# Factors Affecting Enzyme Activity

**Objective:** To study several factors that affect the activity of enzymes

**Background: Catalase** is an enzyme present in most cells and found in high concentration in liver and blood cells. You will use liver homogenate as the source of catalase. Catalase promotes the decomposition of hydrogen peroxide ($H_2O_2$) in the following reaction:

$$\mathbf{2\ H_2O_2 \rightarrow 2\ H_2O + O_2}$$

Hydrogen peroxide is formed as a by-product of chemical reactions in cells. It is toxic and would soon kill cells if not immediately removed or broken down.

**Materials:** (per team of 2)

2 pairs safety goggles
2 lab aprons
50 mL beaker with fresh catalase (yeast) solution
1 ml pipette
10 mL graduated cylinder
100 mL graduated cylinder
Fresh 3% $H_2O_2$
Water pan
Thermometer
Reaction chambers (*Drosophila* vials with 1-hole stoppers)
Stop watch
Ice

*Catalase Solution*: Add 1 pkg yeast to 200 ml warm water and stir. Prepare just before use.

*Available for Inquiry*: buffers, pH paper, NaCl, boiled catalase, hot plate, cooler, testubes/rack

**Procedure:**

In all experiments, make sure that your reaction chamber is scrupulously clean. Catalase is a potent enzyme, and if the chamber is not washed thoroughly, enough will adhere to the sides to make subsequent tests inaccurate. Measure all substances carefully. Results depend on comparisons between experiments, so the amounts measured must be equal or comparisons will be valueless. Before you do the experiment, read through the instructions completely. Make sure that you have all the required materials on hand, that you understand the sequence of steps, and that each member of your team knows his or her assigned function.

**Part A The Time Course of Enzyme Activity**

1. Prepare a table in your data book similar to Table 1.

2. Fill a pan almost full with water.

3. Lay the 50mL (or larger) graduated cylinder on its side in the pan so that it fills with water completely. If any air bubbles are present, carefully work these out by tilting the cylinder slightly while keeping it underwater. Turn the cylinder upside down into an upright position keeping its mouth underwater at all times.

4. Obtain a reaction chamber.

5. Obtain a small amount of stock catalase (yeast) solution in a 50mL beaker. You will need 1.0mL of yeast solution for each trial. When you are ready, you will add it to the vial with the 1 ml pipette. Keep the catalase cold, and stir before using it.

6. Pour 10mL of hydrogen peroxide ($H_2O_2$) into the reaction chamber. Pipette in **1.0mL of stock catalase solution** (yeast solution) and ***IMMEDIATELY*** stopper the reaction chamber tightly, submerge it in the water bath and place it so all the bubbles formed in the reaction chamber are captured by the inverted graduated cylinder.

7. Move the graduated cylinder into a position so that its mouth comes to lie directly over the tip of the dropping pipet. One member of the team should hold it in this position for the duration of the experiment.

8. Measure the gas levels in the graduated cylinder at 30-second intervals for 10 minutes. Record the levels in your data table.

## TABLE 1: Catalase activity over time

| **Time** | **mL $O_2$ evolved** |
|---|---|
| 30 seconds | |
| 1:00 | |
| 1:30 | |
| 2:00 | |
| 2:30 | |
| 3:00 | |
| 3:30 | |
| 4:00 | |
| 4:30 | |
| 5:00 | |
| 5:30 | |
| 6:00 | |
| 6:30 | |
| 7:00 | |
| 7:30 | |
| 8:00 | |
| 8:30 | |
| 9:00 | |
| 9:30 | |
| 10:00 | |