Cambridge Assessment International Education

Teaching Pack

Investigating the effect of temperature on an enzyme-catalysed reaction

Cambridge International AS & A Level Biology 9700

Version 1.0

Cambridge Pathway

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Introduction

This pack will help you to develop your learners' experimental skills as defined by assessment objective 3 (AO3 Experimental skills and investigations) in the course syllabus.

Important note
Our *Teaching Packs* have been written by **classroom teachers** to help you deliver topics and skills that can be challenging. Use these materials to supplement your teaching and engage your learners. You can also use them to help you create lesson plans for other experiments.

This content is designed to give you and your learners the chance to explore practical skills. It is not intended as specific practice for Paper 3 (Advanced Practical Skills) or Paper 5 (Planning, Analysis and Evaluation).

This is one of a range of *Teaching Packs* and each pack is based on one experiment. The packs can be used in any order to suit your teaching sequence.

The structure is as follows:

Briefing lesson (1 hour*)
This lesson reinforces the key concepts, relevant skills, knowledge and understanding linked to the experiment.

↓

Planning lesson (1 hour*)
This lesson focuses on planning an experiment.
It ends with a demonstration video of the experiment.

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Lab lesson (1 hour*)
This lesson focuses on carrying out the experiment including the collection and recording of observations, measurements and estimates.

↓

Debriefing lesson (1 hour*)
This lesson focuses on the analysis and interpretation of data.
This includes making conclusions, evaluating methods and the quality of data, and how improvements could be made.

** the timings are a guide only; you may need to adapt the lessons to suit your circumstances.*

In this pack you will find lesson plans, worksheets and teacher resource sheets.

Experiment: Investigating the effect of temperature on an enzyme-catalysed reaction

This *Teaching Pack* focuses on the effect of temperature on the rate of an enzyme-catalysed reaction. Proteases such as trypsin catalyse the hydrolysis of the protein casein, which gives milk its white colour. This reaction is characterised by the decolorisation of the milk, providing a way to estimate the rate of the reaction easily in a school laboratory. Your learners will carry out the reaction at different temperatures to investigate the effect of temperature on the rate of the reaction.

The experiment covers the following experimental skills, as listed in **AO3: Experimental skills and investigations** (see the syllabus):

- plan experiments and investigations
- collect, record and present observations, measurements and estimates
- analyse and interpret data to reach conclusions
- evaluate methods and quality of data and suggest improvements.

The following techniques are covered:

- denaturing an enzyme by boiling, to act as a control
- measuring time to a colour change / observing a colour change
- calculating a mean
- finding the rate of reaction using 1/time
- drawing a line graph with a smooth curve.

Briefing lesson: Measuring rates of reaction

Resources	• Worksheet A
Learning objectives	By the end of the lesson: • ***all*** learners should know that rates of enzyme-catalysed reactions are measured by substrate disappearance or product formation • ***most*** learners should understand the use of 1/time as a measure of reaction rate • ***some*** learners will be able to describe the advantages and disadvantages of interval sampling versus continuous monitoring.

Timings	Activity
15 min	**Starter/Introduction** Ask your learners to suggest how the rate of each of the following enzyme-catalysed reactions could be measured: 1. Decomposition of hydrogen peroxide by catalase to form oxygen and water 2. Hydrolysis of lipid by lipase to form fatty acids and glycerol 3. Hydrolysis of starch by amylase to form maltose They might need hints to think about how they could measure product formation or disappearance of the substrate in each case. Answers should include 1. Measuring the rate of oxygen production with a gas syringe, or mass loss with a balance; 2. Measuring the rate of fatty acid formation by using a pH indicator; 3. Measuring the rate of starch removal by testing with iodine solution. Discuss details of the different methods and why these are the most practical approaches in each case. Summarise by agreeing that overall, rates of enzyme-catalysed reactions are measured by following either substrate disappearance or product formation. Ask your learners to say which applies to each of the above reactions.
35 min	**Main lesson** Discuss the difference between quantitative and qualitative data in terms of numerical measurements versus descriptive observations. (Qualitative data is observed and cannot be measured using numerical values; quantitative data expresses a number range or quantity.) Give learners Worksheet A. Ask them to explain if the table shows quantitative or qualitative data. Agree that although the table shows numerical data (sample times, temperature) the recorded data are observations not measurements, so the data is qualitative. Discuss the idea that enzyme-catalysed reactions are often monitored using visual observations such as colour changes. Ask learners to suggest how such observations could be used to provide **quantitative** data. They might need prompting to suggest recording how long it takes for the colour change to occur and then using the reciprocal (1/t), in order to convert the time taken to a rate.

