Name_____ Date_____ Period _____

**Honors Biology**

***Floating Disk Enzyme Lab***

**Introduction**

Enzymes are catalytic proteins that speed up the rate of chemical reactions that would otherwise happen more slowly. A catalyst is a chemical agent that changes the rate of a reaction without being consumed by the reaction. You have thousands of different enzymes in each of your cells. Each of these enzymes is responsible for one particular chemical reaction that occurs in the cell.

In this lab, you will study catalase, an enzyme that is found in the cells of many living tissues, including those of plant, animal, and even fungus. The catalase we will be testing in this lab comes from yeast cells which contain a large amount of the enzyme catalase.

Hydrogen peroxide ($H_2O_2$) is produced as a waste product in every cell of your body. It is a byproduct of many normal chemical reactions. If the cells did not break down the hydrogen peroxide, they would be poisoned and die. Catalase speeds up the reaction that breaks down the toxic hydrogen peroxide into 2 harmless substances: water and oxygen:

$$\mathbf{2H_2O_2 \rightarrow\ 2H_2O + O_2}$$

The rate of this reaction can be determined by measuring the amount of oxygen produced. Filter paper disks are dipped into a suspension of yeast cells. These disks are dropped into $H_2O_2$ and the oxygen created as a byproduct in the reaction builds up on the disk, causing it to float. The time it takes for the disks to rise to the surface of $H_2O_2$ is measured. Depending on the catalase solution some of the reactions will happen faster than others, producing oxygen faster and the disk will float sooner.

**Part A: Establishing Baseline Data**

**Materials**

- Forceps
- Filter paper disks
- 100 ml graduated cylinder
- Timer (1 cell phone)
- 3% Hydrogen peroxide
- 100% catalase - Yeast suspension
- Ruler
- Clear plastic cups
- Marker
- small beaker

**Procedure**

1. Prepare a clear cup with 3% $H_2O_2$ that is 4 cm deep.
2. Pour 20 ml of 100% catalase into the small beaker. Shake the bottle of the yeast suspension BEFORE pouring to make sure it is well mixed. You will use this source of catalase for the rest of the lab.
3. Pick up a single filter paper disk with forceps and dip the disk in your catalase enzyme solution in the beaker.
4. Still using the forceps, drop the disk into the $H_2O_2$. Watch the disk carefully and **start timing** when the disk hits the bottom of the cup, **stop timing** when the disk reaches the surface.
5. Run 4 more trials with the $H_2O_2$ at room temperature, using a new filter paper disk each time. Record your data in **Data Table 1** below.
6. When you clean up make sure the filter paper disks do NOT go down the drain.

**Data Table 1**

| Trial | Seconds to Rise |
|---|---|
| 1 | |
| 2 | |
| 3 | |
| 4 | |
| 5 | |
| **Average** | |

a. What is the enzyme in this reaction? _____

b. What is the substrate in this reaction? _____

c. What is/are the product(s) in this reaction? _____

d. What is the gas you see bubbling up? _____

**Part B: For this part of the lab, each group will be assigned ONE of the following Essential Questions to investigate. You will follow the procedure, and report out the results to the class.**

**Essential Question 1: What is the effect of substrate concentration on the rate of enzyme reaction?**

**Essential Question 2: What is the effect of enzyme concentration on the rate of enzyme reaction?**

**Essential Question 3: What is the effect of temperature on the rate of enzyme reaction?**

**Essential Question 4: What is the effect of pH on the rate of enzyme reaction?**

**Essential Question 1: What is the effect of substrate concentration on the rate of enzyme reaction?**

1. Prepare the 3 different $H_2O_2$ solutions using Table 1 as a guide. Pour 4 cm of the diluted hydrogen peroxide ($H_2O_2$) solutions into 3 different clear cups. Label each cup with the marker.
2. Using forceps, dip a filter paper disk into the yeast suspension.
3. Drop the disk into the cup of 1.5% solution. Start timing as soon as the disk touches the surface of the hydrogen peroxide. Keep timing until the disk reaches the surface again. Record data in **Data Table 2** below.
4. Dispose of the disk. Repeat for 4 more trials, using a new filter paper disk each time.
5. Repeat steps #1-4 for the .6% and 0% dilutions, using Table 1 as a guide. Complete 5 trials for each.

