**Catalase Enzyme “DOT” Lab MAKE UP** Name:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

AKA “Sinkers” or “Floaters” Date:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Hour:\_\_\_\_\_\_

\_\_\_\_\_\_\_/4 points

**Introduction**

Enzymes are proteins that SPEED UP chemical reactions in living cells. They are biological catalysts, which speed up chemical reactions by **decreasing** the activation energy need to begin a reaction. In this lab, you will learn about enzyme characteristics and explore several **factors that may affect enzyme FUNCTION**. The chemical reaction we will study in this lab involves the hydrogen peroxide (H202), which naturally decomposes very slowly into water and oxygen gas:

“SLOW Process”

2 H2O2  2 H2O + O2

The reaction happens very slowly, but over a number of years a bottle of peroxide will convert almost entirely into water.

The enzyme we will study in this lab is called catalase and it dramatically speeds up the breakdown of hydrogen peroxide. Catalase exists in the cells of any organism that breaths oxygen and gets energy for cells through aerobic respiration. Thus, catalase is a common enzyme found in humans. It reduces toxic levels of H202that accumulate as waste (or metabolic byproduct) as our cells generate energy. In other words, any cell without catalase enzymes would soon die from the toxic buildup of H202. The rate of the catalyzed breakdown of hydrogen peroxide can be measured by the rate of O2 production. In this investigation the speed with which O2 bubbles cause a paper disk to rise indicates the relative speed of the reaction.

catalase

“QUICK Process”

2 H2O2  2 H2O + O2

Your source of the catalase enzyme will be baker’s yeast. Baker’s yeast, *Saccharomyces cerevisiae*, is a fungus that is used commonly in baking. Yeast is an organism that utilizes the process known as cellular respiration to breakdown glucose (sugar) for energy through a chemical reaction to form water and CO2. During this process, toxic H202 slowly builds up as a byproduct and must be broken down in the cell by catalase enzymes.

**Key Lab Question (keep this in mind as you do this lab):** What are some factors that could affect the rate in which catalase enzymes breakdown hydrogen peroxide? (i.e., What factors could **speed up** or **slow down** how quickly catalase works?)

**Pre-Lab Questions:**

1. What is a catalyst? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

2. What is an enzyme? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

3. What is the purpose of catalase in the breakdown of hydrogen peroxide (H2O2)? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

4. Why is catalase important in the human body? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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**To begin the lab, Frank used**  a sharpie to label his 4 Dixie cups in the following way: 1)Enzyme @ **room temp**, 2) Enzyme + **SALT**, 3) **Boiled** Enzyme, 4) **Chilled** Enzyme. After his instructor poured each of the 4 enzyme solutions into the appropriate Dixie cup, he placed 3 filter paper disks into each enzyme solution and left it to soak for ~ 5 minutes.

**MAKE PREDICTIONS:**

1. What kind of reaction do you think that the hydrogen peroxide will have with the room temperature enzyme:

Rapid bubbling (with the dot rising quickly to the surface) Slow Bubbling (Dot rises slowly to the surface of the enzyme) No reaction

2. What kind of reaction do you think that the hydrogen peroxide will have with the chilled enzyme:

Rapid bubbling (with the dot rising quickly to the surface) Slow Bubbling (Dot rises slowly to the surface of the enzyme) No reaction

3. What kind of reaction do you think that the hydrogen peroxide will have with the boiled enzyme:

Rapid bubbling (with the dot rising quickly to the surface) Slow Bubbling (Dot rises slowly to the surface of the enzyme) No reaction

4. What kind of reaction do you think that the hydrogen peroxide will have with the enzyme plus salt:

Rapid bubbling (with the dot rising quickly to the surface) Slow Bubbling (Dot rises slowly to the surface of the enzyme) No reaction

**Task 1: Testing Catalase enzymes** (Baker’s Yeast) **at Room Temperature**

Frank filled each of his 3 test tubes with **2** dropper squirts of hydrogen peroxide solution (about 1 inch)

After his paper disks were well soaked with enzyme (~5 minutes in solution), he carefully poured the enzyme solution into the LEFTOVER enzyme solutions Dixie cup without losing the 3 paper disks. Using tweezers, he placed one filter paper dot into the first test tube with the hydrogen peroxide solution. The disk sank to the bottom. He began timing at the moment the disk touched the **bottom** of the test tube. The disk eventually floated to the surface as it filled with O2 bubbles produced by the breakdown of H202. Frank stopped timing when the disk reached the **surface**. He repeated this method for three trials.

