SI GOB - Full Discipline Demo

GOB-Enzymes: Temperature, pH, and Specificity

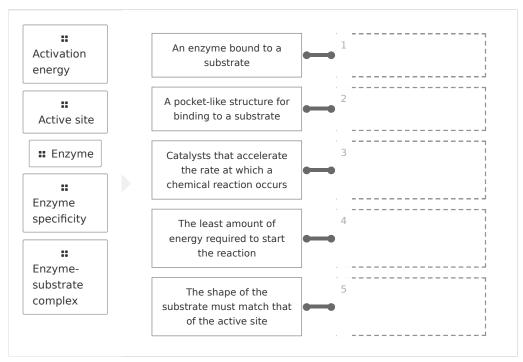
Final Report - Answer Guide

InstitutionScience Interactive UniversitySessionSI GOB - Full Discipline DemoCourseSI GOB - Full Discipline Demo

Instructor Sales SI Demo

Test Your Knowledge

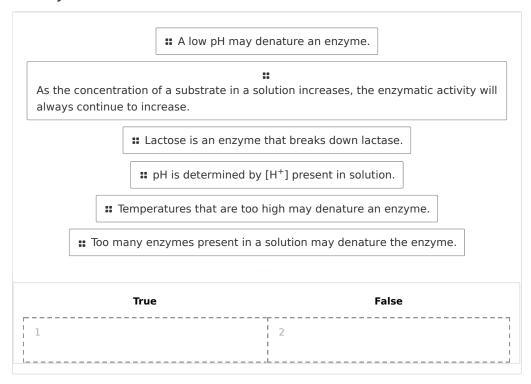
Match each term with the best description.



Correct answers:

- 1 Enzyme-substrate complex 2 Active site 3 Enzyme
- 4 Activation energy 5 Enzyme specificity

Identify each statement as true or false.



Correct answers:

1 Temperatures that are too high may denature an enzyme.

pH is determined by [H+] present in solution.

A low pH may denature an enzyme.

2 Too many enzymes present in a solution may denature the enzyme.

Lactose is an enzyme that breaks down lactase.

As the concentration of a substrate in a solution increases, the enzymatic activity will always continue to increase.

Exploration

Enzymes are proteins that accelerate the rates of chemical reactions.

O True	•
○ False	



Enzymes react with and break the bonds of	
 active sites 	
products	
substrates	~
All of the above	
As the concentration of an enzyme increases, the rate of the reaction	·
decreases	
increases	~
remains constant	
 All of the above 	
The optimum pH of enzymatic reactions varies between enzymes.	
True True	
☐ True ☐ False	~
• raise	
The enzyme lactase facilitates the cleavage of lactose into	
dextrose and sucrose	
galactose and glucose	~
pentose and fructose	•
All of the above	
Exercise 1	
Explain why a glucose test strip may be used to determine the enzymatic activity	of lactase.



Glucose is a byproduct of the catabolism of lactose into glucose and galactose. As lactose and sucrose are both disaccharides, they will not give a positive glucose test result. Thus, the only way that a positive result for glucose will be detected is if the lactase has broken down the lactose into glucose and galactose.

In this exercise, five reactions were performed. Of those reactions, two were negative controls and one was a positive control. Describe which of the reactions were controls and if they provided the expected results. Use the data in Data Table 1 to support your answer.			
In this exercise well A and well B were negative controls and neither well contained the lactase enzyme. It would be expected that both of these wells would not test positive for any glucose as			
there was not any enzyme present to catabolize the reaction for the milk or any enzyme specific to sucrose. The positive control was found in well E, as it contained 20% glucose. The well did test positive for glucose, with a concentration of 100 mg/dl. Thus, all three controls provided the expected results.			
Did the lactase enzyme show specificity? Support your answer by comparing your data for actose and sucrose.			
The lactace enzyme did show specificity for catabolizing lactose and not sucrose. When the			

The lactase enzyme did show specificity for catabolizing lactose and not sucrose. When the lactase was added to the milk, containing lactose, it was able to catabolize the reaction, breaking the lactose into glucose and galactose. The glucose was measured after the reaction occurred at 100 mg/dl. However, the lactase did not catabolize the sucrose as there was not any glucose detected after the reaction. Thus, the lactase is specific for lactose and does not interact with sucrose.