**Table 1: Catalase Dilutions (based off of 3% $H_2O_2$ solution)**

| Final quantity needed | Concentration of final solution | mL of $H_2O_2$ | mL of water |
|---|---|---|---|
| 50mL | 1.5% | 25 | 25 |
| 50mL | .6% | 10 | 40 |
| 50mL | 0% | 0 | 50 |

**Data Table 2: Reaction Rate (in seconds) of $H_2O_2$ Dilutions**

| Trial | Time for 3% (s) (from Part A) | Time for 1.5% (s) | Time for .6% (s) | Time for 0% (s) |
|---|---|---|---|---|
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |
| **Average** | | | | |

**Claim (This answers your Essential Question)**

_____

_____

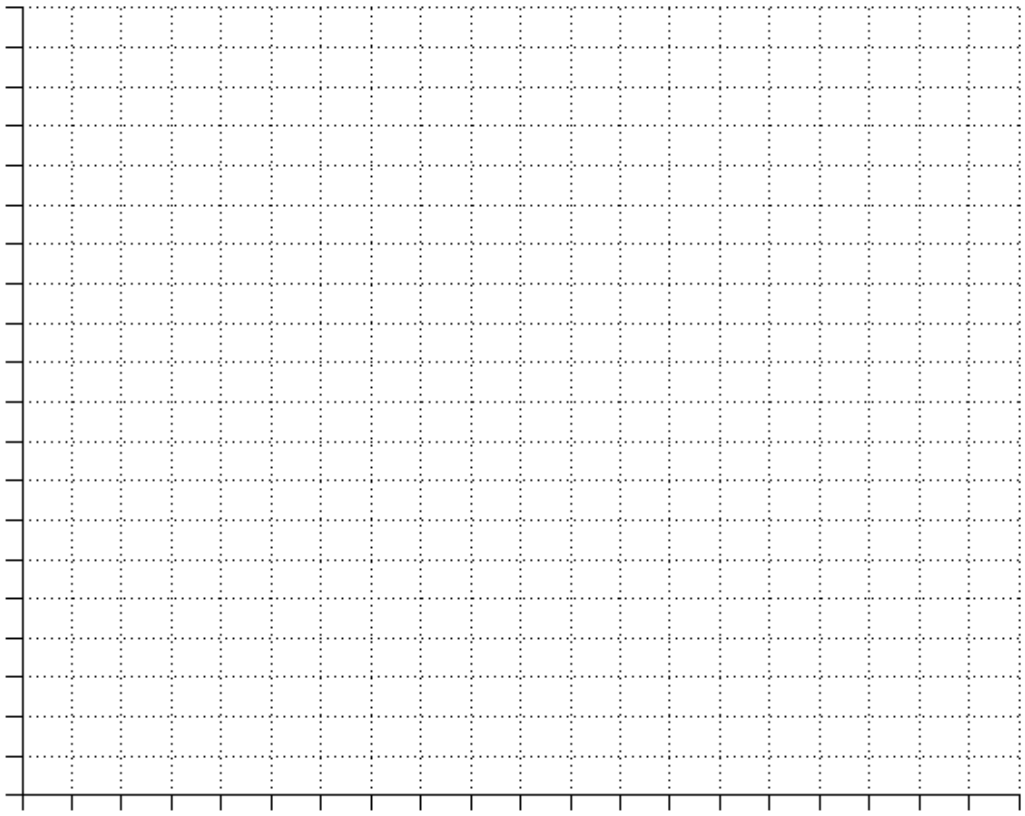
_____

_____

*Created by Robin Bulleri, 2017, modified from Paula Donham and other sources*

**Evidence & Reasoning**

Construct a bar graph showing **average** time versus the substrate concentration. Place substrate concentration on the x-axis and the time on the y-axis.



How does your evidence support your claim? Describe the scientific concepts and principles related to your experiment.

