**AP Biology**  **Enzyme Lab**

**Background:**

What would happen to your cells if they made a poisonous chemical? You might think that they would die. In fact, your cells are always making poisonous chemicals. They do not die because your cells use enzymes to break down these poisonous chemicals into harmless substances. Enzymes are proteins that speed up the rate of reactions that would otherwise happen more slowly. The enzyme is not altered by the reaction. You have hundreds of different enzymes in each of your cells.

In this lab, you will study an enzyme that is found in the cells of many living tissues. The name of the enzyme is catalase; it speeds up a reaction which breaks down hydrogen peroxide, a toxic chemical, into 2 harmless substances--water and oxygen.

**The reaction is: 2 H2O2 ----> 2 H2O + O2**

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. In this lab, you will study the catalase found in potato and liver cells. It might seem strange to use dead cells to study the function of enzymes. This is possible because when a cell dies, the enzymes remain intact and active for several weeks, as long as the tissue is kept refrigerated.

Pre-Lab Questions: answer before the lab

1. pH is measured on a scale of \_\_\_\_ to \_\_\_\_\_.

2. Acids contain an excess of H+ and will measure between \_\_\_ & \_\_\_, while bases contain an excess of OH- and will measure between \_\_\_\_ & \_\_\_\_. A pH of 7 is \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_.

3. What type of macromolecule are enzymes? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

4. Explain the function of enzymes.

5. What is the name of the enzyme that is being used in this lab and in what materials is it found?

6. How do you know when a reaction is taking place? (think about the product of the reaction)

**Hypothesis:** Form a hypothesis about the conditions under which an enzyme works best. Be specific! \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Procedures:**

PART A - Observe Normal Catalase Reaction

Warnings: Keep droppers and graduates clean - don’t mix chemicals! Report spills/rinse immediately. Wear safety goggles throughout the lab!

1. Label two test tubes 1 and 2.

2. Using a plastic syringe, place 2 ml of the 3% hydrogen peroxide solution into test tube #1.

3. Use the temperature probe to measure the temperature of the solution. Record it here:\_\_\_\_\_\_\_\_\_\_\_\_\_\_oC

3. Using forceps razor, cut a piece of potato into very small pieces and place a few pieces and into the test tube. Push the potato into the hydrogen peroxide with a stirring rod. Observe the bubbles. What gas is being released? (consider the equation) \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

4. Throughout this investigation you will estimate the rate of the reaction (how rapidly the solution bubbles) on a scale of 0-5 (0=no reaction, 1=slow, ..... 5= very fast). Assume that the reaction in step 3 proceeded at a rate of "3". Record this information in Table 1.

5. You will also estimate amount of bubbles, also on a scale of 1-5 (0= no bubbles, 5= most bubbles). Assume the bubble level in step 3 is at a level of 4 after a minute or so. Record this is Table 1.

6. Recall that a reaction that absorbs heat is endothermic; a reaction that gives off heat is exothermic. Now, use temperature probe to measure the temperature in the test tube again. Has it gotten warmer or colder \_\_\_\_\_\_\_\_\_\_? Is the reaction endothermic or exothermic? Record this in Table 1.

7. Pour off the liquid into a test tube 2. Assuming the reaction is complete. What is this liquid composed of? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

8. What do you think would happen if you added more potato to this liquid? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

9. Test this and record the reaction rate in Table 1.

10. Add another 2 ml of hydrogen peroxide to the potato remaining in the Test tube 1. Record the reaction rate in Table 1 and finish filling out the table.

Table 1: Normal Catalase with potato

|  |  |  |  |
| --- | --- | --- | --- |
| **Test tube** | **Reaction Rate** | **Bubble level** | **Endo/ Exo thermic?** |
| 1 |  |  |  |
| 2 |  |  |  |
| 1 after first reaction |  |  |  |

Part B - Concentration of Enzyme

You will now test for the presence of catalase in tissues other than potatoes.

