Cambridge Assessment International Education

Teaching Pack

Immobilising enzymes

Cambridge International AS & A Level Biology 9700

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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.

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: Immobilising enzymes

This *Teaching Pack* focuses on the immobilisation of the enzyme lactase.

The immobilisation of enzymes is a technique in industry that is widely used to maximise the efficiency of biochemical reactions. In this experiment, learners will encapsulate the enzyme lactase in alginate beads and show that its ability to hydrolyse its substrate, lactose, is not affected by immobilisation. This illustrates how a continuous process could be used to make useful products in industry, including food and drinks for people who are lactose-intolerant.

This experiment has links to the following syllabus content (see syllabus for detail):

- 3.1. Mode of action of enzymes
- 3.2. Factors that affect enzyme action

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

- 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 technique is covered:

- obtaining qualitative results from observations of colour changes using a number scale for intensity of colour with a key.

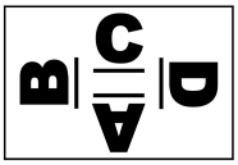
Prior knowledge

Knowledge from the following syllabus topics is useful for this experiment.

- 2.3. Proteins
- 2.4. Water

Briefing lesson: Modelling molecular motion

Resources	• Worksheets A and B • Teacher instructions 1, 2 and 3 • Bucket (or similar large container) • Balloons (1 per pair of learners) • A3 card/paper (10 sheets) • Large piece of card • Sticky tape (3–4 dispensers) • Scissors (3–4 pairs) • Sticky notes of two different colours (including 2 sticky notes per pair) • Strong elastic bands (3–4) • Inflatable beach ball or large cushion
Learning objectives	By the end of the lesson: • ***all*** learners should be able to describe the advantages and drawbacks of immobilising enzymes in specific scenarios • ***most*** learners should be able to explain the advantages and drawbacks of immobilising enzymes in specific scenarios • ***some*** learners will be able to evaluate, using specific terminology, the benefits and drawbacks of using immobilising enzymes.

Timings	Activity
10 min	**Starter/Introduction** Arrange the learners into pairs and give each pair a copy of Worksheet A (visual shown). Ask three multiple choice questions on the enzyme lactase. Each question should have four optional answers (A, B, C or D).  Learners are given 30 seconds to discuss the answer in their pairs, before they hold up Worksheet A with their chosen letter at the top (in the visual here, the chosen answer is 'C'). If possible, show the questions at the front of the class for everyone to see. As a class, discuss which answers are wrong and why, in order to develop a common understanding. This activity encourages pair and whole-class discussion, and brings all learners up to speed with key terminology from prior lessons. Example questions are provided on Teacher Instructions 1.
	Main lesson Models can help learners understand concepts in Biology by aiding their ability to imagine the behaviour of the molecules involved. In this lesson, you will introduce some models of enzyme activity, using everyday equipment, to help develop learners' understanding and encourage independent thinking through the visualization of biological concepts. Before the start of the lesson, you will need to create: paper representations of lactose, glucose and galactose; sticky-tape enzymes; and a reaction vessel. Read Teacher Instructions 2 and Teacher Instructions 3 for details of what is required.

Timings	Activity
	Give pairs of learners the paper 'lactose' for Model 1 (see Teacher Instructions 2) and explain what it is. Ask them to discuss in their pairs how they might use this to demonstrate what would happen to the disaccharide when acted on by the enzyme lactase. They should suggest pulling them apart to show hydrolysis and production of two products (glucose and galactose). Discuss suggestions if necessary. (The lactose molecules are needed for Model 1, so learners should avoid actually tearing them.)
	**Teacher demo** (see Teacher Instructions 2 for instructions)**:** Scrunch up a piece of sticky tape into a ball. Explain that this represents an enzyme and that there are already some in your bucket. Carry out the teacher demo and elicit what the model shows.
	**Model 1** (see Teacher Instructions 2 for instructions)**:** Give pairs of learners a balloon and some sticky tape. Prompt learners to record how this model demonstrates the activity of molecules during an enzyme-catalysed reaction in their notebooks.
	**Model 2** (see Teacher Instructions 3 for instructions)**:** Briefly ask, '*Why do most enzymes denature when the temperature is raised?*' Then explain that they are going to be part of a model to demonstrate what happens. Explain that they are each an amino acid and you want them to think about how they could represent the structure of an enzyme using just their hands and some elastic bands. Explain that the model must show: how a peptide bond is formed with amino acid residues either side of them; the role that hydrogen bonds play within the structure of the protein; how the enzyme binds to its substrate. Take a few suggestions, then run the model using Teacher Instructions 3. If required, display Worksheet B.
	**Plenary** Ask learners to suggest a molecular explanation for the extension activity in part **C** of Model 2, where rope was added to tie the learners together. Elicit that adding a material in this way acts to stabilise the enzyme, making it more likely that the substrate will bind to the active site even during heating. Ask what the drawbacks of binding an enzyme in this way might be. Learners should suggest that the active site is less accessible and enzyme–substrate complex formation is limited, because the matrix (represented by the rope in the model) restricts the ability of the substrate to enter the active site.