_____

_____

_____

_____

_____

_____

_____

_____

_____

**Essential Question 2: What is the effect of enzyme concentration on the rate of enzyme reaction?**

1. Obtain the 3 different enzyme (yeast) solutions from the front counter. Use a small beaker or cup for each solution.
2. Pour 4 cm of 3% hydrogen peroxide ($H_2O_2$) solutions into one clear plastic cup.
3. Using forceps, dip a filter paper disk into the 200% yeast suspension.
4. Drop the disk into the cup of 3% solution. Start timing as soon as the disk touches the surface of the hydrogen peroxide. Keep timing until the disk reaches the surface again. Record data in **Data Table 3** below.
5. Dispose of the disk. Repeat for 4 more trials, using a new filter paper disk each time.
6. Repeat steps #3-5 for the 50% and 25% dilutions for 5 trials of each.

**Data Table 3: Reaction Rate (in seconds) of Yeast (catalase) Dilutions**

| Trial | Time for 200% (s) | Time for100% (s) (from Part A) | Time for 50% (s) | Time for 25% (s) |
|---|---|---|---|---|
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |
| **Average** | | | | |

**Claim (This answers your Essential Question)**

_____

_____

_____

_____

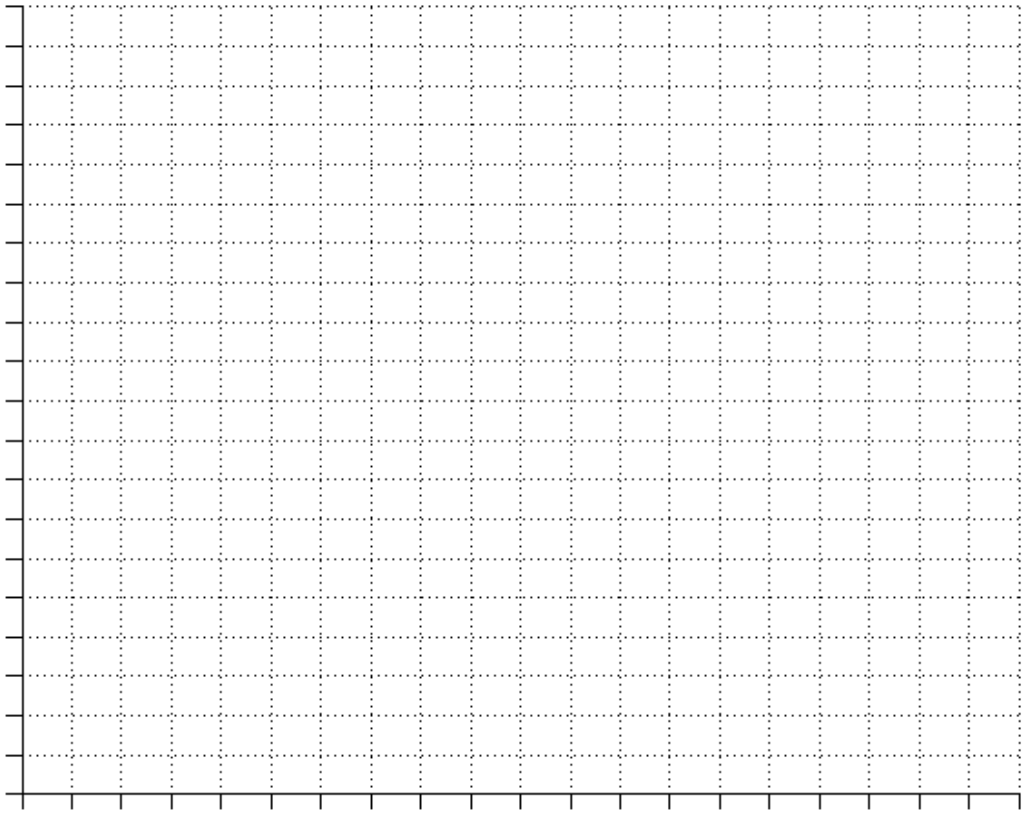
_____

_____

_____

**Evidence & Reasoning**

Construct a bar graph showing **average** time versus the enzyme concentration. Place enzyme concentration on the x-axis and the time on the y-axis.



How does your evidence support your claim? Describe the scientific concepts and principles related to your experiment.