1. Label 3 test tubes 1-3.

2. Place 2 ml of hydrogen peroxide in each of 3 clean test tubes and then add each of the three test substances to the tubes. Try to add about the same amount of each. As you add each test substance, record the reaction rate and bubble level (0-5) for each tube in Table 2.

|  |  |  |  |
| --- | --- | --- | --- |
| **Tube #** | **Substance** | **Reaction Rate (0-5)** | **Bubble Level (0-5)** |
| 1 | Liver |  |  |
| 2 | Apple |  |  |
| 3 | Carrot |  |  |

PART C - What is the Effect of Temperature on Catalase Activity?

1. Label clean test tubes 1-6.

2. Put a small amount of potato pieces into test tube 1 and leave at room temperature.

3. Put equal amounts of potato pieces into the bottom of test tube 2. Place this test tube in a boiling water bath for 5 minutes.

4. Put equal amounts of potato into test tube 3 and put it in a warm water bath for 5 minutes.

5. Put equal amounts of potatoes into test tube 4 and put it on ice for 5 minutes.

6. Put 2mL of hydrogen peroxide into test tubes 5 and 6 and place 5 in the warm water and 6 in the ice.

7. Record the temperatures, in °C, of the environments for test tube 1, 2, 3, and 4 in Table 3.

8. Remove all test tubes and place them in a test tube rack. Add 1-2mL of hydrogen peroxide to test tubes 1 and 2. Pour the content of test tube 5 into 3 (both from warm water) and the pour the contents of test tube 6 into 4 (both from ice environment).

CAUTION: Use a test-tube clamp to move the hot test tubes.

9. Record the reaction rate and bubble level for test tubes 1-4 in Table 3

Table 3: Temperature and Enzyme Activity

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Test Tube** | **Environment** | **Temperature (oC)** | **Reaction Rate** | **Bubble Level** |
| 1 | Room Temp |  |  |  |
| 2 | Boiling |  |  |  |
| 3 | Warm |  |  |  |
| 4 | Ice |  |  |  |

PART D - What is the Effect of pH on Catalase Activity

1. Label 3 clean test tubes 1-3 and add 2 ml hydrogen peroxide to each test tube.

2. Add the following: Tube 1- add 4 drops of acetic acid (vinegar) Tube 2- add 3 drops of water (control) Tube 3- add 4 drops of sodium bicarbonate

3. Using the pH Probe, put the end of the probe into the solution in the test tube.

4. Now add the same amount of potato pieces to each of the test tubes (try to do it all at about the same time, so you can easily compare) and record the reaction rate and bubble level for each in Table 4

Table 4: pH and enzyme activity

|  |  |  |  |
| --- | --- | --- | --- |
| **Test Tube** | **pH** | **Reaction Rate** | **Bubble level** |
| 1: Acetic Acid (vinegar) |  |  |  |
| 2: Water (control) |  |  |  |
| 3: Sodium Bicarbonate |  |  |  |

Clean out all test tubes and pour liquid contents down the drain. Then put the solids in a trash can.

**ANALYSIS:**

1. Describe the relationship between catalase and hydrogen peroxide. Indicate which is the enzyme, which is the substrate and what occurs during the reaction. It may be helpful to write the equation.

2. Is catalase reusable? Use your data to support your answer. (see part A step 7-10)

3. Based on your observations, which tissues from part B contained catalase?

4. Do some contain more catalase than others? How can you tell and how does this relate to reaction rate?

5. Graph temperature verses reaction rate of the potato from Table 3. (Independent variable goes on the xaxis and dependent on the y-axis. Don’t forget units, labels, and a title)



6. What was the optimal temperature and pH for catalase activity? How does temperature and pH affect the reaction rate of catalase?

7. Propose a way to refine your experiment to find the exact, or OPTIMAL pH and temperature of catalase.

8. In 2-3 sentences, summarize the data and information displayed in this graph. (Use the space next to the picture to answer).