Planning lesson: Encapsulating enzymes

Resources	• Worksheets C, D and E • A3 paper (1 per group of learners) • Apparatus as per *Teacher method* (for a teacher demonstration) • *Immobilising enzymes* video
Learning objectives	By the end of the lesson: • ***all*** learners should be able to outline a plan for how to produce beads containing immobilised enzyme and to set up a reaction vessel • ***most*** learners should be able to plan how to improve the efficiency of a reaction vessel containing immobilised enzyme beads • ***some*** learners will be able to evaluate alternative methods that could be used to produce a reaction vessel containing immobilised enzyme beads

Timings	Activity
15 min	**Starter/Introduction** Before the start of the lesson, you need to set up equipment and materials to carry out a teacher demonstration to show how alginate beads can be made. Read the Teacher notes and Teacher method for instructions on how to make up the required solutions; make just enough for this demonstration. At the start of the demonstration, you should have a syringe filled with sodium alginate and a beaker of calcium chloride. Ask learners to gather around, and then demonstrate the process of forming alginate beads. Show that the sodium alginate is slowly released into the beaker of calcium chloride. Ensure that you: • Explain that the calcium ions in the calcium chloride solution cross link the alginate strands, show how this causes the liquid alginate to become a semi-solid when it enters the calcium chloride solution. Pass the beads around the room to prompt further discussion; remove a few beads from the calcium chloride solution with a sieve, wash them thoroughly under a running tap and gently dry them on a paper towel before passing them around. • Illustrate how the rate of fall of the drops of sodium alginate have an effect on the diameter of the beads that form in the calcium chloride solution. • Ask questions and discuss points of importance with regard to the practical activity that will follow later. For example, '*Why is it important that the nozzle of the syringe should not touch the solution?*' and '*Why should we leave the beads in the calcium chloride solution for at least 10 minutes?*' Ask learners to consider how they could immobilise an enzyme using alginate beads. Elicit the idea that the enzyme should be mixed with the alginate, rather than with the calcium chloride solution, and why. Explain that this process is called 'encapsulating' the enzyme. Remind learners of the benefits and drawbacks of encapsulating enzymes that they encountered in the previous lesson. Ask how *encapsulating* is different to the method they modelled with balloons and sticky tape (*adhesion*). What might be the advantages and disadvantages of each method?

Timings	Activity
20 min	**Main lesson** Arrange the class into groups of 3–4, with each group ideally comprising learners of mixed ability, gender and cultural backgrounds. Give each group Worksheet C, which includes a range of laboratory equipment. Explain that in the next lesson, they will carry out their own experiment to show that immobilising lactase in beads does not prevent it from breaking down lactose in milk. Explain to learners that they need to select apparatus to build a reaction vessel capable of producing lactose-free milk in a continuous process. They should be given at least 5 minutes to discuss their thoughts, and then should be provided with a piece of A3 paper to produce a rough labelled diagram. During this activity, circulate to provide support and guidance. Make sure they consider the implications of using each piece of apparatus, for instance, why choose a 50 cm^3 syringe over a 25 cm^3 one? Or why should/shouldn't they use a funnel and filter paper to collect the milk? (Questions will vary depending on the choices learners make.) Remind them of the considerations they discussed during the demonstration. Ask why they might need scissors. Why is it important for all the beads to be as uniform in size and shape as possible? Or does it not matter for the purposes of this experiment because the focus is not the measurement of the rate of reaction?
15 min	Give each learner Worksheet D, which includes an unlabelled diagram of the actual experimental set-up that learners will prepare in the practical lesson. They should be challenged to think carefully about how the arrangement has features that are similar and different to their approach, and to evaluate their approach accordingly. Give each learner Worksheet E, which outlines the method they will use in the practical lesson. They are asked to identify the activity that should be undertaken in each step, or the rationale for taking a step. Learners should attempt this individually. Ask them to compare their answers with a partner once they have finished. They should discuss how they could ensure that the method maximises the yield of product. You may wish to circulate during this activity to provide further prompts to learners who finish the activity earlier than others, in order to (i) remind learners that the milk should to be warmed before pouring it into the column (to ensure that reaction occurs at the optimum temperature of lactase) and (ii) inform learners that the products of the immobilisation column can be tested for glucose (discuss why using semi-quantitative glucose test strips might be better than using Benedict's solution in this case).
10 min	**Plenary** Play the Master video and encourage learners to modify their answers to Worksheet E. The task is intended to ensure that all learners have a clear idea of the method, and the underlying basis of each step, prior to undertaking the practical themselves in the next lesson. The completed worksheet should be read and reviewed for homework alongside the answer sheet, in advance of conducting the practical task. Alternatively, you could collect it for formative assessment, and to ensure that the class has a common understanding of the task ahead. Inform learners that they will begin the practical work immediately at the beginning of the next lesson.

Lab lesson: Getting practical

Resources	• Completed Worksheets D and E • Apparatus as per *Teacher method* • Teacher Instructions 4 and a whiteboard marker pen
Learning objectives	By the end of the lesson: • ***all*** learners should be able to describe how to produce beads containing immobilised enzyme and set up a reaction vessel containing them • ***most*** learners should be able to explain how to produce a yield of lactose-free milk • ***some*** learners will be able to suggest how aspects of the method used to produce an immobilised enzyme column could be improved.

Timings	Activity
50 min	**Main lesson** The practical lesson begins immediately. Learners will need their completed Worksheet E (which has been checked against the answers for homework, or by yourself) and the diagram on Worksheet D. Explain that their experiment is designed to demonstrate that lactase still works even when immobilised. As they go along, they need to write down **three** problems they encounter and how they overcame them. Ask them to also consider, whilst carrying out each step of the method, if there would be any issues with that step if they were to carry out a more quantitative investigation, such as investigating the rate of enzyme-controlled reaction. They should note down where they think there might be issues, and briefly consider how they might improve the method to overcome them. These notes will be used in the *Debriefing lesson*. **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. Teacher Instructions 4 provide a number of questions that you could ask as you circulate the room (these will inform the plenary activity).
10 min	**Plenary** Learners will be at different stages of the practical activity towards the end of the lesson, with some likely to need the full hour to completely finish. To act as an effective differentiator, set up an 'open board' on which learners are encouraged to use whiteboard markers to develop a mind-map-style answer in response to the questions listed on Teacher Instructions 4. (You should also refer learners to this on occasion to promote independent thinking during periods of 'dead time' during the practical and also during the packing away of equipment).

