

Are Enzymes Specific for their Substrates?

Task Information

Subject: Biology

Content:

- MST Framework Reference:
Standard 4- Science: The Living Environment
- Regents Biology Syllabus:
Unit I: Topic III.B.2.1 and Topic III.B.3
- Variance Biology Program Guide:
Energy, Matter, and Organization: Enzymes

Format: Manipulative

Purpose: Collection and analyzation of data about enzymes activity.

Skills:

Primary: Interpreting Data, Investigating
Secondary: Recording Data, Observing

Time: 30-35 minutes

Materials:

- 1% glucose solution
- 3 Unknown Disaccharide Solutions: A, B, and C
"A" = water ; "B" = 1 - 3% Lactose solution; "C" = water
- Enzyme Solution: LACTAID Liquid (over-the-counter in grocery or drug stores)
- Test strip indicator for glucose (over-the-counter in drug stores)
- Spot plate (depression plate) with at least 9 to 12 wells
- China marker to mark spot plates

Preparation:

- Prepare solutions and dispense in either dropper bottles or microscale disposable pipettes. Label all containers.
- Glucose solution: 1 gram of glucose dissolved in 99 ml distilled water.
- Lactose solution: 3 grams of lactose dissolved in 97 ml distilled water.
- Lactase solution: LACTAID Liquid (over-the-counter in grocery or drug stores): 1 small bottle per 100 ml distilled water

Safety:

DO NOT INGEST ANY MATERIALS.

Be certain MSDS information is available for each chemical substance used in this activity.

Extensions/Modifications: N/A

Are Enzymes Specific for Their Substrates?

Task: At this station, you will be collecting and analyzing data about enzymes activity.

Materials

- Glucose solution
- 3 Unknown Disaccharide Solutions: A, B, and C
- Enzyme Solution
- Indicator strips for glucose
- Spot plate (depression plate)

Background

Disaccharides are chemically changed to simple sugar molecules by enzymes. In this exercise, you will determine whether a certain enzyme is specific for only one disaccharide.

Read through all the directions before beginning.

Directions

After reading the directions, set up a rough copy of a data table to record your observations.

1. Add two drops of the known glucose solution to Well #1 of the spot plate. Insert the end of one strip of the glucose test strip indicator. Wait 15 - 30 seconds before reading the results. Record your observations.
2. Add one drop of the enzyme solution to Well #2 of the spot plate. Repeat the above test with the test strips and record your observations.
3. Add two drops of each of the unknown disaccharide solutions to the spot plate as follows:

"A" in spot #3, "B" in spot #4, "C" in spot #5

Test each with a different indicator strip. Record your observations.

4. Add two drops of each of the unknown disaccharide solutions to the spot plate as follows:

"A" in spot #6, "B" in spot #7, "C" in spot #8.

To each sample of unknown disaccharide, add one drop of the enzyme. Wait about two minutes and then test with the indicator strip. Record your observations.

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**Are Enzymes Specific for Their Substrates?
Answer Sheet**

Observations:

In the space below, draw an appropriate table to record your observations.

Analysis:

1. Using complete sentences, explain why testing well #2 is a necessary step in the procedure.

2. Explain in complete sentences, how this experiment shows that enzymes are specific for only one substrate?

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3. If the enzyme used in this experiment was lactase, name the specific substrate on which it acts.

4. If the enzyme used in this experiment was lactase, which spot contained that substrate?

5. Using complete sentences, explain your answer to question 4.

6. Write the **word equation** for the chemical reaction that is catalyzed by lactase.

7. Is the reaction in this activity dehydration synthesis or hydrolysis (digestion)?

8. Using complete sentences, explain your answer to question 7.

Are Enzymes Specific For Their Substrates? - Scoring Rubric

Maximum Score - 15 points

Data Table

3 points total

Sample data table showing correct results of testing the specificity of the enzyme lactase.

Title: Testing for Enzyme Specificity

Spot	Substance Tested	Glucose present yes or no?	
1	glucose solution	x	
2	enzyme solution		x
3	unknown A solution		x
4	unknown B solution		x
5	unknown C solution		x
6	unknown A + enzyme solution		x
7	unknown B + enzyme solution	x	
8	unknown C + enzyme solution		x

Criteria:

Allow 1 point for each of the following;

- Appropriate title present.
- Substances tested are identified.
- Results of tests recorded and paired. Accuracy of results are not considered here.

Question 1 - Indicates need for control

2 points total

Criteria:

- 2 points** Correctly / generally states that well #2 acts as a control to show that the enzyme solution does not contain glucose. Answer should be written in complete sentences.
- 1 point** Correct statement, but not in complete sentences.
- 0 points** Incorrect responses, even if it is in complete sentences, or no response provided.

