

ENZYME ACTION

NAME: Protease

DESCRIPTION: Off-white powder MC 23.8

ACTION: The enzyme protease acts on protein to break it down into amino acids. For example,

Gelatine (firm) $\xrightarrow{\text{Protease}}$ Amino acids (liquid)

Milk (opaque) $\xrightarrow{\text{Protease}}$ Amino acids (clear)

STORAGE: Store in the refrigerator at 4°C.

SAFETY: Enzymes are biologically active proteins and should be handled with care. Proteolytic enzymes in particular may irritate the skin, eye or mucous membranes. Avoid direct contact or inhalation.

TIPS FOR TEACHERS:

Suggested pracs:

1. Action of protease on gelatine (slow)

Add 10g of gelatine to 125mL of cold water and allow the gelatine granules to disperse. When the gelatine granules are fully wetted, heat gently in a microwave for 1.5 minutes at 50% power, or place in a warm water (60°C) bath until the solution becomes clear. Pour the liquid into petri dishes and allow to cool, then place in a refrigerator to set completely.

Prepare a 2% protease solution with distilled water at room temperature.

Using a plastic straw (the wider McDonalds thick shake ones are good), remove a small disc of firm gelatine from the dish leaving a small well. Place two drops of protease enzyme solution into the well. Cover with a sheet of plastic “cling wrap” then leave the gelatine on the bench overnight at 20 - 23°C and note the size of the well the next day. Gelatine liquefaction denotes protein breakdown by enzyme activity. To allow an accurate measure of the degree of liquefaction, run a control by adding two drops of distilled water to a second well that has been cut well away from the first.

2. Action of protease on skim milk (fast)

Prepare a suspension of low-fat milk powder (MC41.3) by adding 100mL of “just boiled” water to 4.0g of milk powder. Stir to homogenise the suspension then allow to cool to 40°C in a water bath. Prepare a 2.0% solution of protease in distilled water at room temperature, then warm to 40°C in a water bath. Combine 5mL of milk suspension with 5mL of 2.0% protease solution and maintain the mixture at 40 °C. The action of protease on the milk suspension results in a clear transparent solution. Run a control along side your experiment by substituting distilled water for the 2.0% solution of protease.

Comments and further Ideas:

If you choose the prac involving the breakdown of milk protein, you can use a data logger with a light sensor (or better still, a colorimeter) to monitor the rate of reaction by recording the increase in light transmission as the reaction proceeds. (See also the experimental notes for MC23.1, Trypsin).

Protease is often present in commercial brands of washing powder. You could investigate the enzyme activity of various brands.

Protease is used in a wide range of industrial processes including;

- Preparation of soymilk and soy sauce.
- Recovery of silver from photographic film.
- Production of leather in the tanning process.
- Treatment of sewage for reduction of solids and improved settling.

Please note: Variations in substrate composition and enzyme activity can mean that the suggested experiment might not work exactly as described in every situation.