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Amylase detection

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Protocol status: Working

We use this protocol and it's working

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Abstract

The iodine test is used to test for the presence of starch. Starch turns into an intense "blue-black" colour upon addition of aqueous solution of iodine, due to the formation of an intermolecular charge transfer complex.

Starch is a polysaccharide consisting of glucose units joined together by glycosidic bonds. The amylase in the starch reacts with iodine to form a dark blue complex while amylopectin develops reddish purple colour. In the presence of amylase the starch is broken down into saccharides, consequently such colour does not develop, when iodine is added.

A common configuration of the method in the radial diffusion assay and agar gel containing starch is prepared. A sample well is created by punching a hole in the gel and extract the questioned sample is then stained using an iodine solution. Clear area in the gel indicates amylase activity and the size of the clear area is proportional to the amount of amylose in the sample.

Quantitative analysis of amylase

- 1 1% agar gel was prepared containing 1% starch
- 2 The gel was poured in the petriplates and was allowed to solidify
- 3 Gel was punched by using gel punchers into five different wells
- 4 20 μ l of amylase enzyme was added in the range of 2 μ l, 4 μ l, 6 μ l, 8 μ l, 10 μ l from the stock of 1mg/ml of amylase in outer wells and unknown enzyme sample in the centre well
- 5 The petriplates were incubated in moist chamber
- 6 1% of iodine solution was prepared in ethanol and it was poured on the starch agarose plate on next day
- 7 White colour zones (transperent) were observed against dark blue colour background
- 8 The diameter of white zone of standard and unknown was measured
- 9 A graph was plotted with the concentration versus diameter of zone
- 10 The unknown concentration was calculated by using a standard graph.



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