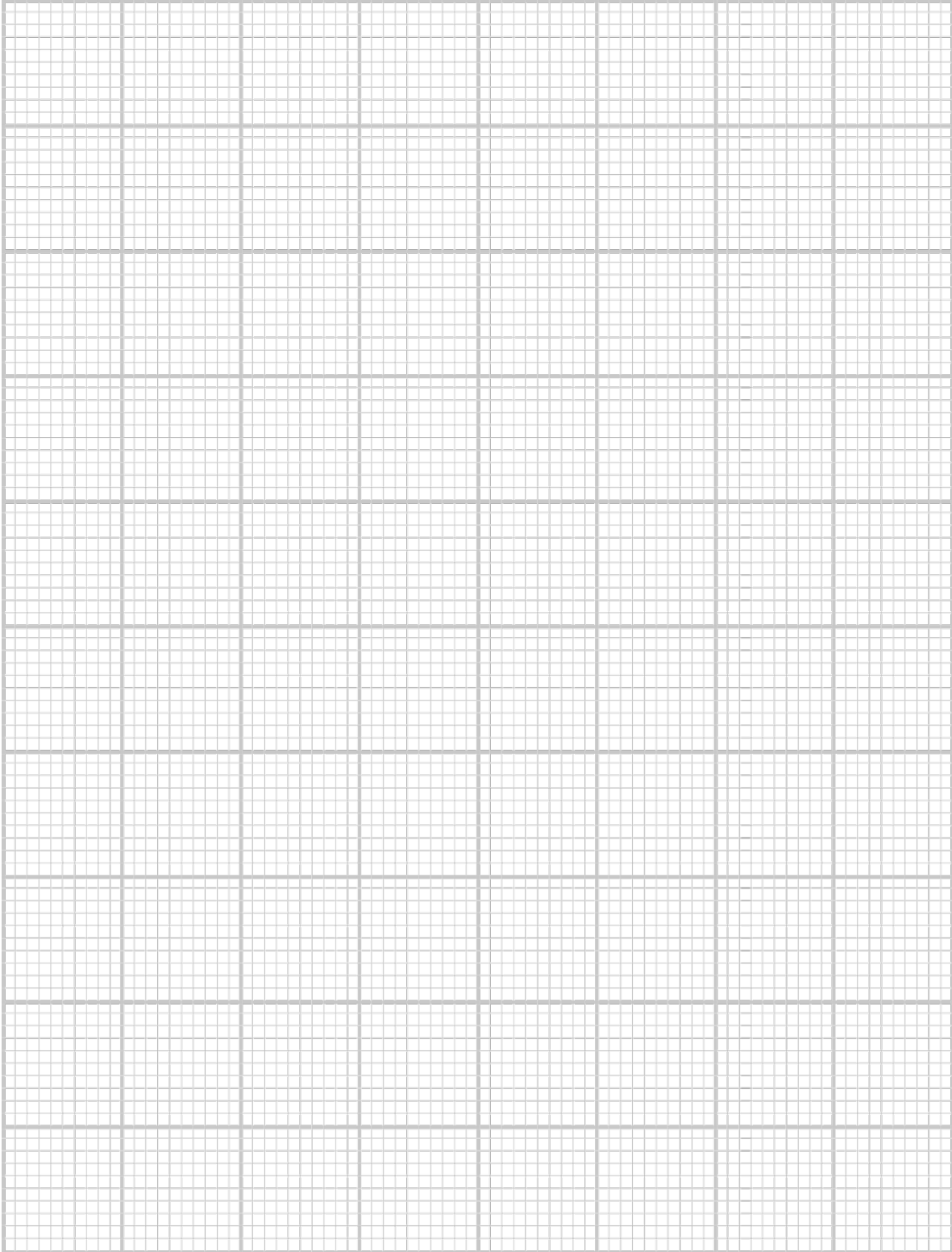
Lab Partner \_\_\_\_\_

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<https://www.vertex42.com/WordTemplates/printable-graph-paper.html>

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## 3D Macromolecules using PyMOL Proteins

### Objectives

- to become familiar with amino acids and primary structure
- to distinguish between the types of secondary structure
- to become familiar with tertiary structure
- to become familiar with quaternary structure

To open PyMOL, click on the PyMOL shortcut on the GGC issued laptop.

