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

Chapter 16- Make-Up Lab (Honors)
Digestive Enzymes at Work

Read about the following experiment and results. When finished, complete all instructions, fill in all tables, and answer the questions.

Students will be analyzing the actions of the digestive enzymes on the following macromolecules: proteins, lipids, and carbohydrates. Before starting this experiment, students had to number microcentrifuge tubes, 1 through 8.

Part A- Protein Digestion

For this experiment the student will be observing the actions of pepsin on albumin (protein). First, the student used a clean pipet and added 3 drops of 2% albumin (protein) solution to microcentrifuge tube 1. Next, the student used the same pipet and added 6 drops of 2% albumin solution to microcentrifuge tube 2. Using another clean pipet, the student then added 6 drops of 1% pepsin solution to microcentrifuge tube 1 and gently swirled the contents. After swirling both microcentrifuge tubes, the student place both centrifuge tubes into a 40°C water bath and waited for 15 minutes. After 20 minutes, the student removed the microcentrifuge tubes from the water bath and used another clean pipet and added 3 drops of Biuret indicator solution to each microcentrifuge tube and observed any reactions. His results were as follows: microcentrifuge tube 1's color changed from blue to pin, and microcentrifuge tube 2's color remained a bluish-dark purple. He recorded these observations on his data table.

Note: Biuret solution is bluish-purple in the presence of polypeptides and lavender-pink in the presence of amino acids.

Part B- Lipid Digestion

For this experiment the student will be observing the actions of lipase on litmus-milk solution. First, the student used a clean pipet and added 3 drops of 1% litmus-milk solution to microcentrifuge tube 3. Next, the student used the same pipet and added 6 drops of 1% litmus-milk solution to microcentrifuge tube 4. Using another pipet, the student then added 3 drops of 1% lipase solution to microcentrifuge tube 3 and gently swirled the contents. After waiting 7 minutes, the student observed any reactions. His results were as follows: microcentrifuge tube 3's color changed from blue to pink and microcentrifuge tube 4's color stayed blue. He recorded these observations on his data table.

Note: Litmus is a pH indicator. Litmus appears blue in basic solutions and pink in acidic solutions.

Part C- Carbohydrate Digestion

For this experiment the student will be observing the actions of amylase on starch. In order to make these observations, the student will be using 2 indicator solutions. To set up carbohydrate digestion, the student used a clean pipet and added 6 drops of 1% starch solution to microcentrifuge tubes 5, 6, and 7. Next, the student used another clean pipet and added 6 drops of 1% amylase solution to microcentrifuge tubes 6 and 7. Finally, the student used another clean pipet and added 6 drops of 1 % glucose solution to microcentrifuge tube 8. He gently swirled all microcentrifuge tubes and let them sit undistrubed for 5 minutes. For the first part of carbohydrate digestion, testing the starch-amylase solution for starch, the student used another clean pipet and added 6 drops of iodine indicator solution to microcentrifuge tubes 5 and 6 and observed any reactions. His results were as follows: microcentrifuge tube 5's color changed from brown to black and microcentrifuge tube 6's color stayed brown. He recorded these observations on his data table.

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.

For the second part of carbohydrate digestion, testing the starch-amylase solution for glucose, the student used a clean pipet and added 6 drops of Benedict's solution to microcentrifuge tubes 7 and 8 and gently swirled the contents. After swirling both microcentrifuge tubes, the student placed the centrifuge tubes in a 100°C water bath and waited 5 minutes. After 5 minutes the student removed the microcentrifuge tubes from the water bath and observed any reactions. His results were as follows: microcentrifuge tube 7's color changed from blue to an orange color and microcentrifuge tube 8's color changed from a blue to a reddish-orange color. He recorded these observations on his data table.

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.

Purpose: **What is the purpose of this experiment?**

Materials: **List all materials used for each part.**

Part A **Part B** **Part C**

Procedures: **List, in numerical order, the procedure for each part of this lab.**

Part A- Protein Digestion

Part B- Lipid Digestion

Procedures:

Part C- Protein Digestion

Data Table

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

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	

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.)

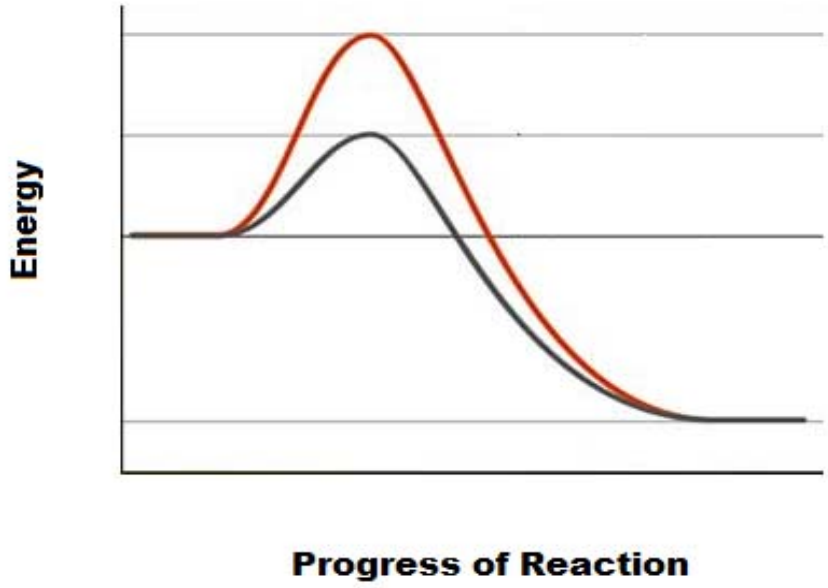
   How do you know? (Be specific)

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?