

Lactase Enzyme Lab  
Biochemistry  
Honors Biology  
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**Purpose:**

This lab will examine the specificity of an enzyme (lactase) to a specific substrate (lactose). Students will observe the actions of the enzyme and how shape is important to enzyme reactions. Students will also observe what will happen when the enzyme is denatured.

**Materials**

- Lactase tablets
- 15ml milk
- Water
- Sucrose (5g per group)
- 100ml graduated cylinder
- 10ml graduated cylinder
- Three 400ml beakers
- Five test tubes
- test tube rack
- marking pencil
- clock
- hot plate with a pyrex test tube
- glucose test strips
- stirring rod

**Introduction**

Lactose, the sugar found in milk, is a disaccharide composed of glucose and galactose (both six sided sugars). Sucrose, ordinary table sugar, is also a disaccharide composed of fructose and glucose, Glucose is a six-sided sugar and fructose is a five-sided sugar.

Lactase is an enzyme that breaks lactose down into galactose and glucose. Lactase can be purchased in pill form by people who are lactose intolerant. These people lack the enzyme, lactase, and cannot break down the sugar lactose into its component parts. Although lactose is similar to sucrose, lactase will break down only lactose because of the shape of the sugar.

In this lab, you will see lactase break lactose down into galactose and glucose. You will also observe what happens if the shape of lactase is changed due to heating.

**Solution preparation**

1. Enzyme solution: Add one lactase tablet to two hundred milliliters of water. Stir until the tablet has dissolved.
2. Skim milk: this solution contains the lactose
3. Sucrose solution: Add 5 grams of sugar to 100ml of water. Stir until the sugar has dissolved.
4. Denatured enzyme solution
  - a. Place twenty milliliters of enzyme solution into a Pyrex test tube.
  - b. Add two hundred ml of water to 400ml Pyrex beaker.
  - c. Place the test tube in the beaker. Gently lay the test tube so it rests on the side of the beaker.
  - d. Place the beaker and test tube on the hot plate
  - e. Boil the water in the beaker for 30 minutes
  - f. Let the solution cool to room temperature

**Lab Procedures:**

1. Gather the materials
2. Label the test tubes with the following labels:
  - a. Test tube with skim milk and enzyme solution
  - b. Test tube with skim milk and water
  - c. Test tube with skim milk and denatured enzyme solution
  - d. Test tube with sucrose solution and enzyme solution
  - e. Test tube with sucrose solution and water
3. In test tube A add 2ml of skim milk and 1ml of enzyme solution
4. Time for 2 minutes and test for glucose with the glucose test tape. Record this data in table 1. If there was glucose present mark a "+" in the table. If glucose was absent, mark a "-" in the table.
5. In test tube B add 2 ml of skim milk and 1ml of water.
6. repeat step 4
7. In test tube C add 2ml of skim milk and 1ml of denatured enzyme solution
8. repeat step 4
9. In test tube D add 2ml of the sucrose solution and 1ml of enzyme solution
10. repeat step 4
11. In test tube E add 2 ml of the sucrose solution and 1 ml of water
12. repeat step 4

**SAMPLE DATA TABLE****Glucose Test Results**

Type of Solution	Glucose Test (+ or -)
Test Tube A: milk and enzyme solution	
Test Tube B: milk and water	
Test Tube C: milk and denatured enzyme solution	
Test Tube D: sucrose solution and enzyme solution	
Test Tube E: Sucrose solution and water	

**Questions: Answer in complete sentences.**

1. Diagram and describe the lactose and lactase reaction
2. Why did the enzyme react to lactose but not to sucrose?
3. What happened when the enzyme was boiled?
4. Another way to affect the enzyme is by lowering the pH of the solution. However, lactase is supposed to be able to work in the stomach. Would lowering the pH of the enzyme solution affect the enzyme? Why or why not?
5. What type of reaction is this? Dehydration or hydrolysis?

## **Lactase Enzyme Lab Teachers Answer Sheet**

Teachers will be able to assess the results table (the only positive reaction should be test tube A) and the teacher will be able to correct the conclusion questions.

### **Answer Key**

1. the students should draw a hydrolysis-induced fit model
2. The shape of sucrose (glucose and fructose) is different from lactose (glucose and galactose). The sucrose will not fit into the active site of lactose.
3. The enzyme denatured. The hydrogen atoms vibrated so much due to the energy added to quaternary structure of the enzyme. Note: as long as the students understand that the bonds broke changing the enzyme shape, they are ok.
4. The enzyme will denature (eventually). The H<sup>+</sup> will interfere with the hydrogen bonds, and denature the enzyme.
5. The reaction is a hydrolysis reaction.

### **Supplemental Information**

Denaturing the enzyme was very difficult. We had to boil the enzyme (placing a test tube with the enzyme in a beaker of boiling water) for 30 minutes.

We thought about lowering the pH of the enzyme solution, but commercial lactase is swallowed and works in the stomach, so lowering the pH was not really an option for us.

We experimented by boiling the lactase for five, ten and fifteen minutes. Thirty minutes worked for us. We suggest that you try boiling the enzyme before the lab, adding some milk (source of lactose) and test for glucose. If glucose is present, boil the lactase for a longer period of time.

You can approach the enzyme specificity in two different ways:

1. Why didn't the sucrose break down in the presence of lactase?
2. Why didn't the lactose break down in the presence of boiled lactase?

This lab is an uncomplicated introduction to enzyme reactions.

### **Comments**

This lab is a relatively simple lab which shows the specificity of enzymes based on shape. You will need to purchase lactaid and glucose test strips. I suggest the glucose test strips from Science Kits and Boreal Labs, which are easy to use.

You might want to set up the solutions before the lab (if you are pressed for time). This will simplify the lab for the students.