Teacher notes

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

Each group will require:

- retort stand, 2 × boss, 2 × clamp
- distilled water (in a wash bottle)
- 50 cm^3 plastic syringe barrel (without plunger)
- 10 cm^3 plastic syringe (with plunger)
- 20–30 cm rubber tubing to fit around the bottom of the syringe nozzle
- 100 cm^3 1.5% calcium chloride solution
- 250 cm^3 beaker
- 100 cm^3 beaker
- 10 cm^3 lactase–sodium alginate solution
- 50 cm^3 milk (not UHT, ideally skimmed /low fat)
- disposable plastic cup
- Hoffman clip (resembles a vice)
- small sieve
- sharp (dissection) scissors
- access to a hot plate/water bath (set at 40°C)
- small piece (about 1 cm^2) nylon gauze e.g. cut from net curtain or nylon clothing
- spatula
- glass rod (blunt-ended stirring rod)
- semi-quantitative glucose test strips, e.g., Roche Diabur-Test 5000 or Ames / Bayer 'Diastix'
- latex or rubber gloves (for safety)

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.

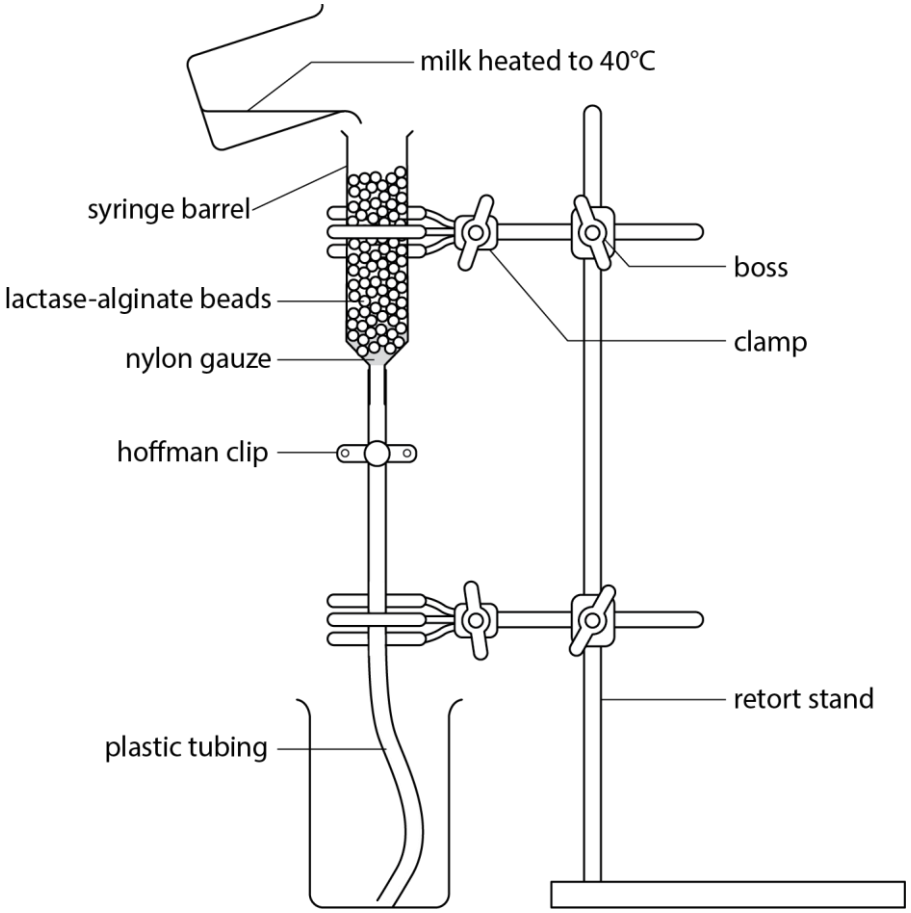
Hazard	First aid
Allergies – latex gloves	Remove the gloves and wash hands under water. Look out for severe allergic reactions such as difficulty breathing and/or swelling of the face, body or tongue. Seek emergency medical attention immediately.
Burns	Flood burnt area with water for at least 10 minutes. For serious injuries see a doctor.

Note that enzymes can trigger allergies in some individuals.

Substance	Hazard	First aid
Calcium chloride (solution)	 **GHS07 (*moderate hazard* MH)**	**In the eye:** Flood the eye (and under the eyelids) with gently running tap water for at least 30 minutes; remove contact lenses if present and easy to do. If eye irritation persists, get medical advice/attention. **Vapour breathed in:** Remove the casualty to fresh air. Loosen clothing as necessary and position individual in a comfortable position. If breathing becomes difficult, give oxygen. Give artificial respiration if necessary. Call a doctor if discomfort or irritation persists. **Swallowed:** Wash out the mouth with water. Do not induce vomiting. Provide the affected individual with sips of water. See a doctor if irritation, discomfort or vomiting occurs. **Spilt on the skin or clothing:** Remove contaminated clothing and shoes; wash before reuse. Wash skin with soap and water. Rinse thoroughly for 15 minutes. If a large area is affected or irritation, discomfort or vomiting occurs, see a doctor. **Spilt on the floor, bench, etc.:** Follow Chemical Hygiene Plan procedures. Collect liquids using vacuum or using absorbents. Place into properly labelled containers for recovery or disposal. Dust deposits should not be allowed to accumulate on surfaces, as these may form an explosive mixture if they are released into the atmosphere in sufficient concentration. Avoid dispersal of dust in the air, i.e. clearing dust surfaces with compressed air. Wash area with plenty of water.
Sodium alginate (solution)	 **GHS07 (*moderate hazard* MH)**	**In the eye:** Check for and remove any contact lenses. Flood the eye, with eyelids open, with gently running tap water for at least 15 minutes. See a doctor. **Vapour breathed in:** Remove the casualty to fresh air. If breathing becomes difficult, give oxygen. If not breathing, give artificial respiration. Call a doctor. **Swallowed:** Wash out the mouth with water. Do not induce vomiting. Loosen tight clothing such as collar, tie, belt or waistband. See a doctor. **Spilt on the skin or clothing:** Remove all contaminated clothing and shoes immediately unless stuck to skin; wash before reuse. Wash skin with soap and water. Cover the irritated skin with an emollient. If irritation develops, see a doctor. **Spilt on the floor, bench, etc.:** Sweep up into shovel. Keep in suitable, closed containers for disposal. Avoid raising powdered materials into airborne dust. Finish by cleaning the contaminated surface with plenty of water.

