

# **SALIVA LAB**

## **(Salivary Amylase Digestion of Starch)**

### **Introduction:**

In the first digestive reaction of starch, a disaccharide called **maltose** is produced. Maltose consists of two glucose molecules joined together. In humans, the production of maltose from starch occurs within the mouth in a reaction catalyzed by amylase, an enzyme found in human saliva.

This exercise is based on the fact that intact starch can combine with an iodine solution to produce a blue-black colored substance. Maltose cannot combine with iodine to produce this color. Therefore in the presence of iodine, the digestion of starch into maltose will be accompanied by the disappearance of the blue-black color.

### **Materials Needed:**

<b>1% maltose solution</b>	<b>Bunsen burner, striker</b>
<b>1% starch solution</b>	<b>Tongs (crucible)</b>
<b>1% iodine solution</b>	
<b>0.5M HCl in dropper bottle</b>	
<b>5 small beakers, 5 disposable pipets, 4 test tubes</b>	

### **Procedure & Discussion:**

1. To give you an idea of the basis for the test you will be performing, combine 2 drops of starch solution and 1 drop of iodine solution in a small test tube and observe the color formed. Then into a second small test tube combine 2 drops of maltose and 1 drop of iodine. Does any color form in this second tube? A positive starch test turns dark blue/black. Negative is an amber color.
2. Label five beakers and five eyedroppers: “Saliva”, “A”, “B”, “C” and “D”; label four test tubes, “A”, “B”, “C” and “D”. Make sure they are all clean.
3. One member of the group should then deposit his or her saliva into the “Saliva” beaker. Tobacco chewers should not volunteer for this task. At least 10 ml are needed. To this saliva add distilled water from the bottle on your table to make a 50:50 solution and mix thoroughly by swirling the beaker. Avoid using saliva from more than one person.
4. Using the “Saliva” eyedropper, add 5 drops of this saliva solution to each beaker A, B, and C.
5. Light the Bunsen burner on the counter. Grasp the edge of the beaker A with tongs and warm the saliva mix over the burner. Do this until the saliva *just begins* to boil and then stop. Extinguish the burner when finished. This beaker will be used to study the effect of heating on the catalytic activity of salivary amylase.

6. Add 2 drops of hydrochloric acid to the saliva in beaker B. Avoid getting acid on either your hands or clothes. This beaker will be used to study the effect of low pH upon the catalytic activity of salivary amylase.
7. Add drops of distilled water as necessary to each beaker to make equal volumes of solution.

Beakers	Saliva	0.5M HCl	H <sub>2</sub> O		1% Starch
A	5 drops	0	12 drops	heat	2 ml
B	5 drops	2 drops	10	no heat	2 ml
C	5 drops	0	12 drops	no heat	2 ml
D	0	0	17 drops	no heat	2 ml

8. From the bottle of starch on your table, add 2 ml of 1% starch to each of the four beakers, A, B, C, and D. Swirl each beaker to mix its contents. Now do the next two steps as quickly as possible.
9. With eyedropper A, remove 5 drops from beaker A and put into test tube A. With eyedropper B, remove 5 drops from beaker B and put into test tube B. With eyedropper C, remove 5 drops from beaker C and put into test tube C. With eyedropper D, remove 5 drops from beaker D and put into test tube D.
10. Add 1 drop of IKI solution to each of the test tubes A, B, C and D to test for the presence of starch. Swirl each test tube and record the solutions' colors in the table below.
11. Thoroughly rinse out all of the test tubes with water. With paper towels, dry the inside of each tube.
12. Repeat steps 9, 10 and 11 at five, ten and twenty minutes.
13. **On completion, using soap, wash out the five beakers and the four test tubes. Rinse well. Throw the plastic pipets away.**

Name \_\_\_\_\_

## Saliva Report Sheet

TABLE 1. Record the color of the solutions in the four tubes:

Tubes	0 Minutes	5 Minutes	10 Minutes	20 Minutes
A				
B				
C				
D				

1. In which beaker did the greatest amount of starch hydrolysis occur?  
How do you know?
  
2. Which beaker served as the control? Explain.
  
3. From your textbook and lecture notes, what type of structural change occurred in the salivary amylase during the heating and cooling?