INSTRUCTIONS

- Answer all questions.
- Use a black or dark blue pen. You may use an HB pencil for any diagrams or graphs.
- Write your name, centre number and candidate number in the boxes at the top of the page.
- Write your answer to each question in the space provided.
- Do not use an erasable pen or correction fluid.
- Do not write on any bar codes.
- You may use a calculator.
- You should show all your working and use appropriate units.

INFORMATION

- The total mark for this paper is 30.
- The number of marks for each question or part question is shown in brackets [ ].
A student was provided with two proteases that hydrolyse the amino acid chains of protein in different ways, producing mixtures of single amino acids and peptides of varying lengths:

- An **endoprotease** that hydrolyses peptide bonds between **specific** amino acids within the protein molecule. The enzyme only functions if there is a minimum of two amino acids on each side of the hydrolysis site.

- An **exoprotease** that hydrolyses protein molecules by removing amino acids one at a time from the terminal carboxyl (–COOH) end. This enzyme only functions if the substrate molecule has a minimum of three amino acids.

The student used these enzymes to hydrolyse a protein formed by the linking of two polypeptides. Fig. 1.1 shows the hydrolysis sites of these two enzymes on this protein.

**Key**

- △ hydrolysis site of the endoprotease
- ↑ hydrolysis site of the exoprotease
- ○ amino acid

![Diagram of protein molecule with hydrolysis sites marked](image-url)
(a) The student investigated the effect of these two enzymes on the hydrolysis of this protein by incubating the protein separately with each of the enzymes.

- Each mixture of enzyme and protein was incubated at 35 °C and a pH of 7.6.
- At intervals of 5 minutes, a sample of each mixture was removed using a capillary tube.
- The products of hydrolysis within each sample were separated by chromatography using the same solvent.
- The products of hydrolysis were located by spraying the chromatogram with a specific dye that stains proteins, peptides and amino acids.
- The student continued sampling the mixtures every 5 minutes and running chromatograms for each sample until hydrolysis of the protein was completed by each enzyme.

(i) Identify the independent variable in this investigation.

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(ii) Identify three variables, other than the chromatography solvent and the specific dye, that the student standardised in this investigation.

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(iii) Describe how the student could have standardised two of the variables you described in (a)(ii).

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(iv) Suggest how the student could have determined when hydrolysis of the protein was complete.

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(b) Describe how the student could prepare and use chromatograms to compare the products of hydrolysis of the protein by the two different proteases.

Your method should be set out in a logical order and be detailed enough to allow another person to follow it.

Details of how the proteins were hydrolysed should not be included.
Fig. 1.2 shows the chromatograms produced when complete hydrolysis of the protein by each protease had occurred. Fig. 1.2 also shows the time taken for complete hydrolysis.

The student concluded that:

1. The endoprotease worked faster than the exoprotease because fewer bonds needed to be hydrolysed.
2. The products of hydrolysis by the exoprotease were all single amino acids giving more spots on the chromatogram.
3. Hydrolysing the protein with a mixture of endoprotease and exoprotease would give the same results as for the exoprotease alone but more quickly.

State and explain whether each of these conclusions is supported or not supported by all of the information provided about these two enzymes, including the evidence in Fig. 1.2.

conclusion 1: ...................................................................................................................................................
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conclusion 2: ...................................................................................................................................................
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conclusion 3: ...................................................................................................................................................
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[3]
(d) Using an internet search the student found that:

- electrophoresis can also be used to separate the products of enzyme hydrolysis of proteins

- a combination of electrophoresis and chromatography has been used to study haemoglobin from people with sickle cell anaemia.

In an investigation, samples of haemoglobin from people with sickle cell anaemia and people without sickle cell anaemia were hydrolysed by an endoprotease. Each sample was placed centrally on one side of a piece of chromatography paper and separated by electrophoresis.

Negatively charged peptides moved to the anode (+) and positively charged peptides moved to the cathode (−).

After electrophoresis, the paper was turned through 90° and chromatography was used to further separate the peptides.
Fig. 1.3 shows the main stages of this experiment.

