

Title: Factors Affecting Enzyme Activity

Purpose: To determine the effects of temperature, pH, and substrate concentration on enzyme activity

Background: See Chapter 6 (Metabolism) in [Campbell *Biology* \(6th ed\)](#) or [BioFact Sheet # 43](#)

Materials:

reaction spot plates	prepared diastase (salivary amylase)	glucose test strips
small cups	distilled H ₂ O	thermometers
plastic pipettes	dilute HCl	labels and markers
starch indicator solution	dilute NaOH	
prepared starch solution		

Procedures:

Activity 1 (Effect of Enzyme Concentration)

1. Obtain 10 mL each of prepared starch solution diastase solution (salivary amylase), and distilled H₂O in plastic cups (*Use the pipettes*). (*Label pipettes*)
2. Place 1 drop of enzyme in each of 12 successive wells of the spot plate followed by 10 drops of H₂O.
3. Immediately add 1 drop of starch indicator to 1st well. (This will indicate amount of starch present at 0 minutes).
4. At 30 second intervals add 1 drop of starch indicator solution to each successive well. Bluish black indicates the presence of starch; orange or yellow indicates the absence of starch. Continue to add the starch indicator until the reaction is complete.
5. Repeat procedure using 2, 3, 4, and 5 drops of the enzyme and 3, 2, 1, and 0 drops H₂O, respectively.
6. Record the reaction time in a data chart (Headings = # Drops Enzyme and Reaction Time (sec)).
7. Graph your results (# Drops Enzyme will be independent variable)

Activity 2 (Effect of pH)

1. Place 2 drops of enzyme and 3 drops H₂O in each of 3 wells of spot plate
2. Add 1 drop HCl to the 1st well, 1 drop H₂O to the 2nd well, and 1 drop NaOH to the 3rd well.
3. Quickly add 2 drops of starch solution to each of the 3 wells.
4. Wait 2 minutes and add 1 drop starch indicator to each well.
5. Observe color/color change in each well and record in a data table.
NOTE: NaOH may decolorize the indicator solution... a light blue color indicates presence of starch

Activity 3 (Effect of Temperature)

1. Place 2 drops of starch solution in 4 wells of spot plate.
2. Place 5 drops of enzyme solution from various temperature water baths (ice water, room temperature water, warm water, and near boiling water (record temperature)
3. Allow reactions to continue 1 minute before adding 1 drop of starch indicator.
4. Record observations in ad data chart.

Activity 4 (Glucose Production from Starch by Enzyme Digestion)

1. Place 5 drops H₂O plus 2 drops starch solution in 2 wells of spot plate.
2. Add drop of enzyme solution to ONLY 1 well.
3. After 3 minutes, test for presence of sugar (glucose) in each well. (Read instructions with glucose test strips).
4. Use starch indicator to test for presence of starch AFTER you test for glucose.
5. Record observations in data chart.

See [Lab Grading Guidelines for Discussion, Conclusion, and Reflection](#).