Timings	Activity
	Ask the learners to work in pairs to use the data in the table to answer the questions on Worksheet A. These questions should get the learners to start thinking about issues they would need to consider when planning their own experiments.
	**Plenary** As a class, discuss the advantages and disadvantages of sampling at intervals versus continuous monitoring when measuring enzyme-catalysed reaction rates. Ideas should include that continuous monitoring would allow for more frequent measurements and more confident conclusions, but that it isn't always possible or practical. For example, biuret solution (and other solutions such as iodine solution) can act as enzyme inhibitors and affect the results, so in these cases samples have to be drawn from the reaction mixture for testing, and so it is not possible to continuously monitor.

Planning lesson: Measuring loss of colour

Resources	• Completed Worksheet A • Worksheets B and C
Learning objectives	By the end of the lesson: • ***all*** learners should be able to describe the appearing cross method • ***most*** learners should be able to write a plan that uses this method • ***some*** learners will be able to explain how the potential error with this method can be reduced.

Timings	Activity
5 min	**Starter/Introduction** Explain to your learners that they are going to plan an experiment to investigate the effect of temperature on the rate at which casein in milk is hydrolysed by a protease. Ask learners in groups of 2–3, to review why temperature affects enzymes (impact on rate of collisions, hydrogen bonding, tertiary structure, shape of active site, complementary fit of substrate). You could ask your learners to draw a concept map.
10 min	**Main lesson** Explain that casein gives milk its white colour. Ask learners to use their knowledge from the previous lesson to suggest ways of measuring the rate of the reaction. They should suggest finding the time taken for the white colour of the milk to disappear – this would be taken as the end-point of the reaction. Ensure learners appreciate that this is a subjective judgement that will vary for different learners. Ask your learners how they might standardise the end-point of the reaction and how accurate their method would be. Give learners Worksheet B. Ask them to take a few minutes to think about how the experiment works and how the appearing cross method could be adapted for the experiment they're going to do, then ask them to discuss their ideas with a partner. Make sure everyone agrees that they would measure the time taken for the cross to appear as the milk decolorises. Learners should understand that they are measuring membrane permeability indirectly by depth of solution.
35 min	Explain that they are now going to plan the experiment. Learners can use their answers to Worksheet A, and Worksheet B for help. Specify that they will use 5 cm^3 of protease solution and 10 cm^3 of milk for each reaction. Tell your learners the optimum temperature for the bacterial protease you plan to use so that they may select an appropriate temperature range. Ask for suggestions for a suitable control experiment and why; do they need to time this one and if not, why not? Emphasise the importance of them being able to justify each of their decisions. You may need to prompt learners during the writing of their plans. In particular, they need to describe how they will ensure that both the substrate and enzyme are at the right temperature before the reaction starts. You may choose to support your learners with Worksheet C, which provides prompts for planning an experiment

Timings	Activity
	effectively, and/or by giving them the suggested responses to Worksheet C. Remind learners to describe the steps they will take in sufficient detail that another person could follow their plan.
	**Plenary** Learners watch the video as a summary of the experiment. Ask your learners to identify any key points they have missed out of their own method. For example, it is likely that some learners will not have recognised the need for pre-incubation of the enzyme and substrate. You may then wish to give them some time to make adjustments to their plan.

Lab lesson: Timing substrate disappearance

Resources	• Teacher notes, Teacher method and *Teacher walkthrough* video
Learning objectives	By the end of the lesson: • ***all*** learners should be able to draw a results table and collect some data • ***most*** learners should be able to draw a results table with correct column headings and units and collect a full set of data • ***some*** learners will check and record actual temperatures, include a column for mean time taken and recognise and repeat anomalous readings.

Timings	Activity
5 min	**Starter/Introduction** Ask learners if they've included repeats in their plan and why they've done this. Explain that they only have 50 minutes to do their experiment and therefore it's not likely they will have time to run repeats. Explain that instead you will pool the class data to find a mean. Discuss the disadvantage of this and what impact that might have on the accuracy of the mean.
50 min	**Main lesson** Learners should collect and set up their apparatus. Make sure you draw their attention to precautions they should take, for example, the care needed when using the enzyme solution and the water baths at higher temperatures (test-tubes should be removed using test-tube holders and not with their hands as shown in the video!). Learners carry out their method for finding the time taken for crosses to become visible at different temperatures. During the lesson, you should check that learners have drawn appropriate tables and have included the correct units in the column headings. Learners should use the time for equilibrating their enzyme and substrate tubes to draw their results table. If necessary, remind learners that their table needs to: • be fully ruled with no units in the body of the table • contain an appropriate number of columns and rows (think whether repeat readings will be recorded and/or whether any processed data will be presented and which variable is being changed; the independent variable should be in the leftmost column or top row) • include headings for the rows and/or columns, with appropriate units. **Safety** Circulate the classroom at all times during the experiment so that you can make sure that your learners are safe and that the data they are collecting is accurate.
5 min	**Plenary** Learners calculate mean times or submit their results to the pooled class data.

