BIL 151 – Enzymes: Reagent Recipes

I. IMPORTANT RULES

Proper lab technique is important for getting good experimental results. Read and heed!

1. **The Platinum Rule: Always label *everything*!**
   You must include chemical name, concentration, pH (if applicable), date and your initials on every reagent vessel.
2. **Do not use beakers for measuring reagents.** They are not accurate. To measure precise amounts of reagents, always use a graduated cylinder or volumetric flask.
3. For very small liquid volumes, you may use a graduated syringe.
4. **NEVER contaminate glassware, spatulas, weighing dishes, etc. with more than one type of chemical reagent.** Use separate, clean glassware, spatula, and weighing dishes for each chemical.
5. If you are not sure a piece of glassware is clean, then wash, rinse with deionized (DI) water and dry. You can then use it with confidence.
6. **Clean up after yourself.** After use, wash all glassware with tap water and soap, rinse 3X with tap water and 3X with deionized (DI) water before placing in a drying rack.
7. **ALWAYS wash dirty glassware or other materials before you leave lab.**

II. INSTRUCTIONS: Mixing Solutions

A buffer is a solution that resists changes in pH. In this lab, a sodium phosphate buffer (pH 7.0) will be used

- by itself
- as a solution in which to suspend live yeast
- as a solution in which to mix hydrogen peroxide (H_2O_2)

If your team plans to use other types of buffers, recipes appear below.

A. General Instructions for Titration

Titration is a process by which one solution is added to another solution such that they react under conditions in which the added volume may be accurately measured. (Titrations are most often associated with acid-base reactions, but may be used for other types of reactions, too.)

While monitoring pH, you will gradually add an acidic component to a basic component to achieve a desired pH of a buffer solution. This process is called **titration**. (The acid and base components will vary, depending on the type of buffer you are making.) To titrate a buffer:

1. Add the base component of your solution to a large mixing beaker.
2. Place a magnetic stir bar in the beaker, and place it on the magnetic stirrer.
3. Turn on the stirrer until you see a small funnel in the center of the solution.
4. Follow the instructions next to the pH meter to properly calibrate the electrodes.
5. Rinse the pH meter electrodes with DI water and place into your base solution.
6. While monitoring pH on the meter, slowly add the acid component of your buffer until the entire solution reaches the desired pH.

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7. Titrate slowly! If you overshoot and have to back titrate, the molarity of your solution could be altered, and this could affect your results.
8. When you have titrated to the proper pH, remove the electrode, rinse it with DI water and place it back in the storage container.
9. Remove the magnetic stir bar with the magnetic retriever stick.
10. Wash and rinse all materials you have used with tapwater and then rinse well with DI water.
11. Seal the container and label with
    a. name of reagent (with pH, if applicable)
    b. your name
    c. the date

B. Recipes for Solutions

Below you will find instructions for mixing the various solutions your team may wish to use for this research project. Read instructions carefully before you start mixing.

1. Sodium phosphate buffer (0.05 M, pH = 7.0)

1. Dissolve 0.69 grams of $NaH_2PO4\text{-}H_2O$ (monobasic sodium phosphate acid) in 100mL of distilled water. This is the **ACID component** of your buffer.
2. Separately, dissolve 1.06 grams of Na_2HPO_4 (dibasic sodium phosphate base) in 150mL of distilled water. This is the **BASE component** of your buffer.
3. Titrate to pH 7.0 by slowly adding acid to base as per the instructions in II-A above. (This will require approximately 2 volumes of acid to 3 volumes of base)
4. If you need a greater volume of buffer, adjust the quantities accordingly.
5. This buffer can be titrated to different pH levels, if you wish to run experiments using the same type of buffer. The limiting factors are the pH levels of your two components.

This solution will be used

- by itself (1L per lab period)
- as the liquid for the live yeast suspension (3L per lab period)
- as the base for the hydrogen peroxide solution (4L per lab period)

2. Glycine-sodium hydroxide buffer (0.05 M, pH 8.5-10.0)

1. Dissolve 0.38 g of glycine in 100mL of distilled water (*acid component*)
2. Separately, dissolve 0.2 g of sodium hydroxide (NaOH) in 100 mL of distilled water (*base component*)
3. Titrate to pH 8.5, or to another basic pH (up to pH 10).

3. Sodium citrate buffer (0.1 M, pH 3.0-6.2)

Use a beaker large enough to accommodate 200 ml to mix the 2 solutions together.

1. Prepare 100 ml of 0.1 M citric acid monohydrate, ($C_6H_8O_7 \cdot H_2O$, FW=210.14), by weighing out 2.10 g and adding it to 100 ml of DI water. *(Citric acid will be your acid component.)*
2. Prepare 100 ml of 0.1 M trisodium citrate dihydrate, ($C_6H_5O_7Na_3 \cdot 2H_2O$, FW=294.12), by weighing out 2.94 g and adding it to 100 ml of DI water. *(Trisodium citrate will be your basic component.)*
3. Use this handy chart to help you achieve your target pH:

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pH	x ml 0.1 M-citric acid	y ml 0.1M-trisodium citrate
3.0	82.0	18.0
3.2	77.5	22.5
3.4	73.0	27.0
3.6	68.5	31.5
3.8	63.5	36.5
4.0	59.0	41.0
4.2	54.0	46.0
4.4	49.5	50.5
4.6	44.5	55.5
4.8	40.0	60.0
5.0	35.0	65.0
5.2	30.5	69.5
5.4	25.5	74.5
5.6	21.0	79.0
5.8	16.0	84.0
6.0	11.5	88.5
6.2	8.0	92.0

4. Yeast suspension

Your model organisms, *Saccharomyces cereviseae*, need a liquid place to live and react. Most teams will be running experiments at pH 7, and so will be using pH 7 sodium phosphate buffer to suspend the yeast. If your team feels it necessary to suspend yeast at a different pH, discuss your protocol with your lab instructor before proceeding.

The yeast suspension available for your team's use was made by mising 70 g of yeast into 1000 mL (1 L) of pH 7.0, 0.05 M sodium phosphate buffer solution (from recipe A, above).