Substance	Hazard	First aid
Lactase	 **GHS08 (*health hazard* HH)**	**In the eye:** Flood the eye, with eyelids open, with gently running tap water for at least 15 minutes. See a doctor. **Vapour breathed in**: Remove victim to fresh air and keep at rest in a position comfortable for breathing. If it is suspected that fumes are still present, the rescuer should wear an appropriate mask or self-contained breathing apparatus. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation. Get medical attention. If necessary, call a poison centre or physician. If unconscious, place in recovery position and get medical attention immediately. Maintain an open airway. Loosen tight clothing such as a collar, tie, belt or waistband. In the event of any complaints or symptoms, avoid further exposure. **Swallowed:** Wash out mouth with water. Remove dentures if any. Remove victim to fresh air and keep at rest in a position comfortable for breathing. If material has been swallowed and the exposed person is conscious, give small quantities of water to drink. Stop if the exposed person feels sick as vomiting may be dangerous. Do not induce vomiting unless directed to do so by medical personnel. If vomiting occurs, the head should be kept low so that vomit does not enter the lungs. Get medical attention if adverse health effects persist or are severe. Never give anything by mouth to an unconscious person. If unconscious, place in recovery position and get medical attention immediately. Maintain an open airway. Loosen tight clothing such as a collar, tie, belt or waistband. **Spilt on the skin or clothing:** Remove all contaminated clothing and shoes immediately and wash before reuse. Wash skin with water and non-abrasive soap. Be particularly careful to clean folds, crevices, creases and groin. Cover the irritated skin with an emollient. If irritation develops, see a doctor. **Spilt on the floor, bench, etc.:** Use appropriate tools to put the spilled solid in a convenient waste disposal container. Finish cleaning by spreading water on the contaminated surface and dispose of according to local and regional authority requirements. Dilute with water and mop up if water-soluble. Alternatively, absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.

Experiment set-up



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

Material	When	How
1.5% calcium chloride solution	Any time before you start the practical	1. Dissolve 15 g calcium chloride-2-water in 1 dm^3 distilled water (in a volumetric flask). This will provide enough for a class of around 8–9 pairs of learners. 2. Cover with cling film. 3. Store in a refrigerator.
Enzyme solution (Lactase (β-galactosidase) enzyme, e.g. *Novozymes Lactozym Pure*®)	Just before start of practical use unless manufacturer's instructions say to let it rest before use.	Dilute to make a 50 cm^3 solution as per the manufacturer's instructions. This will provide enough enzyme for a class of around 8–9 pairs of learners.

Material	When	How
3% sodium alginate solution	At least **one hour before** start of practical	1. Set up a hot plate to 40°C. 2. Dissolve 1.5 g of sodium alginate in 50 cm^3 distilled water (in a 100 cm^3 beaker). This will provide enough solution for a class of around 8–9 pairs of learners. 3. Add a magnetic stirrer and put the beaker on the hot plate. The heat is needed for the solid sodium alginate to fully dissolve. This will take at least an hour. *Note: If the alginate gel is too dense, the substrate cannot enter the bead. Use 3% alginate to mix with equal volumes of lactase. Keep solutions in the refrigerator if they are not to be mixed immediately. This minimises evaporation, which would alter their concentrations.*
Enzyme–alginate mixture	Once the sodium alginate is ready and has cooled; just before the practical starts.	1. Make sure the sodium alginate solution has cooled or you will denature the enzyme. 2. Mix equal volumes of 3% sodium alginate solution and lactase solution.

Teacher method

The method outlined accompanies the *Teacher walkthrough* video.

Before you begin

Think about:

- the number of groups you will need (group size 2–3 learners)
- the amount of equipment/chemicals required
- if groups will each have a beaker of enzyme–alginate mixture, a collecting station or pre-filled syringes
- the questions you could pose to learners during less active periods in the practical task, for example during incubation windows or during the clean-up (some examples are provided on Teacher Instructions 4).

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.	*Asking different learners to collect different items can improve the smooth running of the practical task.*
2. They should find a space where the equipment can be assembled safely.	*Learners should remain standing due to the use of a vertical reaction column and for general safety reasons.*
3. Make sure learners are briefed with regard to aspects of safety, including the hazard warning symbols associated with the reagents, the sharp scissors, and the water bath/hot plate.	*The water bath/hot plate should be set at 40°C, but there is a possibility that it could vary in temperature during the lesson.*
4. Learners should fill a 10 cm^3 syringe with enzyme–alginate mixture.	*You should have prepared the enzyme–alginate mixture before the lesson. Decide if each group will have their own beaker of enzyme–alginate mixture, if they should go to a designated station to collect it, or if the syringes are pre-filled.*
5. Learners should hold a 10 cm^3 syringe containing the enzyme–alginate mixture about 10–15 cm above the disposable plastic cup containing the calcium chloride solution. By slowly forcing down the plunger, small droplets of this mixture are allowed to fall into the cup.	*Holding the syringe too close over the calcium chloride solution will cause 'tadpole tails'. Remind learners not to let the tip of the syringe to come into contact with the calcium chloride solution, as this will cause the alginate to harden, blocking the outlet.*

