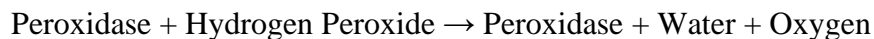# Effect of Temperature on Peroxidase

Peroxide (such as hydrogen peroxide) is a toxic byproduct of aerobic metabolism. Peroxidase is an enzyme that breaks down these peroxides. It is produced by most cells in their peroxisomes. The basic reaction is as follows:

Peroxidase + Hydrogen Peroxide → Peroxidase + Water + Oxygen

To determine the rate of an enzymatic reaction, you must measure a change in the amount of at least one specific substrate or product over time. In a decomposition reaction of peroxide by peroxidase (as described in the formula above), the easiest molecule to measure would probably be oxygen, a final product. This could be done by measuring the *actual* volume of oxygen gas released or by using an indicator. In this experiment, an indicator for oxygen will be used. The compound guaiacol has a high affinity for oxygen, and in solution, it binds instantly with oxygen, turning yellowish to brown in color. The greater the amount of oxygen gas produced, the darker brown the solution will become. The color change can be recorded as a change in the amount of light absorbed by the solution as measured using a spectrophotometer.

A series of 8 test tubes was prepared as follows:
Table 1 – Preparation of tubes

Tube	Distilled water (mL)	0.1% peroxide (mL)	Guaiacol (mL)	Temperature (^{o}C)
1	6	0.3	0.2	5
2	6	0.3	0.2	15
3	6	0.3	0.2	25
4	6	0.3	0.2	40
5	6	0.3	0.2	55
6	6	0.3	0.2	70
7	6	0.3	0.2	100
8	6.3	-	0.2	ambient

To each tube, 1.5 mL of peroxidase was added and timing was begun immediately. After 5 minutes, the absorbance of each tube is measured using the spectrophotometer.

Questions

1. [SP 1] Identify the enzyme, substrate and product in this reaction.

2. [SP 1, SP 3] Explain why we are able to use guaiacol as a measure of enzyme activity.

3. [SP 3] State the purpose of tube 8.

4. [SP 4, SP 6] Graph the sample data from Table 2. Based on the data, make a statement about the effect of temperature on enzyme reaction rate.

Table 2 – Absorbance reading for each tube

Tube	1	2	3	4	5	6	7	8
Temp (^{o}C)	5	15	25	40	55	70	100	Ambient
Absorbance	0.106	0.177	0.251	0.312	0.289	0.164	0	0

5. [SP 1, SP 6] Explain the lack of absorbance in Tube 7.

6. [SP 2] Draw a model of the enzyme and substrate at 25°C and at 70°C.

7. [SP 6] Use the data and your model to propose one adaptive value of endothermy.

8. a) [SP 1] Identify three or four factors that vary in the environment in which organisms live.
   b) [SP 1, SP 6] Identify which of the factors you identified in (a) you would expect to affect enzyme activity. Justify your choice(s).

9. [SP 3] a) Choose one factor you identified in Q. 8 and write a hypothesis about the predicted effect of that factor on enzyme activity.

   b) Design an experiment to test your hypothesis. Describe your protocol and identify the independent variable, dependent variable, controlled variables and control group.

10. [SP 1] Considering your answers to Q. 8, propose an advantage to organisms occupying a particular ecological niche.