Teacher notes

Watch the *Teacher walkthrough* video and read these notes.

Each group will require:

- 2 × test-tube racks (to hold 6 test-tubes each)
- 12 × test-tubes
- 5 × 500 cm^3 beakers (to act as mini-water baths)
- 1 × 200 cm^3 beaker (for use in denaturing the enzyme using boiling water)
- 6 × bungs to fit test-tubes
- 5 × thermometers
- 2 × 10 cm^3 syringes
- 2 × glass rods
- timer
- access to a kettle of boiling water
- access to 3 thermostatically controlled water baths, set at 40°C, 60°C and 75°C
- ice cubes (approximately 10–15, to fill a 500 cm^3 beaker)
- sticky labels and permanent marker pen
- 100 cm^3 3% powdered milk solution (skimmed; do not use fresh or high-fat milk)
- 50 cm^3 5% bacterial (recombinant) trypsin/protease solution
- distilled water in wash bottle

Safety

The information in the table below is a summary of the key points you should consider before undertaking this experiment with your learners. The information is **not** exhaustive and does not include storage or handling instructions.

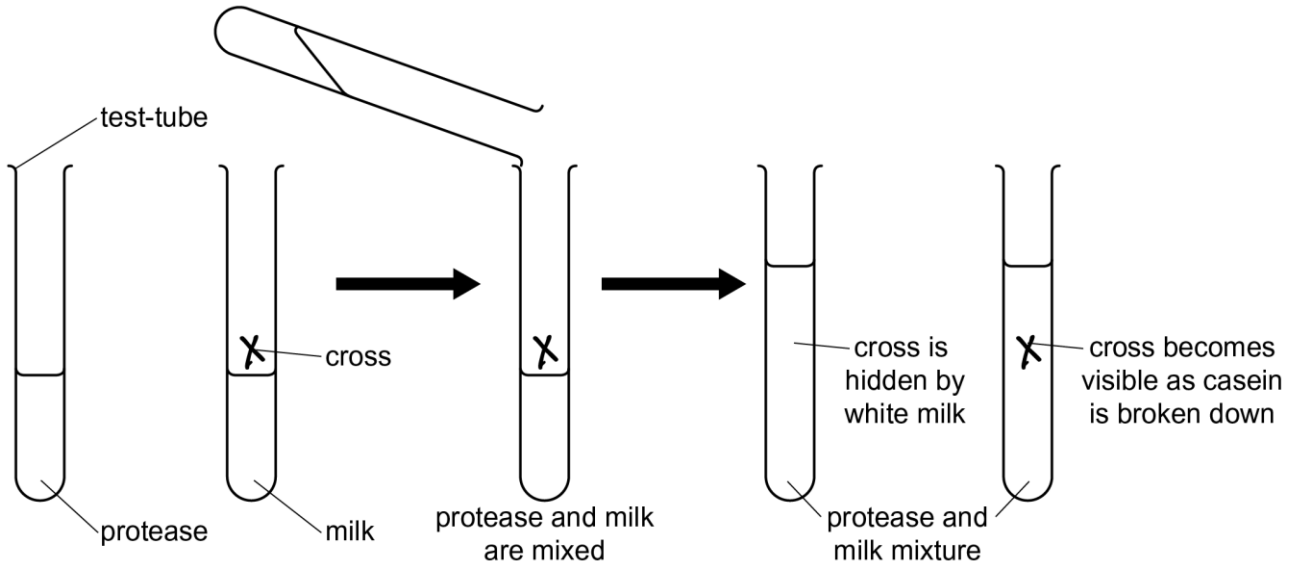
Learners should always wear gloves, eye protection and lab coats. There should not be any eating or drinking in the lab. Hands should be washed thoroughly at the end of the experiment.

It is your responsibility to carry out an appropriate risk assessment for this experiment.

Substance	Hazard	First aid
Enzyme solutions (dilute)	LOW HAZARD	**In the eye:** Flood the eye with gently-running tap water for at least 10 min. See a doctor. **Swallowed:** Wash out the mouth. Give a glass of water to drink. Do not make the casualty vomit. See a doctor. **Dust breathed in:** Remove the casualty to fresh air. See a doctor if breathing is difficult. **Spilt on the skin or clothing:** Remove contaminated clothing. Wash off the skin with soap and plenty of water. Rinse contaminated clothing. **Spilt on the floor, bench, etc.:** Scoop up powders (take care not to raise dust). Wipe up solution spills or any traces of powders with a damp cloth.
Food (generic)	ALLERGENS	Do not consume any foodstuffs in the labs. Gloves should be used if learners have allergies. If discomfort persists, see a doctor.
Latex gloves	ALLERGENS	Remove the gloves and wash hands under water. Look out for severe allergic reactions such as difficulty breathing and/or swelling of the face, body or tongue. Seek emergency medical attention immediately.