Steps	Notes
6. Around 30–40 beads should be produced in this way, which are left to harden in the calcium chloride solution for 10 minutes.	
7. The contents of the plastic cup are then poured into a small sieve and the beads are rinsed thoroughly with distilled water from a wash bottle over a sink.	*Washing removes any calcium chloride solution from their surface. 'Tadpole tails' of sodium alginate may form as the drops fall and residue leaves the nozzle, especially if the syringe was held too close to the solution, but these can be trimmed with sharp scissors later if desired.*
8. A small piece of nylon gauze is put inside an empty 10 cm^3 syringe barrel, so that it rests just above the outlet.	*This prevents beads from blocking the outlet and enables the milk to run through.*
9. The syringe barrel is then clamped to the very top of a retort stand.	
10. The beads are carefully placed inside the syringe barrel using a spatula. A glass rod, or the plunger, can be used to gently ease them down.	*The beads should not be packed too tightly as the milk will need to flow between them.*
11. A piece of rubber tubing is attached firmly to the bottom of the syringe outlet. The rubber tubing should be secured tightly to the nozzle by pushing and gently twisting.	*The rubber tubing will allow the milk to run out of the reaction vessel after passing through the beads.*
12. A Hoffman clip is then attached to the rubber tubing just underneath the outlet of the syringe. It is tightened to its maximum extent to prevent the milk from leaving the syringe barrel.	*The tightness of the Hoffman clip can be adjusted like a tap, and will prevent the milk from flowing through the column too quickly.*
13. A second clamp is loosely fitted towards the end of the rubber tubing near the bottom of the retort stand, to keep it aligned just above a 100 cm^3 beaker. This beaker will collect the milk after it is released through the tubing.	*Ensure that the end of the rubber tubing is positioned above the beaker and that it is clamped just above the opening, to ensure that the run-through will not be spilt on the lab bench.*
14. Learners should warm the milk on a hot plate set to 40°C. They should wait until the milk is at 40°C before adding the milk to the syringe barrel.	*Warming the milk to around 40ºC means that the lactase operates at a temperature closer to its optimum when the substrate is added.*

Steps	Notes
15. The warm milk is then carefully poured into the top of the column. It should reach the top of the vessel without overflowing. The apparatus is then left for 5 minutes, during which time the enzyme has an opportunity to digest its substrate.	*Note that full cream milk or UHT milk should **not** be used. Other substances in full cream milk can affect lactase activity, and glucose may already be present in UHT milk that has been treated with high temperatures.*
16. After 5 minutes, the Hoffman clip is slowly unfastened, which allows the milk to leave the syringe barrel and drip slowly through the rubber tubing into the beaker.	*If the milk does not begin to trickle through the vessel and down the tube, the syringe plunger, or a glass stirring rod, can be used to gently force it to do so.*
17. To determine if the enzyme-catalysed reaction has taken place, the milk in the beaker is then tested for the presence of glucose, one of the products of lactose digestion. This is carried out using a test strip, which changes colour in the presence of glucose.	*Demonstrating the use of a glucose test strip with a concentrated solution of glucose is recommended before learners use this detection method.*
18. A sample of untreated milk should also be tested as a control.	*This is to ensure that untreated milk does not contain any glucose, which would yield a false-positive result.*
19. Comparing the colour obtained on each test strip with the manufacturer's chart will indicate whether digestion has been successful. It also gives a semi-quantitative measure of the effectiveness of lactose digestion.	*Discussing the nature of a 'semi-quantitative' measure is important here, as well as the fact that the process of measurement is subjective and hence the data obtained is limited in its accuracy.*

Clean-up

After the experiment learners should:

- place the beads in a bin
- pour any milk and calcium chloride down the sink and wash down with plenty of water
- bring any leftover sodium alginate solution to you (this could block the sink if discarded)
- clean all glassware
- tidy up their work space
- ensure any spillages have been mopped up
- wash their hands.

Debriefing lesson: Planning further investigation

Resources	• Worksheets F, G and H • Graph paper (1 sheet per learner) • A3 paper (1 sheet per group)
Learning objectives	By the end of the lesson: • ***all*** learners should be able to recognise that the method used to prepare lactose-free milk can be used as the basis of an investigation into the effect of a named variable • ***most*** learners should be able to suggest how the method used to prepare lactose-free milk can be adjusted and developed to investigate the effect of a named variable on enzyme activity • ***some*** learners will be able to evaluate investigations of the effect of a named variable on immobilised enzyme activity using this method.

