Name _____ **Per.** _____ **Date** _____

Chapter 16- Lab
Digestive Enzymes at Work

Purpose: The purpose of this lab is to analyze the actions of enzymes on the digestion of the macromolecules; carbohydrates, lipids, proteins, and nucleic acids.

Pre-Lab Questions:

To complete the table, fill out the monomers for the 4 major macromolecules. A monomer is the smallest unit of a polymer or macromolecule.

Macromolecule	Polymer	Monomer
Carbohydrate	Saccharides/Complex sugars	
Protein	Polypeptides	
Lipids	Fats, Oils, Waxes, Steroids	
Nucleic Acids	DNA, RNA	

Materials: **General Materials**
marker, centrifuge tube rack, test tube rack, test tubes

Part A
2 microcentrifuge tubes
3 pipets
2% Albumin (Protein) Solution
1% Pepsin Solution
Biuret Test Solution
water bath

Part B
2 microcentrifuge tubes
2 pipets
1% Litmus-Milk Solution
1% Lipase Solution

Part C
4 microcentrifuge tubes
5 pipets
1% Starch Solution
1% Amylase Solution
1% Glucose Solution
Iodine
Benedict's Solution
water bath

Procedure:

Part A- Protein Digestion

1. Use a clean pipet to add 4 drops of 2% Albumin (Protein) Solution to centrifuge tube 1.
2 Add 2 drops of 2% Albumin (Protein) Solution to centrifuge tube 2.
3. Use another clean pipet to add 4 drops of 1% Pepsin Solution to centrifuge tube 2. Gently swirl the test tube to mix the contents.
4. Place both centrifuge tubes in a 40°C water bath for 15 minutes.
5. After 15 minutes, remove the centrifuge tubes from the water bath.
6. Using another clean pipet, add 2 drops of Biuret Solution to each centrifuge tube.
7. Observe the solutions in the centrifuge tubes.
   Note: Biuret solution is bluish-purple in the presence of polypeptides and lavender-pink in the presence of amino acids.
8. Record your observations for Part A on the Data Table.

Part B- Lipid Digestion

1. Use a clean pipet to add 4 drops of 1% Litmus-Milk Solution to centrifuge tube 3.
2. Add 2 drops of 1% Litmus-Milk Solution to centrifuge tube 4.
3. Use another clean pipet to add 2 drops of 1% Lipase Solution to centrifuge tube 4. Gently swirl the test tube to mix the contents.
4. After 3 minutes, observe the solutions in both centrifuge tubes.
   Note: Litmus is a pH indicator. Litmus appears blue in basic solutions and pink in acidic solutions.
5. Record your observations for Part B on the Data Table.

Procedure:

Part C- Carbohydrate Digestion

1. Use a clean pipet to add 4 drops of 1% Starch Solution to centrifuge tubes 5, 6, and 7.
2. Use another clean pipet to add 4 drops of 1% Amylase Solution to centrifuge tubes 6 and 7.
3. Use another clean pipet to add 4 drops of 1% Glucose Solution to centrifuge tube 8.
4. Gently swirl the contents of all centrifuge tubes.
5. Allow the centrifuge tubes to sit undisturbed for 2 minutes.
6. Test the Starch-Amylase Solution for Starch (a Complex Carbohydrate).
   a. Use another clean pipet to add 4 drops of Iodine Solution to centrifuge tubes 5 and 6.
   b. Observe the solutions in centrifuge tubes 5 and 6.
      Note: Centrifuge tube 5 is a control for the iodine-starch test. Centrifuge tube 5 will show a positive reaction between iodine and starch. Iodine changes from brown to blue-black in the presence of starch.
7. Test the Starch-Amylase Solution for Glucose.
   a. Use another clean pipet to add 4 drops of Benedict's Solution to centrifuge tubes 7 and 8.
   b. Place centrifuge tubes 7 and 8 in a boiling water bath. Use the foam centrifuge tube rack.
   c. After 3 minutes, remove both centrifuge tubes from the water bath. Ask the teacher to do this.
   d. Observe the solutions in centrifuge tubes 7 and 8.
      Note: Centrifuge tube 8 is a control for the Benedict's test. Centrifuge tube 8 will show a positive reaction. Benedict's Solution changes from blue to a red, orange or mustard colored precipitate when heated and in the presence of glucose.