Hazard	First aid
Burns (hot water)	Flood burnt area with water for at least 10 minutes. For serious injuries, see a doctor.

Experiment set-up



You should make up solutions as per the manufacturer's instructions. However, here are some examples:

Material	When	How
5% protease solution	Immediately before use (The activity of protease declines rapidly over time, so the enzyme solution should be made fresh immediately prior to the activity.)	Follow the manufacturer's instructions for how to make a 5% solution with a pH of 9. If not available, then try: 1. Dissolve 5 g of enzyme powder in 90 cm^3 water. 2. Adjust as necessary to pH 9 (check with the pH probe) by adding 1M sodium hydroxide solution a few drops at a time and stirring with a glass rod. 3. Make the solution up to 100 cm^3 using distilled water.
3% powdered milk solution	Immediately before use	1. Add 3 g skimmed milk powder to 100 ml distilled water. 2. Stir with a glass rod until all the powder has dissolved.

Teacher method

This is your version of the method for this experiment, use this to help support your learners.

Before you begin

Think about:

- the number of groups you will need (group size 2–4 learners)
- the amount of equipment required
- how much time you will need for the water baths to reach temperature before the lesson
- the volume of powdered milk and protease solution needed for the number of groups.

Experiment

Walk around the learners during the experiment in case they encounter any difficulties.

Steps	Notes
1. Learners should collect the equipment they require from the front of the class.	
2. They should make five temperature labels and stick them near the rim of five beakers.	
3. A thermometer is added to each beaker.	
4. 10 to 15 ice cubes and 150 cm^3 of tap water should be added to the beaker labelled 5°C.	*The water temperature in each beaker will reach the target temperature after 10 to 15 minutes. Learners should record the actual temperature of these beakers in their table rather than assuming they are at the temperatures shown on the labels.*
5. Learners add 400 cm^3 of tap water to the beakers labelled 40°C, 60°C and 75°C and place them into three separate water baths set at 40°C, 60°C and 75°C respectively.	
6. Add 400 cm^3 of tap water to the beaker labelled 25°C and leave it on the bench. This beaker will be used for room temperature.	*Learners should record the actual temperature of this beaker in their table rather than assuming room temperature is 25°C.*
7. Learners label test-tubes with each temperature; one pair for each temperature.	*Labels should be placed near the top so they can be read easily when in the beakers.*
8. Learners should prepare a further pair of test-tubes, labelled with the letter ‘C’, for the control.	

Steps	Notes
9. One test-tube in each pair is marked about half way down with a large cross using a permanent marker pen.	*The test-tube with the cross will be referred to as the 'substrate tube.' The test-tube without the cross will be referred to as the 'enzyme tube'.*
10. Learners should add 5 cm^3 of protease to each 'enzyme tube' and 10 cm^3 of milk to each 'substrate tube'.	*It is crucial that learners use separate syringes for the protease and milk to avoid cross contamination.*
11. The enzyme tube labelled 'C' is placed into a beaker of boiling water for 5 minutes.	*The control is the denatured enzyme.*
12. Learners should place the pairs of test-tubes into their respective water baths for 10 minutes. The pair of control tubes are put in the beaker labelled 25°C (or left in the test-tube rack).	*This is to allow both protease and milk to equilibrate at the required temperatures.*
13. During this time, learners should draw a results table.	*If there is time to repeat readings, the tables should contain columns for these and a mean value.*
14. After 10 minutes, one pair of test-tubes should be removed from their water bath, dried, and placed into the test-tube rack.	
15. Learners should then immediately pour the contents of the enzyme tube into the substrate tube, add a bung, invert the tube once to mix it, and then start the stopwatch.	*The stopwatch must be started immediately upon mixing. The mixture must end up in the test-tube with the 'X' marked on it.*
16. With the cross at the rear of the test-tube, learners watch for the cross to become visible through the mixture.	
17. The stopwatch is stopped when the cross becomes visible through the solution.	*A 'fuzzy' cross is not enough – the outline of the cross should be visible. Learners might benefit from setting up a test-tube that standardises the end-point (or you could provide this).*
18. This process is repeated for each pair of test-tubes, one pair at a time, including the control tubes.	*The enzyme and substrate are kept in their water baths until immediately before they are mixed so that they are at the correct temperature when the reaction begins.*

Steps	Notes
19. If time allows, learners repeat the entire sequence to gain repeat readings in order to calculate a mean time for each temperature.	*Repeating readings allows any anomalous results to be recognised and a more accurate mean value to be found.*

Clean-up

After the experiment learners should:

- clean all glassware
- tidy up their work space
- ensure any spillages have been mopped up
- return all equipment and any unused chemicals to you.