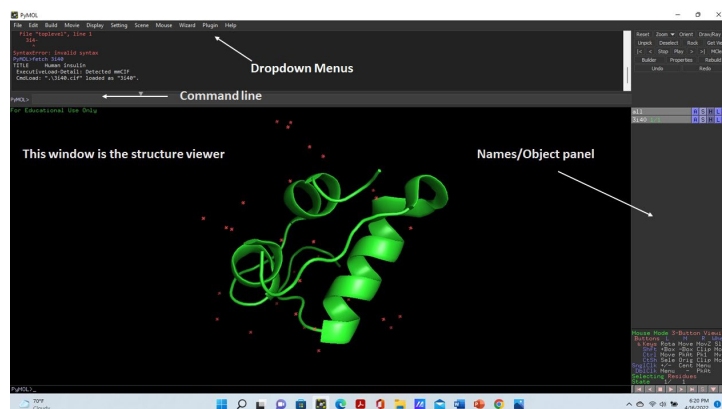
Directions for downloading PyMOL on personal computers:

1. Click on the link: <https://pymol.org/2/>
2. Click on Windows EXE installer
3. Click on next when you see Welcome to PyMOL 2.5.2 (64 bit) Setup
4. Read the License agreement
5. Select install for just me; click next.
6. Choose install directions; click next.
7. If the name of the installation file is too long, remove the words pymol2
8. Click install.
9. When installation is complete, click next.
10. Click finish to close the setup.

### Part 1: Primary Structure

#### *Human Insulin*

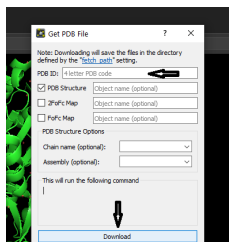
Insulin was the first protein to have its primary structure determined. It has a primary structure of two polypeptide chains linked by disulfide bonds. Chain A has 21 amino acids and B chain has 30 amino acids. Shown below are some of the features of PyMOL that you will use in this activity.



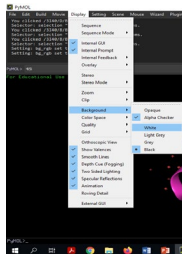
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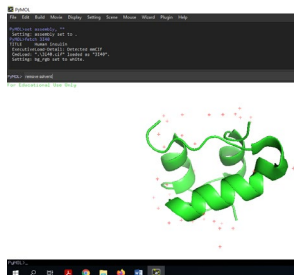
1. To open PyMOL, double click on the PyMOL icon on the desktop. Once PyMOL is open, select **File** from the dropdown menus and choose **Get PDB** from the dropdown menu. In the popup window, type **2hiu** beside PDB id: Click **download**.



2. The protein structure of human insulin will appear in your window. Select **Display** and choose **Background** from the dropdown menu. Select **white** and your background will change color.



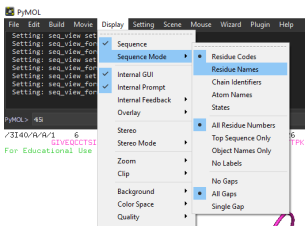
3. The red plus symbols represent water molecules, to remove type **remove solvent** in the command line and click enter.



4. Click anywhere on the protein and use your mouse to rotate the insulin molecule. Pay close attention to the links between the two strands. The links represent the disulfide bonds that exist between the A and B chains.
5. In Names/Object panel, select **C** → **Color** → **by chain** → **by chain** (choose any color)
6. Select **Display** and choose **Sequence** from the dropdown menu. You will see the one letter codes for the amino acids in human insulin. Select **Display** again and choose **Sequence Mode** from the dropdown menu. Select **Residue Names**.

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The numbers of each residue will have the corresponding three-letter code of each amino acid shown below it. Chain A and chain B are shown in PyMOL as A/A and B/B, respectively. The order of amino acids shown is the primary structure of human insulin. Record 8<sup>th</sup> and 10<sup>th</sup> amino acids of chain A and 3<sup>rd</sup> and 30<sup>th</sup> amino acids of chain B in Table 1.

7. To clear the previous, type **Rein** in the command line and hit enter.
8. Repeat steps 1-3 for Porcine (pig) insulin, using PDB id: **4ins**
9. To clear the previous, type **Rein** in the command line and hit enter.
10. Repeat steps 1-3 for Bovine (cow) insulin, using PDB id: **4m4L**

**Table 1: Primary Structure of Insulin**

| Protein         | Chain A   |            | Chain B   |            |
|-----------------|-----------|------------|-----------|------------|
|                 | Residue 8 | Residue 10 | Residue 3 | Residue 30 |
| Human Insulin   |           |            |           |            |
| Porcine Insulin |           |            |           |            |
| Bovine insulin  |           |            |           |            |

*Using complete sentences, write brief answers to the following questions. Be sure to include a reference for any source used to answer the following questions.*

1. All the amino acids in insulin found in pigs, humans, and cows are identical except for the residues in table 1. Using table 1, discuss how primary structure of human insulin differs from porcine insulin and bovine insulin. In your answer, be sure to include the definition of primary structure.
2. Describe how prescribed insulin treats diabetes mellitus.
3. Both porcine and bovine insulin were used to treat diabetes mellitus before the recent development of synthesis of human insulin. Why was this possible?