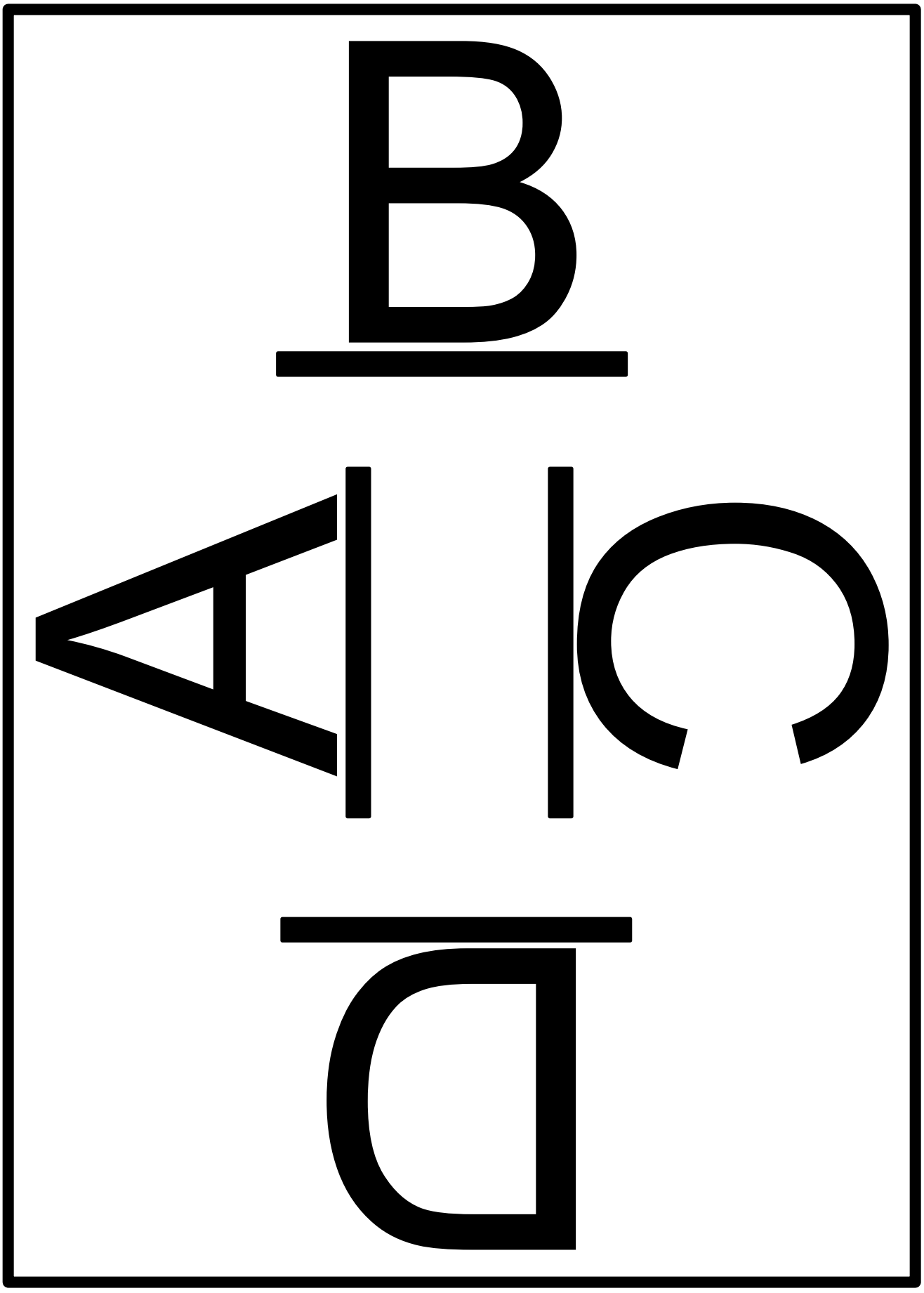
Timings	Activity
10 min	**Starter/Introduction** Give learners Worksheet F and ask them to take a few minutes to plot the graph (Question 1) and think about the answers to the remaining questions *without* writing anything down. They should then discuss the answers with a partner for a few minutes, and then form a group with another pair to continue the discussion – there should be ever-expanding groups forming. For this snowballing technique to be effective, choose the groups so that there is a mix of learners in terms of ability, gender and cultural backgrounds. Each time the group size increases, ask learners to think how they might improve their answers now that they've discussed them with others. Learners then write down their own answers. This task will draw on the work in the previous lessons to reinforce the idea that enzyme-catalysed reactions can be made more efficient using immobilised enzymes, but that the procedure is associated with some drawbacks.
10 min	**Main lesson** Ask learners to make sure they have the notes they made during the practical lesson about problems with the method and how to overcome them. They will also need their notes of how they might adapt each step if the experiment was more quantitative in nature. Give learners 1–2 minutes to discuss with a partner one aspect of the method that presented an obstacle during the last lesson. Choose one learner at random (or ask for a volunteer) and ask for a contribution to get the class discussion started. Ask the other learners in the class '*Who else encountered this problem?*' and elicit various solutions that were used to overcome it. Examples may include: how scissors could be used to trim 'tails' from the beads if they entered the water as a shape other than a sphere; how it was difficult to exert the same pressure/speed when dripping the alginate from the syringe, meaning the beads were not always the same size; how a glass stirring rod or the plunger could be used to gently exert pressure on the beads from above, if the milk did not run through when the Hoffman clip was initially loosened. Conclude the activity by acknowledging the most common areas of difficulty and offer thoughts on how these

Timings	Activity
	issues are resolved in industrial scenarios. Providing a clear analysis of the method is vital in order to inform the activities that follow. Arrange learners in three mixed ability groups. Challenge the groups to develop a full experimental method to investigate a given hypothesis based on the method they carried out in the practical lesson. Give each group one of the following null hypotheses: Hypothesis 1 – There is no significant difference between the concentration of glucose in lactase-treated milk produced by incubation with spherical enzyme–alginate beads of different diameters. Hypothesis 2 – There is no significant difference between the concentration of glucose in lactase-treated milk produced using reaction columns with different rates of milk flow. Hypothesis 3 – There is no significant difference between the concentration of glucose in lactase-treated milk produced using milk of different pH values. Give each learner Worksheet G, which provides prompts for planning their investigation, and each group a sheet of A3 paper on which to write down their plan. Learners will apply their knowledge of last lesson's procedure to an unfamiliar scenario, and in so doing develop their awareness of how to plan an investigation and continue to evaluate good investigative techniques. Key skills include deciding on equipment, identifying variables to be controlled, considering how to collect data and evaluating its quality. To help structure discussions, it is recommended that roles or key responsibilities are assigned to different members of each group, for example: a leader who makes the final decision if opinions differ; someone else to write down the method on the A3 paper; one person to 'defend' their plan during the plenary activity; and so on.
	**Plenary** Pick one learner from each of the groups to be the 'group leader' for that group. Then ask the rest of the learners from each group to move to a different 'group leader', so that each group consists of members from different original groups (a form of the 'rainbow' technique). Each of the three 'group leaders' uses their A3 paper with their plan written on it in the form of a poster, to summarise their plan to their new group. Give each learner a copy of Worksheet H so that they can evaluate the plan being described by the 'group leader'; they should write the null hypothesis at the top of the sheet. Worksheet H also includes a table of example responses that the learners can use as reference when evaluating, to see if the other groups' suggestions are reasonable. Learners will benefit from hearing other groups' approaches on what are very similar investigations, enabling them to appreciate a number of ways of investigating the effect of a named variable on the activity of lactase. Ask the learners to get back into their original groups and discuss each of the other plans that they've learned about. Summarise the lesson by highlighting the most important points, before asking learners to complete the final task on Worksheet H (evaluating their own plan).