Alternative methods

If you do not have access to the required equipment or the suggested method would not work for your class, here are some possible alternatives that you could use.

If thermostatic water baths are not available:

Learners could set up and maintain their own water baths using Bunsen burners. They can add 300 cm^3 of boiling water to a beaker, then add cold/hot water until the desired temperature is reached. A thermometer should be used to monitor the temperature every 5 minutes and cold/hot water is added as required.

Alternative to repeats:
Since the pairs of test-tubes must be processed sequentially time may not allow for repeat readings at each temperature. Instead, class data could be pooled in order to calculate mean times for each temperature.

Example results

In biological experiments, the results can greatly so the results given below are **not** the only possible results but they represent some example data that you can use if you need to.

Temperature / °C	Time taken for cross to appear / s				Rate of reaction / $s^{-1} \times 10^{-3}$
	Trial 1	Trial 2	Trial 3	Mean	
5	88	96	90	91.3	10.9
25	54	51	52	52.3	19.1
40	22	19	20	20.3	49.2
60	163	160	154	159.0	6.3
75	NR	NR	NR	N/A	0.0

Debriefing lesson: Interpreting rate measurements

Resources	• completed data tables from the experiment session • Worksheet D
Learning objectives	By the end of the lesson: • ***all*** learners should be able to plot a line graph and find the optimum temperature of the protease used in their experiment • ***most*** learners should be able to describe the effect of temperature on an enzyme-catalysed reaction from the trend seen in their graph • ***some*** learners will understand that, because of the appearing cross method, they can have confidence in the trend shown on the graph but less confidence in the accuracy of the optimum temperature.

Timings	Activity
15 min	**Starter/Introduction** Review the variables in the experiment. Ask learners to identify the independent and dependent variables and to identify other variables that were standardised. Ask what steps they took to attempt to standardise the end-point. For example, drawing the cross on the test-tube rather than on a piece of card helps to ensure the cross is the same distance from the solution each time. Discuss what else they might have done. Ideas to discuss could include having the same person make the judgement; looking at the cross from the same angle and distance; and ensuring that the light is the same for each judgement, perhaps by illuminating the test-tubes the same way each time with a bench lamp. Ask learners to suggest how confident they are in the accuracy of their time values. They should recognise that whilst the variables were standardised, the determination of the end-point is subjective so they should be cautious about the accuracy of their time values. Revisit the disadvantages of pooling the class times for mean data.
25 min 10 min	**Main lesson** Ask learners to process their data by calculating the reciprocals of their mean times as a measure of rate of reaction. To generate larger values that are easier to plot, you can suggest they multiply the reciprocal by 1000 (1000 / t) provided they indicate this on the axis label with '× 1000'. Learners plot a line graph of rate of substrate disappearance against temperature. Worksheet D provides some help with drawing this particular graph. Ensure that learners draw a smooth curve because they need to find the value of the optimum temperature, which will probably lie between two points. Explain that they should position the peak of the curve where it naturally falls rather than necessarily at any one data point. Ask learners to find the optimum temperature for the enzyme by drawing a vertical line down from their peak to the *x*-axis. Remind them that a bacterial protease was used, so they should not expect the optimum temperature to be mammalian body temperature. In pairs, learners interpret their graph in terms of

Timings	Activity
	the effect of temperature on the rate of enzyme-catalysed reactions. Ensure they understand that the increasing rate of reaction is due to the effect of temperature on the shape of the active site, as well as an increase in rate of collisions, so the line is curved rather than straight. Point out that the rapid decline in rate indicates denaturation, but it also suggests (by not dropping vertically) that not all the enzyme molecules denature at exactly the same temperature.
	**Plenary** Ask them to evaluate how confident they are in their estimate of the optimum temperature compared to the general trend shown on the graph for the effect of temperature on an enzyme-catalysed reaction. As well as reminding your learners about the subjectivity of the end-point judgements, you may wish to include the idea that the fairly large intervals between the temperatures might mean that their drawing of a peak on the curve was also subjective. You could discuss the idea that a more accurate estimate of the optimum temperature could be found by adding some intermediate temperatures to the range. Learners should understand that whilst they can be reasonably confident in the shape of the curve (trend) they cannot be so confident in the individual data points. (Because the trend is just the shape of the curve as opposed to its specific location on the graph relative to the axes.)

