



General Certificate of Education
Advanced Subsidiary Examination
June 2013

Biology

BIO3T/P13/TN

Unit 3T AS Investigative Skills Assignment

Teachers' Notes

Confidential

**A copy should be given immediately to the teacher responsible for
GCE Biology**

Confidential

These notes must be read in conjunction with *Instructions for the Administration of the ISA: GCE Biology* published on the AQA Website.

The effect of pH on the time taken by amylase to hydrolyse starch

Introduction

Amylase is an enzyme that catalyses the hydrolysis of starch. Candidates will investigate the effect of pH on the rate of hydrolysis. The rate of reaction will be determined from how long it takes for starch to disappear from a reaction mixture. Candidates will investigate the rates of reaction at pH 5.0, 6.0, 6.4, 7.0 and 8.0.

Materials

In addition to access to general laboratory equipment, each candidate needs:

- 20 cm³ 0.5 % diastase
- 20 cm³ 1 % starch
- 5 cm³ buffer solutions at pH values of 5.0, 6.0, 6.4, 7.0 and 8.0
- iodine solution in a dropper bottle
- large beaker to use as a water bath
- thermometer
- 2 spotting tiles
- timer
- 10 test tubes
- 2 test tube racks
- syringes or pipettes measuring up to 2 cm³
- marker pen or labels
- dropping pipette
- suitable eye protection.

Candidates will need access to hot water for preparing their water baths.

Technical Information

Technicians/teachers preparing solutions must follow the safety data sheet provided by the supplier when handling reagents.

Amylase solution

Diastase should be used and labelled as amylase. Diastase is amylase derived from plants. Fungal amylases and salivary amylase should not be used.

Diastase from Timstar worked successfully in trials.

Do not use any diastase that has been stored for longer than 12 months in the refrigerator. Diastase that has not been stored in the refrigerator should not be used.

0.5% diastase gave good results in trials. However enzymes can vary considerably, so the investigation should be trialled using the same batch of enzyme to be used by candidates. Adjustments should be made to the concentration to get the investigation to work in a reasonable time, i.e. around about 1 minute for the fastest time, in order to allow candidates enough time to record results.

The buffer solutions

Buffers can be purchased or prepared. The following table gives information on how 100 cm³ of each buffer solution was prepared during trials. If you use the same volumes, check the actual pH values before use.

pH	0.2 mol dm ⁻³ disodium hydrogen phosphate / cm ³	0.1 mol dm ⁻³ citric acid / cm ³
5.0	51.50	48.50
6.0	63.15	36.85
6.4	69.25	30.75
7.0	82.35	17.65
8.0	97.25	2.75

Starch solution

Starch 'solution' is really a suspension. If starch is simply added to water it will not dissolve.

Soluble starch should be used. Starch from Timstar worked successfully in trials.

Make up a 100 cm³ of suspension by adding 1 g of soluble starch to a small amount of cold water and mixing to a paste. Then add about 80 cm³ boiling water and stir. Make the volume up to 100 cm³ with cold water. Allow to cool before refrigerating.

Preparation and storage of solutions

The enzymes, buffers and starch solutions should be made up as short a time as possible before use. The solutions should be stored in the fridge until half an hour before use, when they should be placed in the laboratory to reach ambient temperature.

All solutions should be disposed of after the experiment.

Iodine solution

Iodine solution is harmful to the eyes and eye protection is required. See Hazard 54B.

0.1 mol dm⁻³ iodine solution is advised for this experiment.

Iodine is not very soluble in water. It is soluble in potassium iodide solution. The procedure is slow and it is better to use a mechanical stirrer if available.

To make 250 cm³ of 0.1 mol dm⁻³ solution:

Dissolve 10 g of potassium iodide in 200 cm³ of water then add 6.35 g of iodine. To check that the iodine has dissolved, pour the solution from one beaker to another. Once the iodine is dissolved make up the solution to 250 cm³ with water. The solution will keep for a long time at room temperature so can be made up in advance of the practical.

Turn over ►

Hot water for water bath

Candidates will need access to a supply of hot water. If there is a hot water tap in the lab this will be sufficient. Alternatively jugs of hot water can be used. Kettles should be avoided as boiling water is not needed.

Trialling

The task **must** be trialled before use.

It is essential that the trial is done with the same batch of enzyme to be used by candidates. Adjust the concentration of amylase until the fastest reaction occurs in no less than 40 seconds. If necessary the concentration of starch could be adjusted as well.

Additional Information

AQA might publish Additional Information about an ISA/EMPA practical. This will be placed on e-AQA in Secure Key Materials. We will email Exams Officers who have downloaded the particular Teachers' Notes so they can print a copy for the Head of Biology. Additional information will cover issues such as suitable suppliers or tips on getting a practical to work.

Information to be given to candidates

Candidates must **not** be given information about an ISA assessment until one week before Stage 1. One week before sitting Stage 1 of the ISA, teachers should give their candidates the following information.

You will investigate the effect of pH on the time taken by amylase to hydrolyse starch. In addition, you will need to understand the following topics:

- carbohydrate digestion
- structure of carbohydrates
- factors affecting enzyme reactions.

There **must** be no further discussion and candidates **must not** be given any further resources to prepare for the assessment.

In this investigation, teachers **must not** give candidates the following information:

- when the blue-black colour fails to appear.