ENZYME ACTION - PROTEASE

Enzymes are nature’s catalysts. They speed up reactions to make them useful for metabolic pathways. This protocol describes the action of protease on two proteins, casein and gelatin. Protease 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: MC23.80E
Name: Protease
Description: Brown liquid.
Storage: Store under refrigeration (4 – 5°C).
Source: Derived from Bacteria, Bacillus amyloliquefaciens
Shelf Life: When stored correctly, MC23.80E 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

Protease is able to hydrolyse many different types of protein, so we suggest two alternative procedures to demonstrate its effect.

1. Reaction with casein (milk protein)

Prepare a 1% suspension (w/v) of skim milk powder (MC41.3) in boiling water and allow to cool to 40°C in a water bath.

Prepare a 2% solution (v/v) of protease in distilled water at room temperature, then warm to 40°C in a water bath.

Mix equal volumes of the skim milk suspension and the protease solution and maintain the temperature at 40°C. For small scale student activities, you can mix volumes of 2-5mL in a test tube. The action of protease on the milk protein will cause the suspension to become noticeably clearer. It can help to compare clarity by holding the reaction vessel in front of a white sheet of paper with dark ruled lines. The lines become easier to see as the mixture becomes clearer. To provide a suitable reference point, run a control by substituting distilled water for the 2% protease solution.

2. Reaction with gelatin (meat extract protein)

Prepare 100mL of a 4% solution (w/v) of gelatine by sprinkling the gelatine granules into hot water (75-80°C) and swirling to dissolve. Pour 50mL of the solution into each of two beakers and allow to cool to 45°C. Label one beaker “Control” and the other “Test”.

Prepare a 2% solution (v/v) of protease in distilled water at room temperature. Add 2mL of the protease solution to the “Test” beaker, and add 2mL of distilled water to the “Control” beaker. Mix each well by swirling and allow to stand for 10 minutes, then place in a fridge to set. The “Test” solution should remain as a low viscosity liquid due to the hydrolysis of the gelatine protein, but the “Control” solution will set into a gel.
The optimum conditions for this type of protease are:

- **pH**: 6.0 – 8.0
- **Temperature**: 40 - 50°C

It can be deactivated by exposure to temperatures above 90°C, and pH less than 3.0 or higher than 11.

**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.