Question 2 - Explain enzyme specificity

2 points total

Criteria:

- 2 points** Correctly states that enzyme activity was indicated by the presence of glucose in spot 7. Answer should be written in complete sentences.
- 1 point** Correct statement, but not in complete sentences.
- 0 points** Incorrect responses, even if it is in complete sentences, or no response provided.

Question 3 - Lactose as specific substrate/disaccharide . 1 point total**Criteria:**

- 1 point** Correctly names lactose as the specific substrate/disaccharide on which lactase acts.
- 0 points** Incorrect responses or no response provided.

Question 4 - Identifies substrate 1 point total**Criteria:**

- 1 point** Correctly identifies depression/spot/well 7 as containing the substrate.
- 0 points** Incorrect responses, or no response provided.

Question 5 - Explain chemical activity 2 points total**Criteria:**

- 2 points** Correctly explains that in spot 7 chemical activity occurred as indicated by a positive test for glucose. Answer should be written in complete sentences.
- 1 point** Correct statement, but not in complete sentences.
- 0 points** Incorrect responses, even if it is in complete sentences, or no response provided.

Question 6 - Components in chemical reaction 1 point total**Criteria:**

- 1 point** lactose + lactase + water yields glucose + galactose
or
 lactose + water $\xrightarrow{\text{lactase}}$ glucose + galactose
- 0 points** Incorrect responses, or no response provided.

Question 7 - Type of reaction 1 point total**Criteria:**

- 1 point** Correctly identifies this as a hydrolysis reaction.
- 0 points** Incorrect responses, or no response provided.

Question 8 - Explain hydrolysis 2 points total**Criteria:**

- 2 points** Correctly states that the lactose (disaccharide) is broken down into simpler molecules of glucose and galactose (monosaccharides) by the addition of a water molecule. Answer should be written in complete sentences.
- 1 point** Correct statement, but not in complete sentences.
- 0 points** Incorrect responses, even if it is in complete sentences, or no response provided.

Highest possible score - 15 points

Student ID _____

Scoring Form

Male / Female (circle one) Are Enzymes Specific for Their Substrates?

Directions: Circle the student's score for each question. Add the points for each question and write the total score at the bottom of the scoring form.

Data Table	0	1	2	3
1. Indicates need for control	0	1	2	
2. Explanation of enzyme specificity	0	1	2	
3. Lactose as specific substrate/disaccharide	0	1		
4. Identifies spot 7 as containing substrate	0	1		
5. Explanation of chemical activity in spot 7	0	1	2	
6. Identifies components in chemical reaction	0	1		
7. Identifies type of reaction	0	1		
8. Explanation of hydrolysis	0	1	2	

Total Score _____

Total possible score - 15 points

Student ID _____

Scoring Form

Male / Female (circle one) Are Enzymes Specific for Their Substrates?

Directions: Circle the student's score for each question. Add the points for each question and write the total score at the bottom of the scoring form.

Data Table	0	1	2	3
1. Indicates need for control	0	1	2	
2. Explanation of enzyme specificity	0	1	2	
3. Lactose as specific substrate/disaccharide	0	1		
4. Identifies spot 7 as containing substrate	0	1		
5. Explanation of chemical activity in spot 7	0	1	2	
6. Identifies components in chemical reaction	0	1		
7. Identifies type of reaction	0	1		
8. Explanation of hydrolysis	0	1	2	

Total Score _____

Total possible score - 15 points

Student ID Bio-E.S.- 1

Scoring Form

Male / Female (circle one) Are Enzymes Specific for Their Substrates?

Directions: Circle the student's score for each question. Add the points for each question and write the total score at the bottom of the scoring form.

Data Table	0	1	2	3
1. Indicates need for control	0	1	(2)	
2. Explanation of enzyme specificity	0	1	(2)	
3. Lactose as specific substrate/disaccharide	0	(1)		
4. Identifies spot 7 as containing substrate	0	(1)		
5. Explanation of chemical activity in spot 7	0	1	(2)	
6. Identifies components in chemical reaction	0	(1)		
7. Identifies type of reaction	0	(1)		
8. Explanation of hydrolysis	0	1	(2)	
Total Score				<u>15</u>
				Total possible score - 15 points

Student ID Bio-E.S.- 2

Scoring Form

Male / Female (circle one) Are Enzymes Specific for Their Substrates?

Directions: Circle the student's score for each question. Add the points for each question and write the total score at the bottom of the scoring form.