Data Table

Centrifuge Tube #	Contents	Observations- Color Changes
1	Albumin (Protein) + Biuret	
2	Albumin (Protein) + Pepsin + Biuret	
3	Litmus-Milk Solution	
4	Litmus-Milk + Lipase Solution	
5	Starch + Iodine	
6	Starch + Amylase + Iodine	
7	Starch + Amylase + Benedict's	
8	Glucose + Benedict's	

Conclusion Questions:

Part A

Compare and contrast the observations of the biuret test results for centrifuge tubes 1 and 2.

1. What affect did pepsin have on the Albumin solution?
   (Answer for this question should not be about color change.)

   How do you know? (Be specific)

2. The Pepsin Solution was prepared using .01M Hydrochloric Acid in order to optimize the pepsin enzyme. Why was this necessary.

Part B

Compare and contrast the observations of the test results for centrifuge tubes 3 and 4.

3. What affect did lipase have on the Litmus-Milk solution?
   (Answer for this question should not be about color change.)

   How do you know? (Be specific)

Part C

Compare and contrast the observations of the iodine test results for centrifuge tubes 5 and 6.

4. What affect did amylase have on the Starch Solution?
   (Answer for this question should not be about color change.)

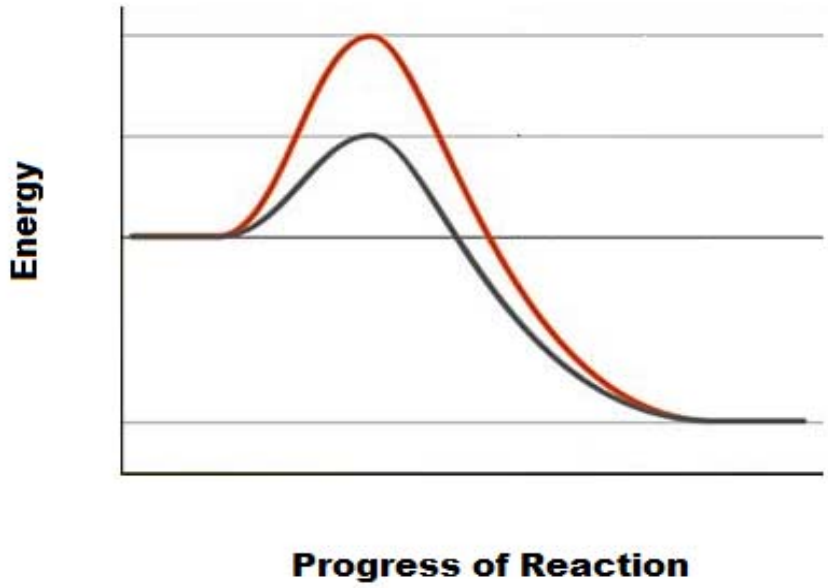
   How do you know? (Be specific)

Compare and contrast the observations of the Benedict's test results for centrifuge tubes 7 and 8.

5. Explain why centrifuge tube 7's solution color is the same as centrifuge tube 8's solution color.

General Questions About Enzymes and Enzyme Actions

6. **Using the list below label the graph of the actions of an enzyme.**



Activation Energy w/out Enzyme
Activation Energy w/ Enzyme
Products
Reactants

7. An enzyme is a biological catalyst that speeds up a chemical reaction by lowering the _____ _____ of the reaction.

8. An enzyme is composed of the macromolecule _____.

9. Explain how temperature and pH can affect the function of an enzyme.

10. The actions of an enzyme on a substrate is similar to a lock and key. Explain why.

11. Why are enzymes necessary in the digestion of food?

12. Why do we want to break down food into monomers?