_____

_____

_____

_____

_____

_____

_____

_____

_____

**Essential Question 3: What is the effect of temperature on the rate of enzyme reaction?**

1. Pour 4 cm of 3% hydrogen peroxide ($H_2O_2$) solutions into 2 different clear cups.
2. Obtain the COLD catalase from your teacher. The temperature should be less than 10°Celsius.
3. Using forceps, dip a filter paper disk into the COLD 100% yeast suspension.
4. Drop the disk into the cup of 3% $H_2O_2$ solution. Start timing as soon as the disk touches the surface of the hydrogen peroxide. Keep timing until the disk reaches the surface again. Record in data in **Data Table 4** below.
5. Dispose of the disk. Repeat steps #2-4 with the boiled catalase for 5 trials of each, using a new filter paper disk each time.

**Data Table 4: Reaction Rate (in seconds) at Various Temperatures**

| Trial | Time for room Temp (s) (from Part A) | Time for Cold Temp (s) | Time for Boiled Catalse (s) |
|---|---|---|---|
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |
| 5 | | | |
| **Average** | | | |

**Claim (This answers your Essential Question)**

_____

_____

_____

_____

_____

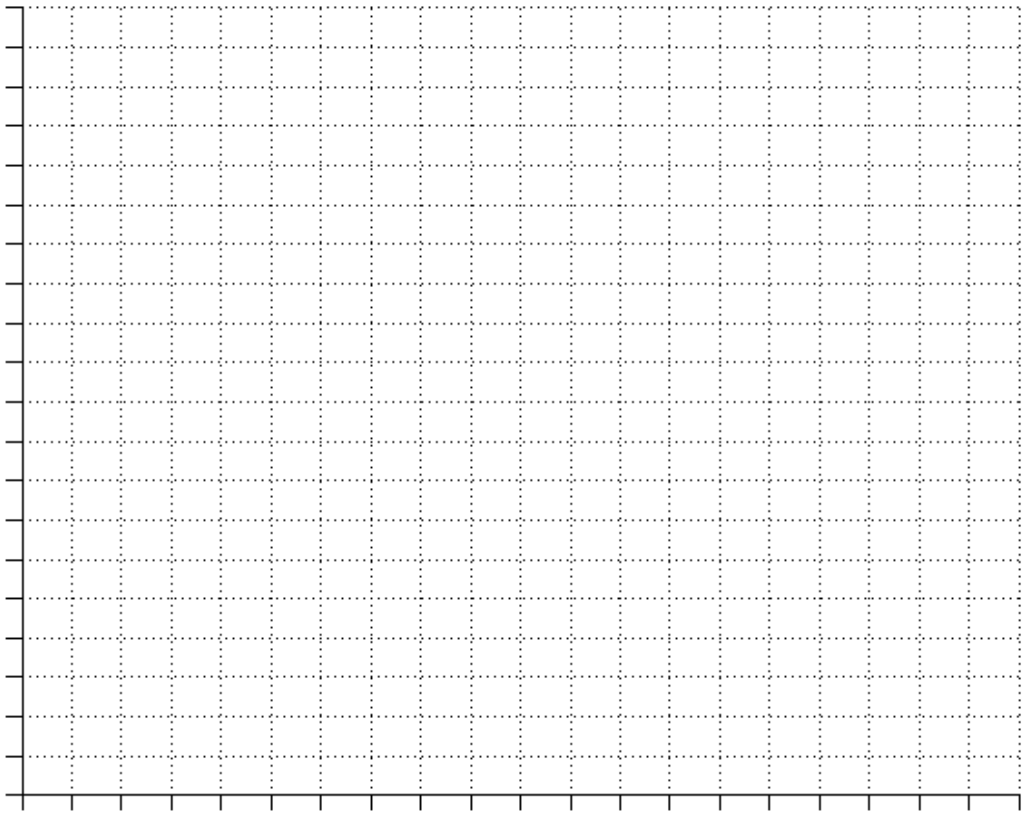
_____

_____

_____

**Evidence & Reasoning**

Construct a bar graph showing **average** time versus the temperature. Place temperature on the x-axis and the time on the y-axis.



How does your evidence support your claim? Describe the scientific concepts and principles related to your experiment.

