GCE A LEVEL – NEW
1400U50-1A
BIOLOGY – A2 unit 5
Practical Examination
Experimental Task
TEST 1
TUESDAY, 4 APRIL 2017
2 hours

ADDITIONAL MATERIALS
In addition to this examination paper, you will require a calculator and a ruler.

INSTRUCTIONS TO CANDIDATES
Use black ink or black ball-point pen. Pencil may be used to draw tables and graphs.
Write your name, centre number and candidate number in the spaces at the top of this page.
Write your answers in the spaces provided in this booklet.

INFORMATION FOR CANDIDATES
The total number of marks available for this task is 20.
Your teacher will directly assess your practical skills.
The number of marks is given in brackets at the end of each question or part question.
You are reminded of the necessity for orderly presentation in your answers.
This practical involves the action of the enzyme **phosphatase** which acts on substrates to release phosphate groups. In this investigation the substrate for the enzyme is phenolphthalein phosphate (PPP). Phosphatase reacts with PPP to produce phenolphthalein (PP) and a free phosphate group as shown below.

![Diagram of phosphatase reaction](image)

Both products are colourless in neutral and acid conditions.

When alkaline sodium carbonate is added, the phenolphthalein turns pink and the phosphatase is inactivated.

You are provided with an extract from mung beans which contains the enzyme phosphatase.

In this investigation you are going to determine how the production of phenolphthalein changes during the course of the reaction.

The experiment will be carried out at room temperature and pH 7.

You are given:
1. 2 syringes – each with a volume of 5 cm³
2. 2 syringes – each with a volume of 1 cm³
3. Enzyme extract (approximately 20 cm³) in pH 7 buffer
4. 15 test tubes
5. Solution of sodium carbonate (approximately 20 cm³)
6. Solution (approximately 20 cm³) of phenolphthalein phosphate (the substrate)
7. Stop clock
8. Colour chart of phenolphthalein solutions (page 3)
9. Glass rod
10. 3 beakers – each with a volume of 100 cm³
11. 250 cm³ beaker of distilled water
12. 250 cm³ beaker for waste
13. Test tube rack
14. Paper towels
15. Safety goggles
YOUR TEACHER WILL BE OBSERVING YOUR EXPERIMENTAL TECHNIQUE.

**Method**

1. Use one syringe to place 1 cm$^3$ of the sodium carbonate solution into each of 5 test tubes. Do not use this syringe again.

2. Use a **clean** syringe to add 5 cm$^3$ of substrate (PPP) to a 100 cm$^3$ beaker.

3. Use a **different** syringe to add 5 cm$^3$ of enzyme extract to the substrate. Mix and **immediately** start the stop clock.

4. **After 1 minute** and then at 1 minute intervals, use a 1 cm$^3$ syringe to remove 1 cm$^3$ of the enzyme and substrate mixture, and add it to one of the test tubes which contains sodium carbonate solution.

5. Before taking the next sample, fill the syringe with distilled water and discard the waste into the beaker provided.

6. Use the colour chart provided below to estimate the concentration of phenolphthalein (PP) in each of your 5 test tubes, in mol dm$^{-3}$. Record your results clearly.

7. Repeat the whole investigation twice to give three readings for each time interval.

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**Colour chart for phenolphthalein solutions**

<table>
<thead>
<tr>
<th>Concentration of PP</th>
<th>0.0 × 10$^{-6}$ mol dm$^{-3}$</th>
<th>1.0 × 10$^{-6}$ mol dm$^{-3}$</th>
<th>2.0 × 10$^{-6}$ mol dm$^{-3}$</th>
<th>3.0 × 10$^{-6}$ mol dm$^{-3}$</th>
<th>5.0 × 10$^{-6}$ mol dm$^{-3}$</th>
<th>1.20 × 10$^{-5}$ mol dm$^{-3}$</th>
<th>2.50 × 10$^{-5}$ mol dm$^{-3}$</th>
<th>7.50 × 10$^{-5}$ mol dm$^{-3}$</th>
</tr>
</thead>
<tbody>
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</tr>
</tbody>
</table>
(a) Below, record your results accurately and clearly.
(b) Plot the results from your table on the grid below.
(c) (i) With reference to your graph discuss the consistency of your readings. [2]

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(ii) Suggest two sources of inaccuracy in this investigation and an improvement for each. [2]

Inaccuracy 1: .........................................................................................................................................................

Improvement ......................................................................................................................................................
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Inaccuracy 2: .........................................................................................................................................................

Improvement ......................................................................................................................................................
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(d) Suggest how the experiment could be modified to investigate the effect of pH on the activity of phosphatase. [2]

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END OF PAPER