# TEACHER NOTES FOR ENZYME ACTION: TESTING CATALASE ACTIVITY

**TIME TO COMPLETE LAB:**

~80 minutes; Two 40 minute periods with a stopping point after the students have tested the effect of different enzyme concentrations. If each lab group is assigned a different condition, the lab may be completed in 40 min.



**Westminster College**

# TARGET GRADE LEVEL:

This lab is intended for AP Biology or upper level high school students. It correlates with Lab 2 in the 2001 College Board’s AP Biology Lab Manual.

# OBJECTIVE/MAJOR CONCEPTS:

* Measure the effects of changes of temperature, pH, and enzyme concentration on the reaction rates of an enzyme catalyzed reaction in a controlled experiment.
* Explain how environmental factors affect the rate of enzyme-catalyzed reactions.

# PREPARATION:

1. To prepare the yeast solution, dissolve 7 grams (1 package) of dried yeast per 100 mL of 2% glucose solution. A 2% glucose is made by adding 20 g of glucose to enough distilled water to make 1 L of solution. Incubate the suspension in 37 – 40°C water for at least 10 minutes to activate the yeast. Test the experiment before the students begin. The yeast may need to be diluted if the reaction occurs too rapidly. The reaction in Step 11 (with 10 mL of 1.5% hydrogen peroxide and 5 drops of suspension) produces enough oxygen to exceed a measured concentration of 22% in 40 to 60 seconds.
2. In general, the different pH buffers are provided by SIM. Buffers at pH 2, 4, 6, 7, 8, 10, and 12 are available for additional testing.

If you wish to prepare your pH buffers, you may use the following recipes:

* 1. pH 4.00: Add 2.0 mL of 0.1 M HCl to 1000 mL of 0.1 M potassium hydrogen phthalate.
  2. pH 7.00: Add 582 mL of 0.1 M NaOH to 1000 mL of 0.1 M potassium dihydrogen phosphate.
  3. pH 10.00: Add 214 mL of 0.1 M NaOH to 1000 mL of 0.05 M sodium bicarbonate.

1. Prepare 100 mL of 1.5% H O

(for Parts I and II) by adding 50 mL of distilled water to

2 2

50 mL of 3% H O .

2 2

1. 3% H O

can be purchased from any supermarket. If kept refrigerated, bring it to room

2 2

temperature before starting the experiment.

# EXTRA INFORMATION:

1. This experiment may take a single group several lab periods to complete. A good breaking point is after the completion of Step 12, when students have tested the effect of different enzyme concentrations. Alternatively, if time is limited, different groups can be assigned one of the three tests and the data can be shared.
2. Your hot tap water may be in the range of 50 – 55°C. If not, you may want SIM to supply water baths where students need to maintain warm water temperatures. Warn students not to touch the hot water.
3. Many different organisms may be used as a source of catalase in this experiment. Living yeast is the easiest for SIM to provide and should be prepared just prior to use. If enzymes from an animal, a protist, and a plant are used by different teams in the same class, it will be possible to compare the similarities and differences among those organisms. Often, beef liver or beef blood are used.

5. You may need to let students know that at pH values above 10, enzymes will become denatured and the rate of activity will drop. If you have pH buffers higher than 10, have students perform an experimental run using them.

# EXTENSION ACTIVITY SAMPLE DATA:

## Part I Spontaneous Decomposition of H2O2

|  |  |
| --- | --- |
| Table 2 | |
| # drops H2O2 | Slope, or rate (% O2/s) |
| 10 | TBD |

**Part II Effect of Enzyme Concentration**

|  |  |
| --- | --- |
| Table 3 | |
| Test tube label | Slope, or rate (% O2/s) |
| 5 Drops | 0.27 |
| 10 Drops | 0.73 |
| 20 Drops | 1.59 |

**Part III Effect of Temperature**

|  |  |
| --- | --- |
| Table 5 | |
| Test tube label | Slope, or rate (% O2/s) |
| 0-5 °C | 0.58 |
| 20-25 °C | 0.82 |
| 30-35 °C | 1.43 |
| 50-55 °C | 0.36 |

**Part IV Effect of pH**

|  |  |
| --- | --- |
| Table 6 | |
| Test tube label | Slope, or rate (% O2/s) |
| pH 4 | 0.36 |
| pH 7 | 0.89 |
| pH 10 | 0.97 |

**ANSWERS TO QUESTIONS:**

Part I and II Effect of Enzyme Concentration

1. How does changing the concentration of enzyme affect the rate of decomposition of H2O2?

*The rate should be highest when the concentration of enzyme is highest. With higher concentration of enzyme, there is a greater chance of an effective collision between the enzyme and H2O2 molecule.*

1. If you increase the concentration of enzyme to 25 drops, what do you think will happen to the rate of reaction? Predict what the rate would be for 30 drops.

*Roughly, the rate doubles when the concentration of enzyme doubles. Since the data are somewhat linear, the rate is proportional to the concentration. Predictions should be based on the students’ actual data. There is the possibility that the reaction is slowing down (becoming non-linear) at 20 drops because the substrate is used up; the student should be able to explain this result.*

1. Does the rate of the reaction change when the hydrogen peroxide is allowed to spontaneously decompose for 24 hours? What does this indicate about the rate of spontaneous decomposition?

*In theory, the rate of the reaction with spontaneously decomposed H2O2 will be somewhat slower because there is less substrate with which the catalase enzyme can interact.*

Part III Effect of Temperature

1. At what temperature is the rate of enzyme activity the highest? Lowest? Explain.

*The temperature at which the rate of enzyme activity is the highest should be close to 30°C. The lowest rate of enzyme activity should be 60°C.*

1. How does changing the temperature affect the rate of enzyme activity? Does this follow a pattern you anticipated?

*In general, the rate increases as the temperature increases, until the temperature reaches about 50°C. Above this, the rate decreases. Individual results may vary.*

1. Why might the enzyme activity decrease at very high temperatures?

*At high temperatures, enzymes lose activity as they are denatured.*

Part IV Effect of pH

1. At what pH is the rate of enzyme activity the highest? Lowest?

*Answers may vary. Activity is usually highest at pH 10.0 and lowest at pH 4.0.*

1. How does changing the pH affect the rate of enzyme activity?

*Student answers may vary. Usually, the enzyme activity increases from pH 4.0 to pH*

*10.0. At low pH values, the protein may denature or change its structure. This may affect the enzyme’s ability to recognize a substrate or it may alter its polarity within a cell.*