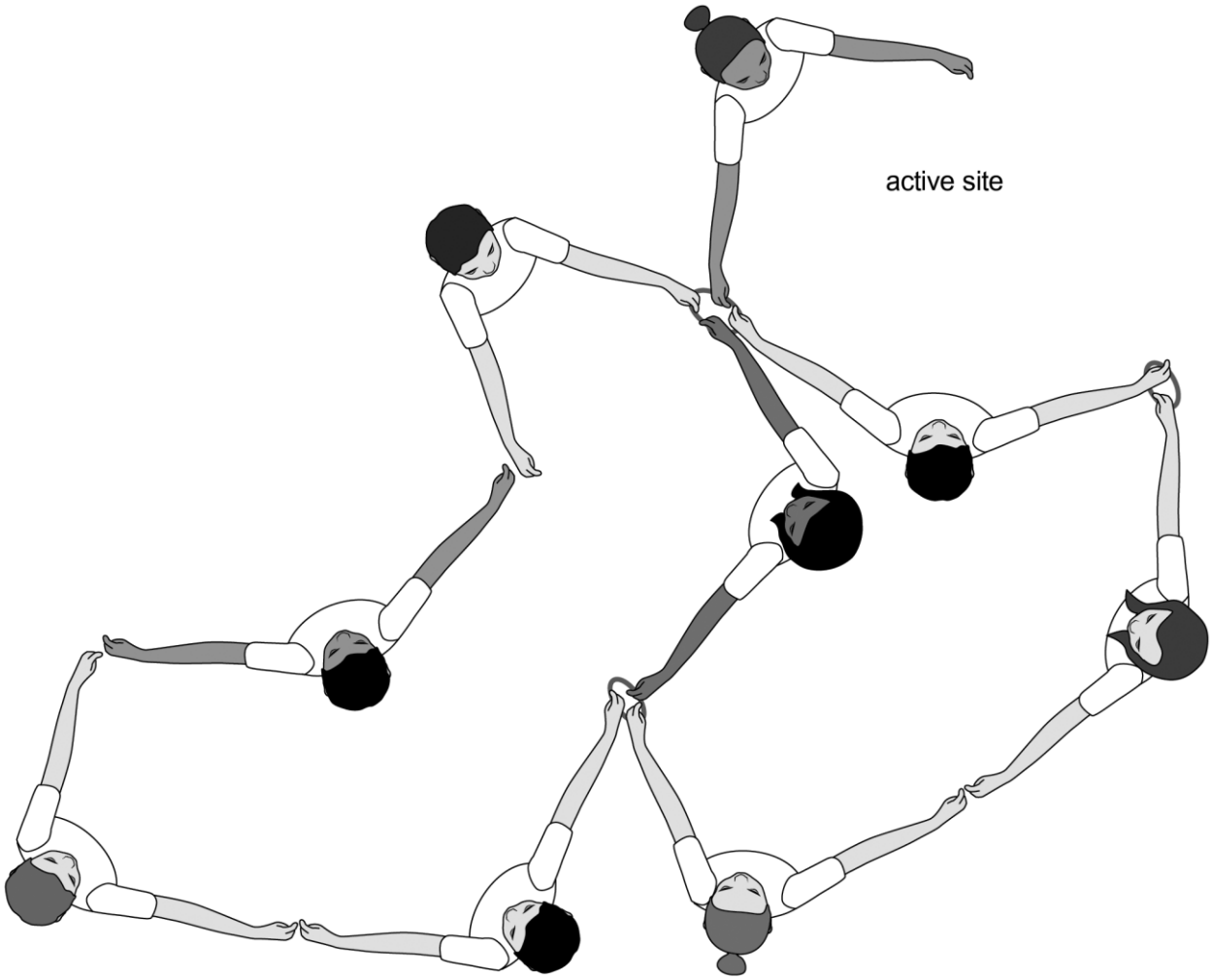
Worksheets and answers

	Worksheet	Answers
For use in *Briefing lesson*:		
A: ABCD choice card	**22**	**–**
B: Model 2	**23**	**–**
Teacher Instructions 1: Starter multiple choice questions	**36**	**–**
Teacher Instructions 2: Teacher demo and Model 1	**37–38**	**–**
Teacher Instructions 3: Model 2	**39**	**–**
For use in *Planning lesson*:		
C: Making enzyme–alginate beads	**24–25**	**42–43**
D: An immobilised enzyme reaction vessel	**26**	**–**
E: The method	**27–28**	**44–45**
For use in *Lab lesson*:		
Teacher Instructions 4: Practical questions	**40–41**	**–**
For use in *Debriefing lesson*:		
F: Free versus immobilised enzymes	**29**	**46**
G: Planning an investigation	**30**	**–**
H: Evaluating methods	**31–35**	**–**