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## Part II: Secondary Structure

Secondary structures describe the type of protein structure that forms when amino acids form hydrogen bonds within a single polypeptide chain or between polypeptide chains

### ***$\beta$ -pleated sheet***

Beta-pleated sheet forms between adjacent polypeptide chains or within the same polypeptide chain when the rigid structure of the amino acid proline causes a bend in the polypeptide chain. The following protein is a  $\beta$ -barrel found in *Pseudomonas aeruginosa* OprF and is classified as an outer membrane porin.

1. To clear the previous session, type **Rein** in the command line and hit enter.
2. To get the next pdb, type **Fetch 4rlc** in the command line.
3. The protein structure of the  $\beta$ -barrel found in *Pseudomonas aeruginosa* will appear in your window. Select **Display** and choose **Background** from the dropdown menu. Select **white** and your background will change color.
4. The red plus symbols represent water molecules. To remove the water molecules, select H in the Names/Object panel, **select H  $\rightarrow$  Hide  $\rightarrow$  waters**.
5. Click anywhere on the protein and use your mouse to rotate the protein.

*Using complete sentences, write brief answers to the following questions. Be sure to include a reference for any source used to answer the following questions.*

Describe what you see as you rotate the molecule.

6. To view the protein in a different view, select H in the Names/Object panel, **select S  $\rightarrow$  Show as  $\rightarrow$  sticks**
7. Click anywhere on the protein and use your mouse to rotate the protein.

*Using complete sentences, write brief answers to the following questions. Be sure to include a reference for any source used to answer the following questions.*

You have just added the side chains of the amino acids to the protein. Describe what you see as you rotate the molecule. What happened to the size of the opening?

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What is a porin?

### ***$\alpha$ -helix***

An alpha-helix has a coiled shape held in place by hydrogen bonds between the amide groups and the carbonyl groups of the amino acids along the chain. The following protein is an apolipoprotein that is found in the structure of a lipoprotein.

Repeat steps 1-4 for the **protein**, using PDB id: **1av1**

*Using complete sentences, write brief answers to the following questions. Be sure to include a reference for any source used to answer the following questions.*

Describe what you see as you rotate the molecule.

What is a lipoprotein?

### ***Triple helix***

A triple helix consists of three alpha helices woven together. Structural proteins such as collagen, connective tissue, skin, tendons, and cartilage contain triple helices.

Repeat steps 1-4 for collagen, using PDB id: **1bkv**.

5. In Names/Object panel, **select C → Color → by chain → by chain** (choose any color)

Click anywhere on the protein and use your mouse to rotate the collagen and identify the three helices.

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*Using complete sentences, write brief answers to the following questions. Be sure to include a reference for any source used to answer the following questions.*

Describe what you see as you rotate the molecule.

What is the difference between an  $\alpha$ -helix and a triple helix?

The disease scurvy is caused by a vitamin C deficiency. Describe scurvy.

### **Part III: Tertiary Structure**

Tertiary structure gives a specific three-dimensional shape to the polypeptide chain. It involves the attractions and repulsions of the R groups of the amino acids of the peptide chain. Myoglobin is a transport protein that carries oxygen.

Repeat steps 1-4 for sperm whale myoglobin, using PDB id: **1vxa**. Click anywhere on myoglobin and use your mouse to rotate it and identify the eight helices.

5. In Names/Object panel, **select C → Color → by chain → by chain** (choose any color)

*Using complete sentences, write brief answers to the following questions. Be sure to include a reference for any source used to answer the following questions.*

How many chain(s) do you see? \_\_\_\_\_

In the center myoglobin, there is a flat planar structure. What is it and why is it necessary?

List all the R group interactions responsible for tertiary structure?

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#### Part IV: Quaternary Structure

Quaternary structure is the combination of two or more polypeptide chains stabilized by the same interactions found in tertiary structures. Hemoglobin consists of two alpha chains and two beta chains with heme groups in each subunit that transport oxygen in the blood to the tissues.

Repeat steps 1-4 for hemoglobin shown without oxygen bound, using PDB id: **2hhb**.

5. In Names/Object panel, **select C → Color → by chain → by chain** (choose any color)

6. Click anywhere on hemoglobin and use your mouse to rotate it.

*Using complete sentences, write brief answers to the following questions. Be sure to include a reference for any source used to answer the following questions.*

How many chain(s) do you see? \_\_\_\_\_

Describe what you see as you rotate the molecule.

Describe sickle cell anemia and how it affects hemoglobin.

#### Clean Up

Make sure to wipe down your work area and remember to log out of the computers.

#### Calculations

No calculations

#### Analysis & Discussion

Questions are located in each section.

**References:** Please list any references, including your textbook, that you used to answer the questions in the lab.