(i) Sickle cell haemoglobin and normal haemoglobin have a difference in amino acid sequence.

On Fig. 1.3, draw a circle around the spot in each chromatogram that shows that the two types of haemoglobin have different amino acid sequences. [1]

(ii) Explain your answer to (i).

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[Total: 18]
Hybridisation can be used by plant breeders to develop new varieties of crop plants.

A student investigated the ability of one species of grass, species A, to hybridise with two other species of grass, species B and species C, by studying the chromosomes of the hybrids.

The diploid number of chromosomes for each species was:

- species A 12
- species B 20
- species C 14

Fig. 2.1 shows part of a grass flower stalk with several flowers.

These are the main steps in the procedure used by the student:

1. The anthers were removed from 40 flower stalks of species A.
2. Separate plastic bags were placed around each of the flower stalks, still attached to the plant.
3. Pollen from species B was transferred onto the stigmas of 20 of the flower stalks of species A.
4. Pollen from species C was transferred onto the stigmas of the other 20 flower stalks of species A.
5. The grains formed by fertilisation of species A, each containing a single seed, were collected.
6. Hybrid embryo plants were removed from the grains and the number of chromosomes in the cells of each of the hybrid embryo plants was counted.

(a) (i) Identify the dependent variable in this investigation.

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(ii) Identify the variable that was controlled by the use of plastic bags on the flower stalks of species A.

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(b) Table 2.1 shows the results that the student obtained from counting the number of chromosomes in the cells of each of the hybrid embryo plants.

<table>
<thead>
<tr>
<th>hybrid embryo from cross</th>
<th>number of chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A × B</td>
<td>16</td>
</tr>
<tr>
<td>A × C</td>
<td>26</td>
</tr>
</tbody>
</table>

Explain the results of the student’s investigation, as shown in Table 2.1.

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In a second investigation, the student repeated the procedure to hybridise the grasses.

- After hybridising the grasses, 50 grains containing hybrid embryo plants were collected from each of the two crosses, \( A \times B \) and \( A \times C \).
- Hybrid grass plants were grown from the grains collected.
- When the hybrid grass plants flowered, pollen containing the male gametes was collected.
- For each of the two crosses, the number of chromosomes in a random sample of 30 male gametes obtained from the pollen of the hybrid grass plants was counted.

Table 2.2 shows the results of this investigation.

**Table 2.2**

<table>
<thead>
<tr>
<th>number of chromosomes in 30 male gametes from the hybrid grass plants produced from:</th>
<th>cross ( A \times B )</th>
<th>cross ( A \times C )</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>16</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>16</td>
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<tr>
<td>6</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>13</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>

(i) The student also counted the number of chromosomes in the female gametes of each hybrid grass plant.

Predict the number of chromosomes you would expect the student to find in the female gametes of the hybrid grass plants.

- hybrid from the cross \( A \times B \) ...........................................................................................................................................
- hybrid from the cross \( A \times C \) ........................................................................................................................................... [1]

(ii) The student concluded that hybrids from cross \( A \times B \) could not be bred successfully.

State one piece of evidence in Table 2.2 that supports this conclusion.

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Question 2 continues on page 12
(d) The student carried out a third investigation to find out if the difference in the number of hybrid grains germinating from the two crosses was significant. The student counted the number of grains that germinated from 11 samples of 50 grains from each cross.

Table 2.3 shows the results of the investigation.

Table 2.3

<table>
<thead>
<tr>
<th>sample number</th>
<th>number of grains that germinated</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cross A × B</td>
<td>cross A × C</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>31</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>34</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>34</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>36</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>38</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>37</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mode</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>median</td>
<td>34</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The student calculated the mean, mode and median for the data from the cross of A × B and recorded these in Table 2.3.

(i) Complete Table 2.3 by writing in the values of the mean, mode and median for the results from cross A × C.

(ii) The student used the t-test to compare the means.

State one feature of the data that allows use of the t-test.

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(iii) State a null hypothesis for this $t$-test.
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(iv) State why the number of degrees of freedom for this $t$-test is 20.
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[Total: 12]