" a world of learning"

ENZYME ACTION

NAME: **Amylase (diastase)**

DESCRIPTION: Clear yellow/brown liquid, MC23.35P.

Reducing sugars are not detectable in MC23.35P when a 0.5% solution is tested with Benedict's Solution.

ACTION: Amylase acts on a starch substrate and breaks it into soluble sugars.

$$\text{Starch} \xrightarrow{\text{Amylase}} \text{Simple sugars}$$

STORAGE: Store in the refrigerator at 4ºC.

SOURCE: This product is derived from Fungi, *Aspergillus sp.*

SAFETY: Enzymes are biologically active proteins and should be handled with care. Avoid direct contact or inhalation.

TIPS FOR TEACHERS:

Suggested pracs:

Prepare a 1% starch solution (use a soluble starch, e.g. MC39.7, that has been boiled and cooled), then prepare a 1% solution of amylase (diastase) by dissolving 1mL of MC23.35P in 99mL of distilled water. Place 5mL of each solution into separate test tubes and equilibrate the temperature in a water bath set at 50ºC. Pour the starch solution into the enzyme solution and shake to mix. Maintain the temperature.

To monitor the progress of the reaction:

Place one drop of iodine solution (1.5% / 3% I/KI, MC26.1) onto a watchglass or the well of a Handy Tray (G11.38). Using a clean pipette, add two drops of the starch/amylase solution to the drop of iodine solution. Compare the colour to that obtained when two drops from a starch control solution are added to a drop of iodine solution. Prepare the control by mixing 5mL of the starch solution with 5mL of distilled water and equilibrate to temperature.

The strong blue/black colour that indicates the presence of starch will become less intense as the enzymic reaction progressively breaks down the starch. This is a rapid reaction and you can expect most of the starch to be consumed within a few minutes.

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To confirm the formation of simple reducing sugars:

When the reaction is complete, transfer 3mL of the starch/amylase mixture to a clean test tube. Add an equal volume of Benedict's Solution and shake to mix. Heat in a boiling water bath for several minutes. A colour change from blue to orange is a positive indication for the presence of reducing sugars.

To eliminate the possibility of a significant amount of reducing sugars being present in the starting reagents as contaminants, test both the starch and the amylase with Benedict's solution. Before testing, dilute each with an equal volume of water to match the *in situ* concentration.

Comments and further Ideas:

Organisms including humans use amylase to break down dietary starch to render it soluble and to allow it to be absorbed during digestion. Many industrial processes also use amylase. For example, it is sometimes used in brewing to remove haziness caused by residual (insoluble) starch in the beer.

Experimental variables to consider include

- Temperature - vary the temperature of the water bath and compare the different rates of reaction. Note, MC23.35P shows high activity over a wide temperature range. This feature can be important for industrial processes, but is not normally found in enzymes derived from mammalian sources.
- pH – vary the pH of the reaction by adding (a) a weak acid such as aqueous ethanoic acid and (b) a weak base such as aqueous Na_2CO_3 to the starch solution. Note any differences in the rate of reaction.
- Concentration – due to the high activity level of MC23.35P, you will need to increase the starch concentration and decrease the amylase concentration to extend the time of reaction beyond a few minutes.

Always run a control alongside your test solution by substituting distilled water for amylase solution.

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

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