# Pre-Lab Information

Purpose Determine how different factors influence the rate of enzyme activity.

Time Approximately 90 minutes

Question How do different factors influence the enzyme rate of reaction for peroxidase?

Summary In this investigation, you will collect data to determine the baseline reaction for the decomposition of hydrogen peroxide using the peroxidase enzyme. Then, you will change the pH of the reaction and determine how pH affects the reaction rate. Finally, you will choose your own variable to test.

# Safety

* Behavior in the lab needs to be purposeful.
* Wear safety goggles when working with substances
* Report all accidents—no matter how big or small—to your teacher.

# Lab Procedure

1. **Gather materials.**

|  |  |
| --- | --- |
| * Peroxidase solution * Timer * Graduated pipettes * Hydrogen peroxide (0.1%) * Color chart | * Guaiacol * Distilled water * Test tubes and test tube rack * pH solution * Safety goggles |

1. **Determine the baseline.**
   1. Obtain two test tubes. In one test tube, add 7 mL of distilled water and 0.3 mL of .1% hydrogen peroxide using a pipette. Then, add 0.2 mL guaiacol.
   2. In the other test tube, add 6.0ml of distilled water and 1.5 mL of peroxidase.
   3. Combine the two test tubes and gently mix. Place the tube in your test tube rack and begin timing.
   4. Observe the color changes for the next five minutes. Record the observed color every 30 seconds in Table A. Use the color chart to help you quantify changes in color.
2. **Determine the effect of pH.**
   1. Determine which pH your group is responsible for from your teacher.
   2. Obtain two test tubes and prepare them as follows.
      * 1. 7 mL of distilled water, 0.3 mL of hydrogen peroxide, and 0.2 mL of guaiacol
        2. 6.0 mL of pH solution (see teacher for your assigned pH) and 1.5 mL of peroxidase
   3. Combine the two test tubes and gently mix. Place the tube in your test tube rack and begin timing.
   4. Observe the color changes for the next five minutes. Record the observed color every 30 seconds in Table B. Use the color chart to help you quantify changes in color.
3. **Prepare your own variable to test.**
   1. Determine another variable to test (temperature, substrate concentration, or enzyme concentration). Once you have determined your experimental design, show it to your teacher for approval.
   2. Obtain materials from your teacher.
   3. Set up test tubes and record the data in Table C.
   4. Test each substance, using the methods outlined in your experimental design. Record your results in Table B.
   5. Prepare a graph of your data that compares the baseline to your experimental results.
4. **Present your data to the class.**
   1. Be prepared to share your graph with the class and include the following.
      * 1. Graphical representation of your data
        2. The factors you tested
        3. How the reaction rate compares to the baseline
5. **Clean up your area.** 
   1. Return unused materials and dispose of any trash according to your teacher’s directions.

# Data

Record your data either in your lab notebook or in the space below.

**Table A: Baseline Data**

|  |  |  |  |
| --- | --- | --- | --- |
| **Time (Minutes)** | **Color** | **Time (Minutes)** | **Color** |
| **0** |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |

**Table B: pH: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

|  |  |  |  |
| --- | --- | --- | --- |
| **Time (Minutes)** | **Color** | **Time (Minutes)** | **Color** |
| **0** |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |

**Table C: Factor you are testing: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

|  |  |  |  |
| --- | --- | --- | --- |
| **Time (Minutes)** | **Color** | **Time (Minutes)** | **Color** |
| **0** |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |

# Follow-Up Questions

Answer the following questions:

1. What patterns did you see based on the data presented by your classmates?
2. Why does changing the pH outside the optimum have a negative effect on the reaction rate?
3. Pepsin is an enzyme that is inactive at a pH greater than 4. Pepsin works to break down proteins in your digestive system. What would be the effect of taking antacids on the enzymatic activity of pepsin? Note that antacids increase pH.