Data Table	0	1	2	3
1. Indicates need for control	0	1	(2)	
2. Explanation of enzyme specificity	0	1	(2)	
3. Lactose as specific substrate/disaccharide	0	(1)	2	
4. Identifies spot 7 as containing substrate	0	(1)		
5. Explanation of chemical activity in spot 7	0	1	(2)	
6. Identifies components in chemical reaction	(0)	1		
7. Identifies type of reaction	0	(1)		
8. Explanation of hydrolysis	0	1	(2)	
Total Score				<u>12</u>
				Total possible score - 15 points

Student ID Bio E.S. - 3

Scoring Form

Male / Female (circle one) Are Enzymes Specific for Their Substrates?

Directions: Circle the student's score for each question. Add the points for each question and write the total score at the bottom of the scoring form.

Data Table	0	①	2	3
1. Indicates need for control	①	1	2	
2. Explanation of enzyme specificity	0	1	②	
3. Lactose as specific substrate/disaccharide	0	①		
4. Identifies spot 7 as containing substrate	①	1		
5. Explanation of chemical activity in spot 7	0	①	2	
6. Identifies components in chemical reaction	①	1		
7. Identifies type of reaction	0	①		
8. Explanation of hydrolysis	0	①	2	
Total Score			<u>7</u>	
				Total possible score - 15 points

Student ID _____

Scoring Form

Male / Female (circle one) Are Enzymes Specific for Their Substrates?

Directions: Circle the student's score for each question. Add the points for each question and write the total score at the bottom of the scoring form.

Data Table	0	1	2	3
1. Indicates need for control	0	1	2	
2. Explanation of enzyme specificity	0	1	2	
3. Lactose as specific substrate/disaccharide	0	1		
4. Identifies spot 7 as containing substrate	0	1		
5. Explanation of chemical activity in spot 7	0	1	2	
6. Identifies components in chemical reaction	0	1		
7. Identifies type of reaction	0	1		
8. Explanation of hydrolysis	0	1	2	
Total Score				
				Total possible score - 15 points

Observations:

In the space below, draw an appropriate table to record your observations.

ENZYME Reaction Test.

Well	Solution Tested	Results
1	Glucose	Positive
2	Enzyme	Negative
3	Solution A	Negative
4	Solution B	Negative
5	Solution C	Negative
6	Solution A + Enzyme	Negative
7	Solution B + Enzyme	Positive
8	Solution C + Enzyme	Negative

15
15

Analysis:

- Using complete sentences, explain why testing well #2 is a necessary step in the procedure.

Testing well #2 is necessary because it acts as a control to show that there is no glucose already in the enzyme solution.

- Explain in complete sentences, how this experiment shows that enzymes are specific for only one substrate?

This experiment illustrates that enzymes are specific since the enzyme would only act on one of the three disaccharides to cause it to breakdown.

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Are Enzymes Specific for Their Substrates? Answer Sheet

Observations:

In the space below, draw an appropriate table to record your observations.

Well	Solution	Observation
1	Glucose	Dark Green
2	Enzyme	Yellow
3	Sol. A	Yellow
4	Sol. B	Yellow
5	Sol C	Yellow
6	A + Enzyme	Dark Green Yellow
7	B + Enzyme	Green
8	C + Enzyme	Yellow

$$\frac{12}{15}$$

Analysis:

- Using complete sentences, explain why testing well #2 is a necessary step in the procedure.

This was necessary to be sure that the enzyme doesn't have any glucose in it.

- Explain in complete sentences, how this experiment shows that enzymes are specific for only one substrate?

because only one solution was affected by the enzyme.

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Observations:

In the space below, draw an appropriate table to record your observations.

~~1 - dk green
2 - dk green
3 - yellow
4 - yellow
5 - yellow~~

Spot plate #	color of test tape
1	dk green
2	dk green yellow
3	yellow
4	yellow
5	yellow
6	green
7	yellow
8	yellow

7/15

Analysis:

- Using complete sentences, explain why testing well #2 is a necessary step in the procedure.

This is necessary because it is the enzyme.

- Explain in complete sentences, how this experiment shows that enzymes are specific for only one substrate?

This experiment showed that enzymes are specific because only one of the unknown disaccharides change.

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3. If the enzyme used in this experiment was lactase, name the specific substrate on which it acts.

lactose

4. If the enzyme used in this experiment was lactase, which spot contained that substrate?

Six

5. Using complete sentences, explain your answer to question 4.

Because this depression changed the test paper
from yellow to green

6. Write the word equation for the chemical reaction that is catalyzed by lactase.

lactose + lactase \longrightarrow lactose + water

7. Is the reaction in this activity dehydration synthesis or hydrolysis (digestion)?

hydrolysis

8. Using complete sentences, explain your answer to question 7.

Because your breaking down lactose