

Looking into Lactase

Understanding Enzyme Specificity and Activity

Enzymes are proteins that catalyze chemical reactions by lowering the reaction's activation energy.

Every enzyme has an active site that binds to another molecule called the substrate. Once bound to the substrate an enzyme can catalyze the reaction up to 10 billion times faster than the comparable, non-catalyzed reaction. In other words, a reaction that only occurs once every ten billion seconds (316 years) will occur once every second.

Enzymes are not consumed in the reaction and can bind to an infinite amount of substrates.

You are working in the quality control department of a local

Milk	Disaccharide Sugar	Monosaccharide Sugars
Cow	Lactose	Galactose & Glucose
Rice		Glucose
Soy	Sucrose	Glucose & Fructose

bioscience company that produces a lactase enzyme product. It is your job to verify the activity of a new drug that uses the enzyme lactase to treat lactose intolerance. In addition, you have been given the task of verifying the optimal pH conditions of the enzyme's activity.

pH is a measure of the acidity or basicity of a solution. Solutions with a pH less than seven (7) are said to be acidic and solutions

with a pH greater than seven are said to be basic or alkaline.

You will be given three different types of milk, each of which contains a different sugar. Both cow milk and soymilk contain different disaccharides that can be broken into two monosaccharides, (Table 1). Rice milk, on the other hand, contains the monosaccharide glucose. You will use glucose test strips to determine the activity of the lactase enzyme.

EXPERIMENT

You will need the following materials and equipment to do this experiment:

Marker	Glucose test strips	Micropipette & tips (100 – 1000 μ L)
Lactase solution	Test tubes	Milk (cow, rice, soy)
pH buffer solutions		

PART ONE: IDENTIFY THE COW MILK

1. **Locate** the three test tubes that contain unidentified milk samples and **record** any qualitative observations on Table 2.

A	
B	
C	

- 2. Locate the packet of glucose test strips at your station. Glucose test strips are used to test for glucose levels and will change color in the presence of glucose. **Which type of milk should test positive for the monosaccharide glucose?**

- 3. Label three glucose test strips A, B, and C. Dip each strip into the corresponding test tube.

TABLE 3: Quantitative Milk Analysis – Glucose Content		
	Test strip color	Relative amount of Glucose (mg/dl)
A		
B		
C		

- 4. Locate the lactase enzyme product you are testing (the pill has been crushed and added to distilled water). Add 250uL of the enzyme to each test tube.
- 5. Locate your mini-vortex and set the dial to eight. Vortex each tube for two seconds. **What do you observe in the milk samples? Has anything changed after adding the enzyme?**

- 6. Label three new glucose test strips A, B, and C. Dip the labeled strips into the appropriate test tube. Immediately lay the test strip on the paper towel. Record your quantitative observations in Table 4 in the post-lactase columns below and place a check in the enzyme activity box if the enzyme lactase was active in that sample. **Which types of milk will test positive for glucose after adding the enzyme?**

TABLE 4: Quantitative Milk Analysis – Enzyme Activity			
		Post-Lactase Glucose Test Strip	
	Test strip color	Relative amount of Glucose (mg/dl)	Enzyme Activity (Change from Table 3 = Yes)
A			<input type="checkbox"/>
B			<input type="checkbox"/>
C			<input type="checkbox"/>

Based upon your analysis what type(s) of milk did the enzyme effect and why is it used to treat lactose intolerance

PART TWO: Verifying Optimal Enzyme Activity

Verify the lactase enzyme’s effectiveness under different pH conditions. Use the same lactase enzyme from part one and determine the enzyme’s activity at five (5) different pH levels.

Perform the following steps to continue your experiment to determine the effects pH may have on lactase activity.

- 1. Locate five (5) empty test tubes. Label the test tubes pH **2, 4, 7, 10** and **12**.
- 2. Locate the pH buffer solutions pH 2, 4, 7, 10 and 12. Add 500uL of each pH buffer to the appropriate test tube.
- 3. Locate the lactase enzyme product lactase and mix by inversion.
- 4. Add 250uL of the lactase enzyme to each pH tube and the water control. Vortex each tube for two (2) seconds.
- 5. Incubate all experimental samples at room temperature for two minutes.
- 6. Add 500uL of cow milk to all experimental samples. Vortex each tube for two (2) seconds.
- 7. Incubate all experimental samples for two minutes.
- 8. Label five new glucose strips and dip the labeled strips into the appropriate test tube. Immediately lay the test strip on the paper towel. Record your observations in Table 5.

TABLE 5: Enzyme Activity at Different pH Levels		
	Color of Test Strip	Relative amount of Glucose (mg/dl) after adding lactase
pH 2		
pH 4		
pH 7		
pH 10		
pH 12		

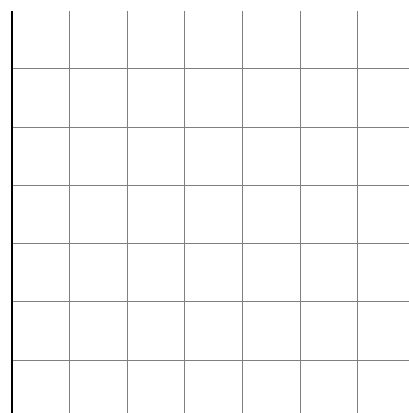
DATA ANALYSIS

Graph the relative amount of glucose to the corresponding pH on the graph. Be sure to label the axes.

What is the independent variable? _____

What is the dependent variable? _____

What effect (if any) does pH have on lactase activity?



CONCLUSION

The drug that you have tested was designed to break down the sugar _____ into two smaller sugars at a pH of _____.

Lactose intolerance is a result of the small intestine's inability to absorb disaccharides. In the absence of an enzyme the disaccharide is passed into the colon where bacteria metabolizes the sugar and then releases the gas as a product of fermentation. Refer to Figure 1 and determine if the pH level's that were explored in Part 2 of the experiment play a role in the design of the drug.

Can you think of any other environmental factors that could alter the drugs activity?

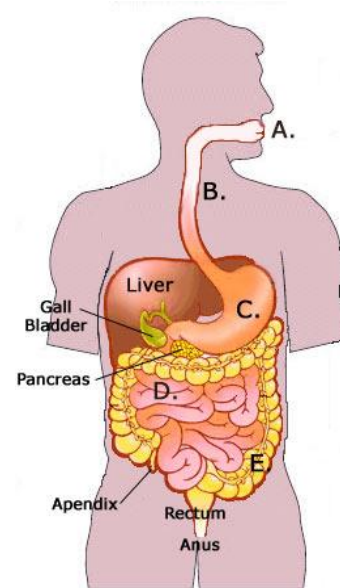


FIGURE 1