1. Calculate the average time for the three trials:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time (seconds)to surface | | | | |
|  | Trial #1 | Trial #2 | Trial #3 | Average Time |
| Enzyme @ rm.temp | 12 | 13 | 11 |  |

**Task 2-Measuring Catalase enzymes** (Baker’s Yeast) **+ Salt**

* In this investigation, Frank repeated the steps above, but this time, using a solution of yeast AND salt.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time (seconds)to surface | | | | |
|  | Trial #1 | Trial #2 | Trial #3 | Average Time |
| Enzyme + Salt | 49 | 55 | 51 |  |

**Task 3-Measuring Catalase enzymes** (Baker’s Yeast) **that has been BOILED**

* In this investigation, Frank repeated the steps above, but this time, he used a solution of yeast that had been boiled.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time (seconds)to surface | | | | |
|  | Trial #1 | Trial #2 | Trial #3 | Average Time |
| Boiled Enzyme | No Reaction | No Reaction | No Reaction |  |

**Task 4-Measuring Catalase enzymes** (Baker’s Yeast) **that has been CHILLED**

* In this investigation, Frank repeated the steps above, but this time, he used a solution of yeast that had been chilled.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time (seconds)to surface | | | | |
|  | Trial #1 | Trial #2 | Trial #3 | Average Time |
| Chilled Enzyme | 30 | 40 | 35 |  |

**Graphing Practice:**

Create a graph of Frank’s results from data table 1 that will COMPARE the **average time** for H2O2 to breakdown when catalyzed by a catalase enzyme experiencing 4 different cellular conditions (room temp, salt, boiled, chilled). Graph his data found in data table 1 on the graph provided below. Remember to give your graph an appropriate title, label your axis correctly and completely, and plot your data accordingly. If you need to construct a key, please do so in the space provided to the left of your graph.



**Post-Lab Questions**  *Answer the following questions.*

1. Think back to measuring catalase enzymes + SALT…What was Frank testing in this particular investigation?

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2. Think back to measuring catalase enzymes that had been boiled…What was Frank testing in this investigation?

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3. Think back to measuring catalase enzymes that had been chilled…What was Frank testing in this investigation?  
\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

4. Which enzyme and cell conditions took the **least** amount of time for the disk to float? \_\_\_\_\_\_\_\_\_\_\_\_\_

Using Frank’s results and prior knowledge, provide an explanation for your findings:

\_\_\_\_\_\_\_\_\_\_\_\_\_

5. Which enzyme and cell conditions took the **longest** amount of time for the disk to float? \_\_\_\_\_\_\_\_\_\_\_\_

What are some possible reasons? \_\_\_ \_\_\_ \_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_

6. Did any of the enzyme and cell conditions cause the disk **not to float**? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Explain WHY? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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Dot Lab Review **Matching**:

catalase

2 H2O2 ------> 2 H2O + O2

**4 Choices:**

Hydrogen Peroxide (H2O2) water (H2O)

Catalase oxygen (O2)

1. Write the name of the **REACTANT** from the DOT lab? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
2. Write the name of the **PRODUCTS** from the DOT lab? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
3. Write the name of the substance contained in the “**bubbles**”? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
4. Write the name of the **liquid** left after the reactions are finished? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
5. Write the name of the **enzyme** that catalyzes the reaction? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
6. Write the name of the **substrate** in the reaction? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
7. Write the name of the substance with the **active site**? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
8. Write the name of the substance **made from amino acids**? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
9. Write the name of the substance that can be **denatured**? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
10. Explain what would happen to your “float time” data if your 3 test tubes did not have the same exact height of hydrogen peroxide? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

WHY do you think that is? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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1. Explain what would happen to your “float time” data if we used old hydrogen peroxide that had been stored on a shelf for 10 years? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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1. Explain WHY bubbles form when you put hydrogen peroxide on a cut? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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1. Explain what would happen to your skin cells over time if they did NOT make catalase enzymes? \_\_\_\_\_\_\_\_\_\_\_\_

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Teacher Dot Lab Demo: **Measuring Catalase enzymes** (Baker’s Yeast) **with different pH conditions (ACID or BASE)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Time (seconds)to surface | | | | |
|  | pH | Trial #1 | Trial #2 | Trial #3 | Average Time |
| Enzyme in **ACID** |  |  |  |  |  |
| Enzyme in water (neutral) |  |  |  |  |  |
| Enzyme in **Base** |  |  |  |  |  |

1. Interpret these results …. Explain how different pH conditions affect the function of catalase? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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1. The human stomach is filled with a strong acid, yet the small intestine has basic chemical conditions. Circle where catalase enzymes would function the best (stomach or small intestine) and explain why?

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