

ENZYME ACTION - PEPSIN

Enzymes are nature's catalysts. They speed up reactions to make them useful for metabolic pathways. This protocol describes 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 proteins down via hydrolysis into their component amino acids.

Code:	MC23.2M
Name:	Pepsin
Description:	Fine powder, off-white in colour.
Storage:	Store at room temperature (15 – 30°C).
Shelf Life:	When stored correctly, MC23.2M has a nominal shelf life of 12 months.
Safety:	Enzymes are biologically active proteins and should be handled with care. Avoid direct contact or inhalation.

Suggested Protocol

Prepare a 1% suspension of albumin flakes (MC41.1). Weigh the required amount of dry flakes into a beaker and add a small amount of cold distilled water. Mix into a paste then add boiling distilled water in small amounts with stirring to make up to the required volume.

Prepare a 1% solution of pepsin in distilled water at room temperature.

Line up 4 test tubes numbered 1 to 4 and add 5mL of the albumin suspension to each, then add the following:

- No. 1 3 drops* of 2M HCl and 1mL of pepsin solution (test mixture)
- No. 2 3 drops* of 2M HCl and 1mL of distilled water (control for effect of HCl alone)
- No. 3 3 drops of distilled water and 1mL of pepsin solution (control for effect of pepsin at high pH)
- No. 4 3 drops plus 1mL of distilled water (primary control)

* see tip below for a recommendation on the number of drops of acid to use.

Stand the four test tubes in a beaker of water at 40-50°C. You should see a noticeable increase in the clarity of the test mixture within about 15 minutes.

Tip

Being a digestive protease, pepsin works best in an acidic environment. To optimise the activity to suit your particular circumstances, we recommend that you run a trial after preparing the reagents. Compare the way the reaction proceeds with 3, 4, 5 and 6 drops of 2M HCl then choose the number that works best when your students run the experiment.

Please Note:

Batch-to-batch variations in substrate composition and enzyme activity can mean that the suggested experiment might not work exactly as described in every situation.