### **Liver Lab**

Most biological processes in living systems involve metabolic pathways of chemical reactions, which occur under different physiological conditions. Fortunately, not all chemical reactions occur spontaneously. If they did, our metabolism would be chaotic. Instead, most metabolic reactions are controlled by biological catalysts known as **enzymes**. Enzymes are proteins that speed up the rate of reactions that would otherwise happen more slowly. The enzymes are not altered by the reaction. You have hundreds of different enzymes in each of your cells. Each of these enzymes is responsible for one particular reaction that occurs in the cell.

Enzymes bind to reacting molecules called the **substrate(s)**, to form an **enzyme-substrate complex**. The area on the enzyme where the substrate(s) bind(s) is called the **active site**. Enzymes are made of long chains of amino acids that form complex shapes. All can be affected by temperature, pressure, and pH. Denaturation (unfolding) of an enzyme caused by changes in pH or temperature can affect the activity of the enzyme. Inhibitors that can competitively bind to the active site on the enzymes and block the actual substrate from binding can also affect the enzyme's activity.

The activity of an enzyme can be determined by measuring the reaction capacity between the enzyme and its target substrate. Such reaction may result in the appearance of a product which can be detected qualitatively using estimation of "bubbliness".

In this lab, you will study enzymes that are found in the cells of many living tissues. The enzyme in today's lab is **catalase**, found in liver cells. Catalase speeds up a reaction which breaks down hydrogen peroxide (H2O2), a toxic chemical, into two harmless substances — water and oxygen.

The reaction is as follows:

$$2 H_2 O_2 \rightarrow 2 H_2 O + O_2$$

This reaction is important to cells because hydrogen peroxide (H2O2) is produced as a byproduct of many normal cellular reactions. If the cells did not break down the hydrogen peroxide, they would be poisoned and die. We also will examine the effect of temperature and pH on catalase activity.

If you are absent: watch the video linked below and complete the questions on the next pages https://www.youtube.com/watch?v=WDFfEX3LV5M

### <u>Data:</u>

As you conduct the experiments, draw a line on each test tube saying how high the bubbles rose. Also write a note or two about what you observed.



## **Control Group:**

#### **Procedure 1: Observe the Normal Catalase Reaction** (Test Tube #1)

Tube 1 is used to determine a baseline for the reaction.

Add a piece of liver to test tube #1 (use the glass stirring rod to push down the liver). Add 2 mL of Hydrogen Peroxide and add it to the same test tube.

- 1. What happened?
- 2. What are the bubbles made of? Refer to the equation.
- 3. Was this reaction endothermic (feels cold) or exothermic (feels warm)?
- 4. What liquid is now in the tube with the liver? (hint: refer to the overall equation)

### PART A:

#### Procedure 2: Is used hydrogen peroxide reusable? (Test tube #2)

Pour the liquid product from test tube #1 into test tube #2. Get a new piece of liver and place it in test tube #2.

- 5. Did a reaction occur?
- 6. Why or why not? Think about the equation and if you have all the parts.

#### **Procedure 3: Is catalase reusable** (Test Tube #3)

Go back to test tube #1, use the stirring rod to remove the liver from this test tube and place it in test tube #3. Measure and add 2 mL of Hydrogen Peroxide and add it to this test tube.

- 7. Did a reaction occur?
- 8. Why or why not? Think about the equation and properties of enzymes.

# **PART B: Effect of Temperature**

**Procedure 4: Observe the effect of temperature on catalase activity** (Test Tube #4)

Boiled liver and add 2 mL of fresh hydrogen peroxide.

### **Procedure 5: Temperature (continued)** (Test Tube #5)

Get a piece of chilled liver and add 2 ml of chilled hydrogen peroxide.

11. Did a reaction occur?

12. Why or why not?

## PART C: Effect of pH

<b>Procedure 6: Observe the effects of p</b>	oH on catalase activity	(Test Tube #6
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Take your test tube and tweezers to the front of the room and get a piece of liver from the container in the front of the room labeled ACID. Place the liver in the test tube, push it down with the stirring rod. Add 2 mL of Hydrogen Peroxide.

13. Summarize your observation of the reaction and explain

#### **Procedure 7 : pH (continued)** (Test Tube #7)

Repeat the previous steps, however this time use test tube #7 and the liver in the container labeled BASE.

14. Summarize your observation of the reaction and explain

## **Conclusion**

15. Summarize how temperature (higher and lower), pH (higher and lower), substrate concentration (high and low), and enzyme concentration (high and low) affects how well an enzyme works (Enzyme Activity) (8 POINTS!)

16. Sketch a graph showing the curve "pH's Effect on Enzyme Activity" (make sure to label denaturation and optimal)

17. Sketch a graph showing the curve "temperature's Effect on Enzyme Activity" (make sure to label denaturation and optimal)