# Lab 2 Protocol: Cell metabolism

## **ACTIVITY 1 – PROTEIN DIGESTION**

**STEP 1**

* Label your test tubes (PA – PF)

**STEP 2**

* Add the reagents in the test tubes as directed in Table 3.
* **Pancreatin should be added last to each tube. This is the starting time.**
* Record the pH of each tube in Table 3.
* Place tubes in a 37 C water bath for about **2 hours**. Swirl the tubes every 15 minutes.
* While you are waiting you can perform the **control biuret and ninhydrin assays.**

**CONTROL BIURET AND NINHYDRIN ASSAYS**

**BIURET ASSAY:** Place 3 ml of 1% albumin solution in a test tube and add 3 ml of 2.5% Sodium Hydroxide (NaOH) and 3 drops of biuret reagent. If the solution does not turn violet, continue adding and counting drops of biuret reagent until the solution turns violet. This will be the number of drops you will need to add to your experimental tubes.

**POSITIVE CONTROL = VIOLET**

**NINHYDRIN ASSAY:** Place 5 ml of 0.1% amino acid and 0.5 ml of 0.5% ninhydrin(**CAUTION**) in a test tube. Place the tubes in a boiling water bath for 5 minutes. Observe and record the color.

**POSITIVE CONTROL = BLUE – VIOLET**

**STEP 3**

* After the 2 hours of incubation perform a biuret and ninhydrin assay on the contents of tubes (PA – PF).

**BIURET ASSAY:** put 3 ml of solution from each tube (PA – PF) into a new labeled test tube and 3 ml of 2.5% NaOH to each tube. Add the number of drops you determined above in the control test of Biuret reagent to each tube. Record results in Table 3.

**NINHYDRIN ASSAY:** put 5 ml of solution from each test tube (PA – PF) into new labeled tubes. Add 0.5 ml of 0.5% Ninhydrin (CAUTION) into each tube and place them in boiling water bath for 5 minutes. Record result in Table 3.

## **ACTIVITY 2 – CARBOHYDRATE DIGESTION**

**STEP 1**

* Label your test tubes (CA – CD)
* Label spot plate wells (A – D)

**STEP 2**

* Add the reagents in the test tubes as directed in Table 4**. Starch should be the last substance added to each tube. This is the starting time.**
* Place tubes in a 37 C water bath for 30 minutes.
* While you are waiting you can perform the control Iodine and Benedict’s assays.

**CONTROL IODINE AND BENEDICT’S ASSAYS**

**IODINE ASSAY:** Place 1 drop of 1% starch solution in a spot plate. Add 1 drop of the iodine solution to the plate.

**POSITIVE CONTROL = BLACK**

**BENEDICT’S ASSAY:** Place 2 ml of 5% maltose solution and 2ml of Benedict’s reagent in a test tube. Place the test tube in boiling water bath for 3 minutes maximum.

**POSITIVE CONTROL = GREENISH BLUE +**

 **YELLOW ORANGE ++**

 **RED +++**

**STEP 3**

* After 30 minutes of incubation perform Iodine and Benedict’s assay on the contents of tubes (CA – CD).

**IODINE ASSAY:** place one drop from each tube content (CA – CD) into the appropriately labeled spot plate well. Add 1 drop of iodine solution to each well. Record results in Table 4.

**BENEDICT’S ASSAY:** Place 5ml of each tube content (CA – CD) into a new labeled tube (BA – BD). Add 2ml of Benedict’s reagent to each tube and boil them for 3 minutes maximum. Record your results in Table 4 (pg. 3.9).

**STEP 4**

* Label 3 test tubes (CF, CG, CH).
* Add the reagents as directed in Table 5. STARCH SHOULD BE ADDED LAST. This is the starting time.
* Incubate these test tubes at room temperature and using the spot plate start perform Iodine test every 30 seconds.
* Record the time when negative iodine test occurs. Some of the tubes may not produce negative results. If this is the case, stop observing them after 5 minutes.

**NEGATIVE CONTROL = YELLOW/AMBER**

## **ACTIVITY 3: EMULSIFICATON OF LIPIDS, THE PROCESS OF DIGESTION, AND DEMOSTRATION OF THE DIRECTION OF DIGESTION**

**EMULSIFICATION OF LIPIDS**

1. Obtain two clean test tubes.
2. Add 5ml of water to both test tubes and 1ml of olive oil.
3. Add a couple of drops of soap to one of the test tubes **only.** Observe the micelles.

**PROCESS OF DIGESTION**

1. Work in small groups. Obtain a large glass of water and a stethoscope.
2. Swallow a large gulp of water. Note the movement of your tongue.
3. Describe the movement \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
4. Would this movement be the same if solid food were swallowed?
5. Watch student swallow and notice the movement of the Larynx (Adam’s apple).
6. Describe what you observe.
7. What could happen if these movements did not occur? \_\_\_\_\_\_\_\_\_\_\_\_\_
8. Place a stethoscope on the student’s upper abdomen, directly below the rib cage, a little to the left of center. Listen to the sounds made as your partner swallows a large gulp of water.

As the water falls against the junction of the esophagus and stomach, you will hear a splashing sound. As the water enters the stomach, you will hear a gargling sound. Have the student swallow several times until you are certain that you can distinguish the two sounds.

1. How much time passes between the two sounds?\_\_\_\_\_\_\_\_ This is a good estimation of how long it takes a single wave of peristalsis to move along the esophagus.
2. Assuming the esophagus is 11 inches long, at what rate does peristalsis occur? Use the following equation:

Rate = length/time = \_\_\_\_\_\_\_\_\_\_ inches per second

What factors or conditions do you think could affect the rate of peristalsis?

**DEMOSTRATION OF THE DIRECTION OF DIGESTION**

1. Obtain a small cup (mouth), small piece of banana, water (saliva), orange juice, and a small piece of sandwich.
2. Mix all these food items in the cup to represent mechanical digestion in the mouth.
3. Transfer the mixture to a small bag (stomach) and keep mixing the contents inside the bag (stomach mixing the contents).
4. Obtain one pantyhose (small intestine) and small tray. Transfer all the contents from the small bag to the pantyhose and squeeze it. Liquid collection on the tray (water full of nutrients is reabsorption back into the body).
5. Obtain a small towel (large intestine). Transfer the contents from the pantyhose to the towel (water reabsorption).
6. Obtain a red bag (Anus) and transfer the contents from the small towel to here. Cut a hole on the bag and squeeze……….

POPS PROJECT:

In a 24-hour period follow exactly what you eat. Total calories, total grams of carbohydrates, total grams of proteins, total grams of fat, and gram of saturated fats vs unsaturated fats.