_____

_____

_____

_____

_____

_____

_____

_____

_____

**Essential Question 4: What is the effect of pH on the rate of enzyme reaction?**

1. Prepare the 2 different pH solutions using 25 ml of $H_2O_2$ and 25 ml of the ph 4 or pH 10 buffer. Pour 4 cm of each pH solution into 2 different clear cups. Label each cup with the marker.
2. Using forceps, dip a filter paper disk into the 100% yeast suspension.
3. Drop the disk into the cup of pH 4 solution. Start timing as soon as the disk touches the surface of the hydrogen peroxide. Keep timing until the disk reaches the surface again. Record data in **Data Table 5** below.
4. Dispose of the disk. Repeat for 4 more trials, using a new filter paper disk each time.
5. Repeat steps #2-4 for the pH 10 solution. Complete 5 trials for each.

**Data Table 5: Reaction Rate (in seconds) of $H_2O_2$ Dilutions**

| Trial | Time for pH 7 (s) (from Part A) | Time for pH 4 (s) | Time for pH 10 (s) |
|---|---|---|---|
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |
| 5 | | | |
| **Average** | | | |

**Claim (This answers your Essential Question)**

_____

_____

_____

_____

_____

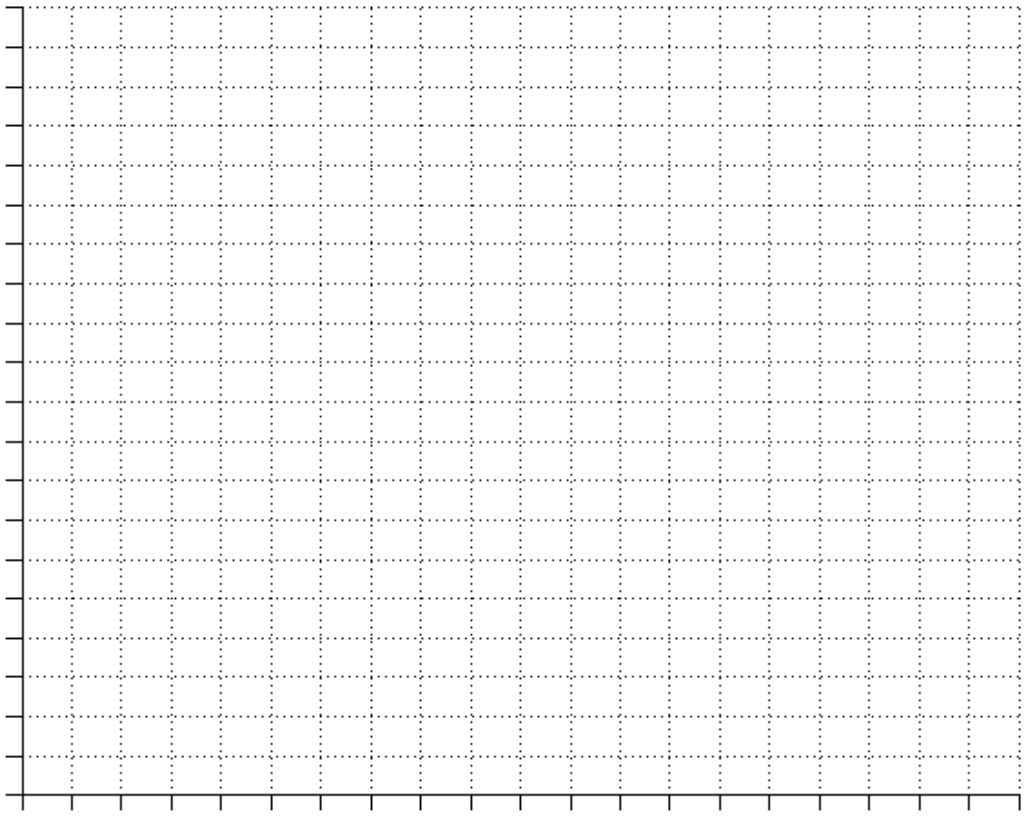
_____

_____

_____

**Evidence & Reasoning**

Construct a bar graph showing **average** time versus the pH. Place pH on the x-axis and the time on the y-axis.



How does your evidence support your claim? Describe the scientific concepts and principles related to your experiment.

_____

_____

_____

_____

_____

_____

_____

_____

_____