Data Table 1: Enzyme Specificity (SAMPLE ANSWER BELOW)

Well Plate	Contents	Glucose Concentration (mmol/L)
Α	3 drops 5% sucrose + 3 drops dH ₂ O	Negative
В	3 drops milk + 3 drops dH ₂ O	Negative
С	3 drops 5% sucrose + 3 drops lactase	Negative
D	3 drops milk + 3 drops lactase	15
Е	3 drops 20% glucose + 3 drops dH ₂ O	110



Exercise 2

Describe the relationship between temperature and the enzymatic activity of lactase. Of the three temperatures tested, which is the optimal temperature for enzyme activity? Use the results in Data Table 2 to support your answer. Hypothesize how the structure of the lactase relates to the results in Data Table 2.
Of the three temperatures tested, the optimal temperature for enzymatic activity of lactase is room temperature (19°C). When the temperature of the enzyme was reduced to -1°C, it was inactive, as glucose was not detected from the reaction. As the temperature of the lactase began to rise to room temperature, the enzyme became more active, peaking at room temperature. From this set of data, it is not clear whether the lack of enzymatic activity was the result of structural changes or the result of the enzyme not colliding with the substrate. When the lactase was heated to 90°C it became inactive. This suggests that the heating of the enzyme caused it to denature and lose its active site to such an extent that it is not able to regain its structural shape as it cools. This is supported by the data, as the glucose results were negative for all time periods of the heated enzyme. As lactase is an enzyme that occurs in the body, one would expect the maximum enzymatic activity to occur around 37°C, which is between the room and hot temperatures tested.
Describe the relationship between pH and the enzymatic activity of lactase. Of the pH values tested, which is the optimal pH? Use the results in Data Table 3 to support your answer. Hypothesize how the structure of the lactase relates to the results in Data Table 3.
Of the four pH values tested, the optimal pH is 3.5, as it had the largest amount of glucose tested after the reaction had occurred. The enzyme was still active at pH 5.0 and pH 6.8, but was inactive at pH 11.5. This suggests that at pH of 11.5, the structure of the enzyme had denatured, rendering the active site of the enzyme inactive. The reduced activity of the enzyme at pH 5.0 and pH 6.8 suggests that the enzyme's structure may not be ideal, but is still functional.
In the body, the lactase enzyme functions to break down lactose during digestion. In light of this, what approximate pH would you expect lactase to be most active? Did your results in Data Table 3 agree or disagree with your expectations for the optimal pH?
As the lactase enzyme functions during digestion, one would expect lactase to be active at a low pH, as the pH of stomach acid is 2-3. This correlates well with the results in Data Table 3, as the



enzyme was most active at a pH of 3.5. This also suggests that the optimal pH may be even lower than the pH range tested in the experiment.

In the background, the presence of naturally-occurring enzymes in fruits and vegetables was discussed. Using your results from this exercise as a basis to guide your thoughts, do you think that cooked, frozen, or raw fruits and vegetables would contain the most active enzymes?

Using the experience of this exercise, and the information in the background section of the experiment, cooked fruits and vegetables would contain the smallest amount of active enzymes, and the frozen and raw would contain the same amount of active enzymes when they are at room temperature.