Worksheets and answers

	Worksheet	Answers
For use in *Briefing lesson*:		
A: Testing for protein hydrolysis	**19**	**23**
For use in *Planning lesson*:		
B: The appearing cross	**20**	**–**
C: Planning sequence	**21**	**24**
For use in *Debriefing lesson*:		
D: Drawing and interpreting the graph	**22**	**–**

Worksheet A: Testing for protein hydrolysis

An experiment is carried out to investigate the effect of temperature on the complete hydrolysis of a protein by the protease pepsin. Protein can be tested for using the biuret test – biuret reagent is pale blue and turns purple in the presence of protein. The experiment involves measuring how long it takes for the protein in a solution to be completely hydrolysed. The following steps are taken:

1. Egg white is mixed with water to make a substrate solution; egg white contains the protein albumen.
2. 20 cm^3 of the albumen solution (in one container) and 3 cm^3 of pepsin solution (in a separate container) are left in a water bath for five minutes at 40°C.
3. After five minutes, the albumen and pepsin solutions are mixed and a few drops are immediately removed from the mixture and added to 1 cm^3 of biuret reagent using a dropping pipette.
4. The colour of the solution is recorded.
5. The albumen–enzyme mixture is left in the water bath and the sampling and testing is repeated every minute until a positive reaction for protein is no longer observed.
6. This is repeated for a further four temperatures.

The results of the experiment are shown in the table below.

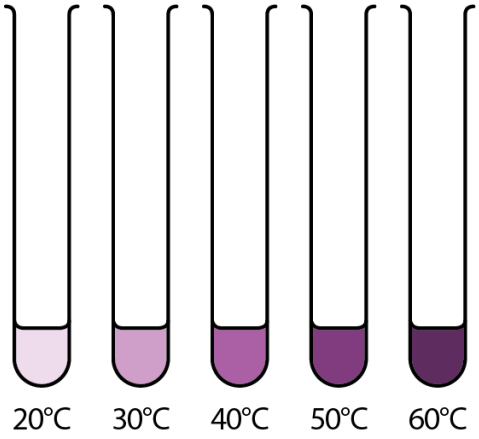
Time / min	biuret test results				
	10°C	20°C	30°C	40°C	50°C
0	purple	purple	purple	purple	purple
1	purple	purple	pale blue	purple	purple
2	purple	purple		purple	purple
3	purple	pale blue		purple	purple
4	purple			pale blue	purple
5	purple				purple
6	purple				purple
7	purple				pale blue
8	purple				
9	pale blue				

1. Why were the albumen solution and pepsin solution left in a water bath for five minutes before mixing?
2. Why was it important to add the same volume of biuret reagent to each sample?
3. Biuret solution can sometimes act as an enzyme inhibitor. Suggest why the biuret test is carried out on samples rather than directly on the reaction mixture.
4. Convert the data in the above table into a rate of reaction for each temperature. Record the data in a new table.
5. Why is the first sample taken immediately after mixing enzyme and substrate?
6. How accurately can you find the optimum temperature for pepsin from this data? Explain your answer.
7. How could you represent the data in a way that allows you to estimate the optimum temperature of pepsin more accurately?
8. Describe **one** way you could adapt the **method** to find a more accurate value for the optimum temperature.

Worksheet B: The appearing cross

An experiment was carried out to investigate the effect of temperature on permeability of plant cell membranes. Tissue from a type of plant that contains a coloured pigment was used. Samples of this plant were placed in water at different temperatures for 20 minutes.

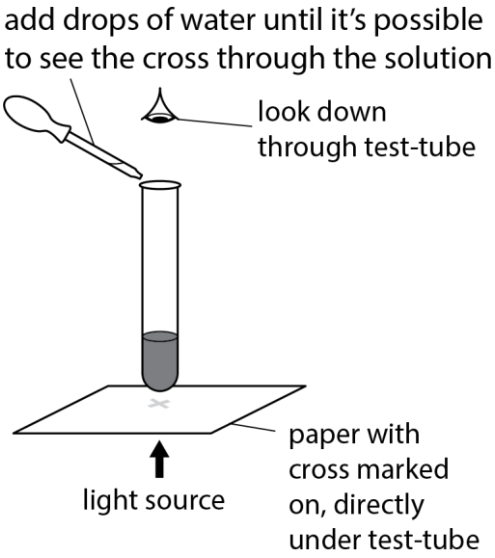
The water looked like this after the 20 minutes:



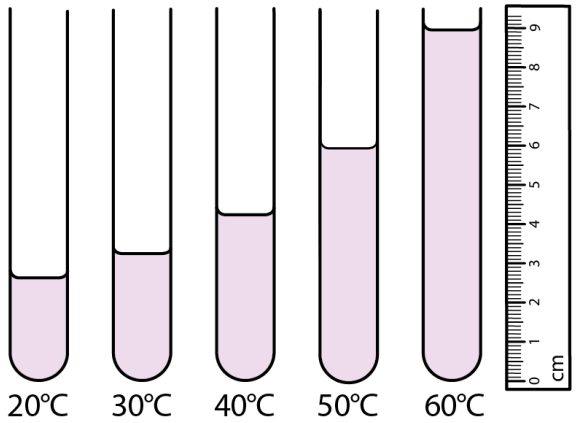
A **depth dilution method** was used to measure how much of the colour pigment had diffused from the plant cells into the water, at each temperature.

