## Enzymatic activity lab

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## Introduction

Enzymes are biological catalysts and are usually proteins. They greatly increase the rate of chemical reactions by lowering the activation energy, which is the energy required to start a reaction. The metabolism of a cell depends upon enzymes in order to function correctly. Enzymes are sensitive to environmental conditions. If the conditions deviate too much, enzymes may stop functioning.

To examine the effects of environmental changes on enzymatic activity, we will work with the enzyme catalase. This is a very common enzyme that is present in most living organisms. Catalase catalyzes the breakdown of hydrogen peroxide to water and oxygen:

2 H2O2  2 H2O + O2

We will measure catalase activity somewhat indirectly. The reaction tubes will contain a detergent, Triton X-100. When O2 is generated in our reactions, it will create foam in the tubes because of the detergent. After the reactions are over, we will measure the height of the foam column as a way of determining how active the enzyme was under the conditions tested.

## Materials

* + Test tubes
  + Filter paper discs (presoaked in catalase)
  + Forceps
  + TritonX-100
  + Manual pipetter
  + 1mL serological pipettes
  + Hydrogen peroxide
  + pH buffers

## Procedure 1

**Part 1: Effect of temperature on catalase activity**

1. Label 4 tubes A, B, C, and D. Also put your group name on each tube.
2. Add a catalase-soaked filter paper disc in the bottom of each tube

*Use forceps to handle the discs. Do not touch them with your hands as oil from your skin will interfere with the assay.*

1. Pre-incubate the tubes for 5 minutes

Place Tube A in a rack in an ice water bath Place Tube B in a rack at room temperature Place Tube C in a rack in the 37˚C water bath Place Tube D in a rack in the 80˚C water bath

1 Protocol adapted from Iwase, T, et al. A Simple Assay for Measuring Catalase Activity: A Visual Approach. Scientific Reports 3:3081, 1-4 (2013).

1. After the pre-incubation step, add 1mL of Triton X-100 to each tube
2. Add 1mL of H2O2 to each tube and swirl to mix
3. Quickly return each tube to the appropriate rack
4. Allow the reactions to run for 10 minutes, then retrieve the tubes from their racks.

*While the reactions are in progress, answer the following questions.*

* 1. What is the independent variable in this experiment?
  2. What is the dependent variable in this experiment?
  3. How do you predict that the enzyme activity will be affected by the different incubation temperatures?

1. Measure the height of the foam in each tube and record your data.

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| **Tube** | **Temperature** | **Foam height (mm)** |
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1. Use your data to construct a graph.

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## Part 2: Effect of pH on catalase activity

1. Label 3 tubes A, B, and C. Also put your group name on each tube.
2. Add a catalase-soaked filter paper disc in the bottom of each tube

*Use forceps to handle the discs. Do not touch them with your hands as oil from your skin will interfere with the assay.*

1. Add 1mL of the appropriate buffer to each tube Tube A – pH 4 buffer

Tube B – pH 7 buffer Tube C – pH 10 buffer

1. Add 1mL of Triton X-100 to each tube
2. Add 1mL of H2O2 to each tube and swirl to mix
3. Place all 3 tubes in the 37˚C water bath
4. Allow the reactions to run for 10 minutes, then retrieve the tubes

*While the reactions are in progress, answer the following questions.*

* 1. What is the independent variable in this experiment?
  2. What is the dependent variable in this experiment?
  3. How do you predict that the enzyme activity will be affected by the different pH buffers?

1. Measure the height of the foam in each tube and record your data.

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| **Tube** | **pH** | **Foam height (mm)** |
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1. Use your data to construct a graph.

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## Questions

1. What was the optimal temperature for catalase activity? Was the prediction you made correct?
2. What happened to catalase activity at 80˚C? Based on what you know about protein structure, how would you explain that result?
3. What was the optimal pH for catalase activity? Was the prediction you made correct?
4. Can you identify any potential problems or sources of error with the experimental design? How could it be improved?
5. What is the substrate for catalase? What are the products of the reaction?
6. How is the substrate of an enzyme different from the active site?
7. The pH in the stomach is pH 2.0. What do you think the optimum pH is for pepsin, an enzyme that is secreted in the stomach?