Data Table 2: Enzymes and Temperature (SAMPLE ANSWER BELOW)

Lactase Tube	Temperature(°C)	Time (minutes)	Glucose Concentration (mmol/L)
Cold	-1	5 minutes	Negative
		10 minutes	Negative
		15 minutes	5
		20 minutes	15
	19	5 minutes	15
Deem		10 minutes	15
Room		15 minutes	15
		20 minutes	15
	90	5 minutes	Negative
Hot		10 minutes	Negative
Hot		15 minutes	Negative
		20 minutes	Negative

Data Table 3: Enzymes and pH

(SAMPLE ANSWER BELOW)

Well Plate	Contents	Glucose Concentration (mmol/L)
1	3 drops pH 3.5 buffer + 2 drops lactase + 2 drops milk	15
2	3 drops pH 5.0 buffer + 2 drops lactase + 2 drops milk	15
3	3 drops pH 6.8 buffer + 2 drops lactase + 2 drops milk	5
4	3 drops pH 11.5 buffer + 2 drops lactase + 2 drops milk	Negative

Competency Review Enzymes lower the required ____ of a reaction. activation energy induced fit specificity All of the above The presence of an enzyme increases the overall energy released during a reaction. True False impacts enzymatic activity. Enzyme concentration pH Temperature All of the above Enzymes may denature outside the optimal ranges for temperature and pH. True False ____ is an enzyme that breaks down the disaccharide sugar found in milk. Galactose Glucose Lactase



Lactose

Lactase catalyzes the reactions that break down both sucrose and lactose.	
TrueFalse	~
•	
Lactase is active at a temperature of	
○ 0° C	
○ 20° C	✓
○ 90° C	
All of the above	
Lactase functions within a pH range of 3.5-6.8.	
○ True	✓
○ False	
Conditions within the human digestive system lie outside the optimal temperature and pH ranges for the enzyme lactase.	
○ True	
○ False	~

Extension Questions

Emily is a student in chemistry lab. She conducts several experiments to investigate the properties of amylase, an enzyme that converts starch into glucose. Benedict's reagent is a blue-colored chemical that changes color according to the amount of glucose in a sample. Benedict's reagent remains blue in the presence of starch. Emily fills seven test tubes with an equal amount of amylase and water. Next, she places each tube in a different water bath for 10 minutes. She adds an equal amount of starch to each tube and performs a Benedict's test.



Increasing amount of glucose in solution

green, orange, red, brown

Tube	Contents	Water Bath Temperature	Color after Benedict's Test
1	amylase + water + starch	0°C	Blue (no change)
2	amylase + water + starch	20°C	Green
3	amylase + water + starch	30°C	Orange
4	amylase + water + starch	35°C	Red
5	amylase + water + starch	40°C	Green
6	amylase + water + starch	45°C	Blue - Green
7	amylase + water + starch	50°C	Blue (no change)

According to experimental results, what is the optimal temperature for amylase? Tube 1 and Tube 7 have the same results for the Benedict's Test. How do you interpret the color results with regard to enzyme activity?

Tube 2 and Tube 5 have the same results for the Benedict's Test.

- a. How do you interpret the color results with regard to enzyme activity?
- b. What would happen to the activity level in each tube if the temperature was changed to the optimum temperature?

(SAMPLE ANSWER BELOW)

The optimal temperature for amylase is 35°C.

Tube 1: The temperature is too low for the enzyme to be active; the starch is not catalyzed into glucose. Benedict's test shows no change because Benedict's reagent will not react with starch. Tube 7: Excessive temperature denatured enzyme, the starch is not catalyzed into glucose, Benedict's test shows no change because Benedict's reagent will not react with starch.

a. The color results suggest that both tubes have the same level of enzyme activity. However, the temperature of Tube 2 (20C) is much lower than the temperature of Tube 5 (40°C). The enzyme in



Tube 5 has already passed the optimum temperature and is partially denatured, meaning that its activity level cannot increase beyond its current level.

b. The enzyme in Tube 2 will remain active and increase in activity as the temperature increases towards the optimum temperature of 35°C. Even if Tube 5 is cooled to 35°C, the activity level will not increase. The enzyme is permanently damaged (denatured) due to excessive heat.