The method was as follows:

1. A cross was marked onto a piece of white card using a pencil – the cross was drawn very lightly so it was only just visible on the card.
2. The card was placed beneath each tube in turn and was illuminated using a bench lamp.
3. The card was observed from above, down through the sample.
4. Water was added to the tube until the cross was just visible.
5. The depth of the liquid in each tube was used as a measure of how much pigment had diffused from the plant cells.



When all the tubes had been treated in this way, they looked like this:



Temperature / °C	Depth of solution at which cross appears / cm
20°C	2.6
30°C	3.2
40°C	4.2
50°C	5.9
60°C	8.9

Worksheet C: Planning sequence

Here is a list of things to consider when planning an experiment. Number them from 1 to 10 to create a suitable sequence. Then use them as prompts to write your own plan.

Remember that a good plan requires you to focus on
what will be investigated and **how** this will be achieved

Decide how to measure the dependent variable, how often to measure it and consider whether you need to try to standardise the measuring procedure. ☐

Write a clear title for the experiment to clearly define the purpose of the experiment. ☐

Use the title to write a hypothesis. ☐

Identify any variables you should standardise. These are variables that may alter the results if they change. Decide how to keep them the same. ☐

Assess the risks of the procedure as low, medium or high by considering the hazards. ☐

Identify the independent and dependent variables, in other words, consider what you will change and what you will measure. The title and hypothesis can help with this – consider how the experiment will test your hypothesis. ☐

Decide how many times you will repeat each measurement to identify any anomalous results and to get a mean value. ☐

Decide how to change and measure the independent variable, the range of values to use, how many values to use and the intervals between the values. ☐

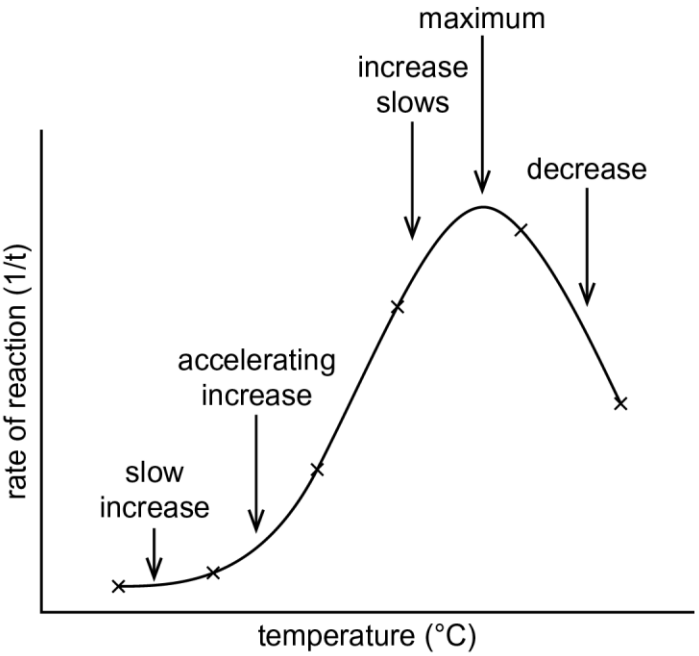
Work out the order in which you would need to do everything. ☐

Decide on a control that removes the effect of the independent variable. ☐

Worksheet D: Drawing and interpreting the graph

When drawing your graph, remember to:

- display the independent variable on the *x*-axis and the dependent variable on the *y*-axis
- use a small cross to mark each data point
- make sure the intersection of the crosses are exactly on the required point
- make sure the plotted points are connected with a clear, sharp and unbroken smooth curve passing through all the points (assuming there are no anomalous points, in which case a line of best fit should be drawn)
- not extrapolate (extend) the curve beyond the plotted points
- draw the peak where it naturally falls on the curve rather than at the highest point.



When interpreting your graph:

- identify specific sections of the curve to talk about by looking for changes in the gradient (examples are shown by arrows on the diagram)
- provide a description of what is happening to the rate of reaction in each section (examples are shown by the text labels above each arrow)
- explain what is happening to the rate in each section in terms of the effect of increasing temperature on the enzyme molecules (active site and frequency of collisions).

Worksheet A: Answers

1. To allow both solutions time to reach the correct temperature before starting the reaction. This is called pre-incubation. Temperature is the independent variable in this experiment. If one or other solution were not at the same temperature when mixed, the reaction would take place at a different temperature and give an inaccurate result.

