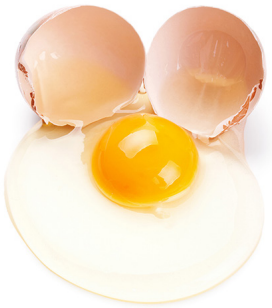
BIO LAB:
Enzyme Eggsploration

TEACHER

LEVEL: Year 11&12 | TOPIC: Biochemistry | TIME REQUIREMENT: 45 mins



CURRICULUM ALIGNMENT

- *Cells require inputs of suitable forms of energy, including light energy or chemical energy in complex molecules, and matter, including gases, simple nutrients, ions, and removal of wastes, to survive (ACSBL044)*
- *Photosynthesis is a biochemical process that in plant cells occurs in the chloroplast and that uses light energy to synthesise organic compounds; the overall process can be represented as a balanced chemical equation (ACSBL052)*

BACKGROUND

Enzymes are biological molecules that act as nature's catalysts. They speed up chemical reactions within cells to make them useful for metabolic pathways and are essential to life. Enzymes have a variety of functions within the body, such as, playing a key role in digestion and metabolism. During digestion, enzymes break down larger molecules into smaller components that can be easily absorbed by the body. Enzymes are even able to produce new molecules by binding two molecules together.

In this practical, students observe the action of Pepsin on albumin, a globular protein. Pepsin is a digestive enzyme that is found in many organisms. It comes in many different forms, but in every case, its function is to aid digestion by breaking down proteins via hydrolysis into their component amino acids. In this practical, students are required to conduct a number of tests on an Albumin suspension to observe how it reacts with Pepsin. As Pepsin, a protease, breaks down the Albumin, the suspension turns from a cloudy, milky appearance to clear. This provides an excellent opportunity for students to observe how enzymes function optimally at conditions similar to those within mammalian bodies. The third test tube effectively models the conditions of an animal stomach during digestion and the test results in this tube show that Pepsin is most effective under these conditions. Students see Pepsin functions with greatest efficiency at an acidic pH. This practical is the perfect accompaniment to theoretical enzyme studies.

PREPARATION - BY LAB TECHNICIAN

Preparing Albumin Suspension

1. To prepare an Albumin suspension, pour 5g of dry Albumin flakes into a 1L beaker, and add a small amount of cold distilled water.
2. Mix into a paste.
3. Slowly add boiling distilled water to make up to 500mL for a 1% solution. Stir continuously as you add the water.

Preparing 1% Pepsin Solution

1. Prepare 1% Pepsin solution by dissolving 1g of powdered Pepsin into 100mL of distilled water. Scale up or down to get the volume required.

General Preparations

1. Prepare 0.1M Hydrochloric Acid solution.
2. Provide each student with a test tube containing 5mL of 1% Pepsin solution and a beaker with 25mL of Albumin.

MATERIALS

- 6 Test Tubes
- Test Tube Rack
- Bunsen Burner
- Test Tube Holder
- 0.1 M Hydrochloric Acid Solution
- Albumin Powder
- Pepsin
- Distilled Water

SAFETY PRECAUTIONS

- Wear appropriate personal protective equipment (PPE).
- Know and follow all regulatory guidelines for the disposal of laboratory wastes.
- Wash hands thoroughly before and after handling any chemicals.
- Sterilise work surfaces before and after the practical.
- Under no circumstances are the materials used in this practical to be consumed as food.

SOUTHERN Biological

METHOD - STUDENT PRACTICAL

1. Label 4 of your test tubes 1-4 respectively, label the fifth “Boiled” and the 6th “Water”.
2. Pour 5 mL of the Albumin suspension into each of the 4 numbered test tubes.
3. Add 5 mL of distilled water to the tube labelled “Water”.
4. Add 2 mL of your Pepsin to the tube labelled “Boiled” and bring to the boil over a Bunsen Burner flame. Exercise caution as the glass will become hot.
5. Add 3 drops of dilute Hydrochloric Acid to “Test Tubes 2, 3 and 4”.
6. Add 3 drops of distilled water to “Test Tube 1”.
7. Set water bath to 40°C.
8. Sit your 4 tubes of Albumin, the “Water” tube and your remaining stock of 1% Pepsin in the water bath until warm. If your boiled Pepsin has cooled down, place it into the water bath as well.
9. Add 1mL of your warmed water to “Test Tube 2”.
10. Add 1mL of your boiled Pepsin to “Test Tube 4”.
11. Add 1mL of your warmed Pepsin solution to “Test Tubes 1 and 3”.
12. Set your timer for 6 minutes. After this time, remove the test tubes from the water bath and place them in the test tube rack.
13. Observe the contents of the test tubes and record the results in Table 1.

OBSERVATION AND RESULTS

Below are the expected results for the 5 test tubes.

Tube	Contents	Results
1	Albumin + pepsin + 3 drops water	Clearing
2	Albumin + 1mL water + HCl	Cloudy
3	Albumin + pepsin + HCl	Clear
4	Albumin + Boiled Pepsin + HCl	Cloudy

Table 1: Eggsploring Enzymes Test Results

INVESTIGATIONS

- Ask your students to consider the quantity of water that was added to “Test Tubes 1 and 2”. Students should understand that the 3 drops of water that are added to “Tube 1” are a substitute for the HCl, and the 1mL of water that is added to “Test Tube 2” is as a substitute for the Pepsin added to the other tubes. This is done to remove any question of whether any change in appearance is due to the extra dilution caused by the addition of those reagents.
- Challenge your students to consider why the reagents are added to “Test Tubes 2 and 4” before they are added to “Test Tubes 1 and 3”. The reagents are added to “Test Tubes 2 and 4” first as no reaction is expected to occur, and adding the reactants earlier ensures that timing is not considered a reason for the lack of reaction.
- Ask your students why the Albumin suspension is cloudy, and what is suggested by the clearance of the cloudiness. They should be able to identify that the Albumin is not completely soluble until the Pepsin has broken it down into smaller molecules.
- From the lack of change in “Test Tube 4”, students should be able to surmise that the enzyme has not worked. Being a protein, Pepsin is denatured by very high temperatures and so boiling has rendered it unable to digest the Albumin.
- Ask your students what the result in “Test Tube 2” suggests. They should be able to surmise that the acid by itself is not enough to account for the clearing of the Albumin in “Test Tube 3”.
- A comparison of the results of “Test Tubes 1 and 3” should indicate that Pepsin will work without the acid, but not as efficiently.