2. The volume of biuret reagent must be a standardised variable. This is because the colour change from purple to pale blue would occur at different times if different volumes of reagent were used. For the results at each temperature to be comparable, the volume of reagent used must be the same every time.

3. Biuret reagent may act as an enzyme inhibitor so cannot be added to the main reaction mixture; it must be used to test samples drawn from the mixture.

4.

Incubation temperature / °C	10	20	30	40	50
Time taken for colour change / min	9	3	1	4	7
Rate of reaction / 1/t	0.11	0.33	1.00	0.25	0.14

5. In case all the protein disappears and the colour change occurs within the first minute. (You need to take a measurement as soon as possible after the reaction has started in case the protein is hydrolysed really quickly, i.e. faster than one minute.)

6. The data in the table only allows you to say that the optimum temperature lies between 20°C and 40°C.This is because the temperature intervals of 10°C are quite large and so the time taken for a colour change might be even quicker at some temperature between 20°C and 30°C or between 20°C and 40°C, than at 30°C.

7. Plot a line graph of the time taken for a colour change against temperature. Draw a smooth curve and then draw a vertical line down to the temperature axis from the peak. This would give a more accurate value for the optimum temperature.

8. Repeat the biuret test at closer temperature intervals, for example every 2°C, over 10°C either side of the peak and re-plot the graph. Then draw a vertical line down to the temperature axis from the new position of the peak.

Worksheet C: Suggested responses

The order of some items can vary, use your discretion to determine if learners' orders are sensible.

1. Write a clear title for the experiment to clearly define the purpose of the experiment.

   Example response: Investigating the effect of temperature on the rate of casein hydrolysis by a protease.

2. Use the title to write a hypothesis.

   Example response: As the temperature increases the rate of the reaction will increase until it reaches the optimum temperature of the protease. After this point, the rate of the reaction will decrease as more and more enzyme molecules become denatured by the higher temperatures.

3. Identify the independent and dependent variables, in other words, consider what you will change and what you will measure. The title and hypothesis can help with this – consider how the experiment will test your hypothesis.

   Example response: The independent variable is temperature and the dependent variable is how long it takes for the casein to be hydrolysed.

4. Decide how to change and measure the independent variable, the range of values to use, how many values to use and the intervals between the values.

   Example response: The temperature at which each reaction takes place will be changed by heating the milk and protease solutions in a water bath until they are both at the required temperature. Five temperature values at 15°C intervals from 5°C to 75°C ensures that the range includes the probable optimum for a bacterial protease which will be 50°C – 60°C.

5. Decide on a control that removes the effect of the independent variable.

   Example response: A suitable control is to mix denatured protease with a sample of milk at room temperature; the enzyme is denatured by leaving it in boiling water (100°C) for 5 minutes. Denatured enzyme should result in no hydrolysis of casein, confirming that temperature is not responsible for any hydrolysis that occurs in other samples.

6. Identify any variables you should standardise. These are variables that may alter the results if they change. Decide how to keep them the same.

   Example response: The volumes of milk (10 cm^3) and protease solution (5 cm^3) need to be the same for each sample. The pH should also be kept the same, but is unlikely to change in this reaction, so can be assumed to be standardised.

7. Decide how to measure the dependent variable, how often to measure it and consider whether you need to try to standardise the measuring procedure.

   Example response: The time taken for casein to be hydrolysed can be measured by timing how long it takes for the white colour of the milk to disappear. This can be found by timing how long it takes for a cross viewed through the solution to become visible. Using the first end-point as a standard tube with which to compare the others will help standardise the measurement. Starting the stop clock immediately on mixing the solutions will also be important to ensure accurate measurements.

8. Decide how many times you will repeat each measurement to identify any anomalous results and to get a mean value.

   Example response: The time taken at each temperature should be found three times to check for any anomalous results. Three times is sufficient because any one result out of line with the other two can be identified.

9. Work out the order in which you would need to do everything.

   Example response:
   1. *Measure standardised volumes of milk and protease solution into test-tubes.*
   2. *Draw a cross onto one of each pair of test-tubes.*
   3. *Place each test-tube into the appropriate water bath until they are all at the required temperature.*
   4. *Add the contents of the unmarked test-tube to the marked test-tube for one temperature, and immediately start the timer.*
   5. *Measure and record how long it takes for the cross to become visible through the solution.*
   6. *Repeat this process for the pairs of test-tubes in each water bath.*

10. Assess the risks of the procedure as low, medium or high by considering the hazards.

    Example response: There is a risk of scalding from the water in the hottest water baths above 50°C. Enzymes can cause allergic reactions but only in sensitive individuals. Overall this is a low-risk procedure.

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