Worksheet A: ABCD choice card

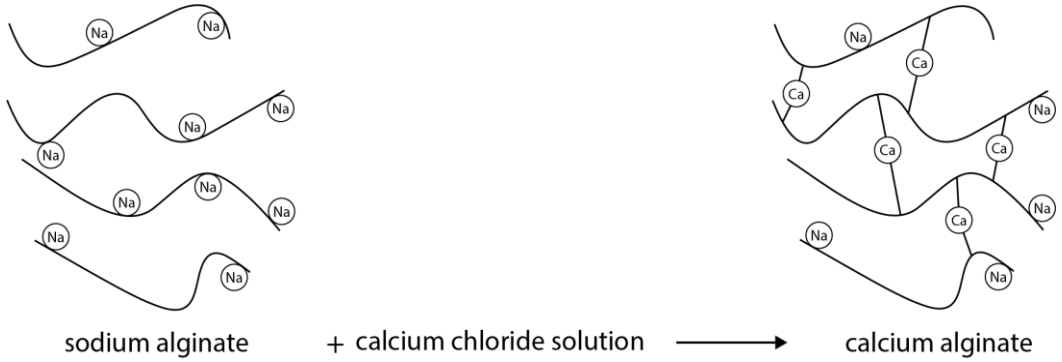


Worksheet B: Model 2

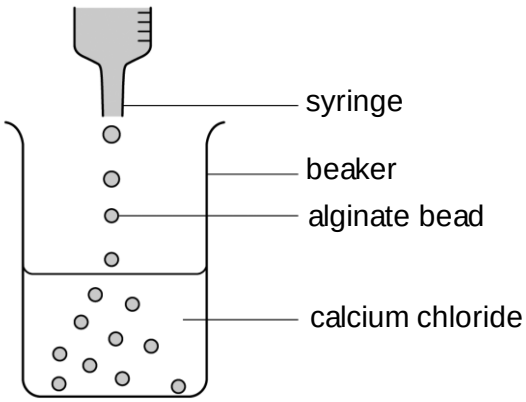


Worksheet C: Making enzyme–alginate beads

1. Use the diagram below and the teacher demonstration to help you to describe and explain what happens when sodium alginate is added to a solution of calcium chloride. Explain how this method can be used to produce enzymes encapsulated within beads.



2. The diagram below shows the procedure demonstrated by your teacher.

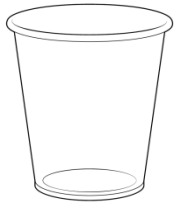
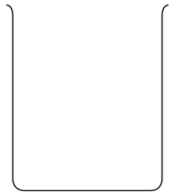
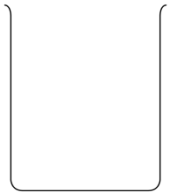
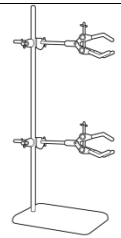
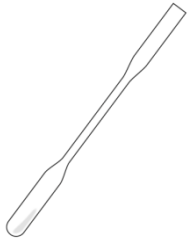
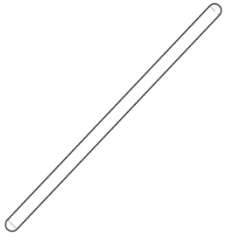
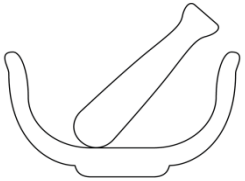
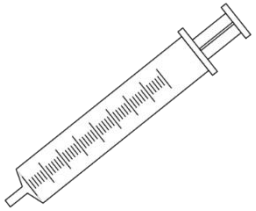
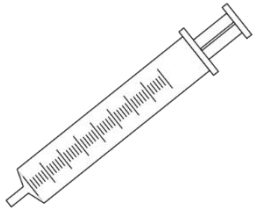
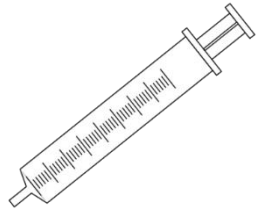
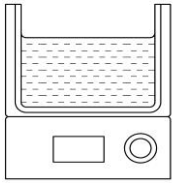
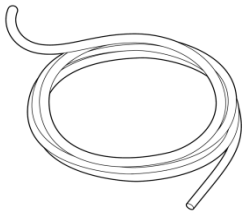
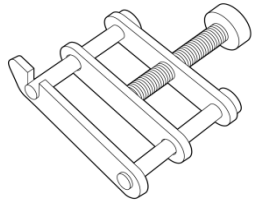
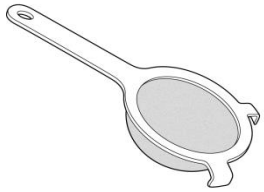
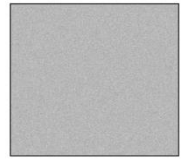
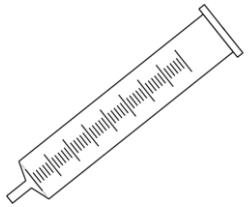
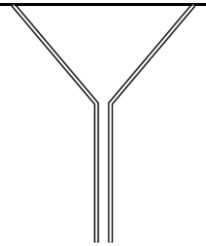
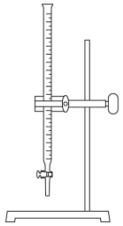
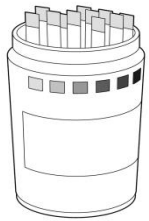
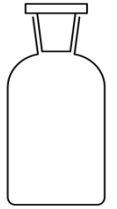


The enzyme lactase breaks down lactose in milk to produce lactose-free milk that is safe for consumption by people with lactose intolerance. Commercial arrangements use immobilised enzymes to create lactose-free milk in a continuous process.

Use the diagram of your teacher demonstration, above, and the list of apparatus on the next page to help you plan a method for demonstrating the continual production of lactose-free milk in a school laboratory using beads containing lactase enzyme. You will also be given the following solutions:

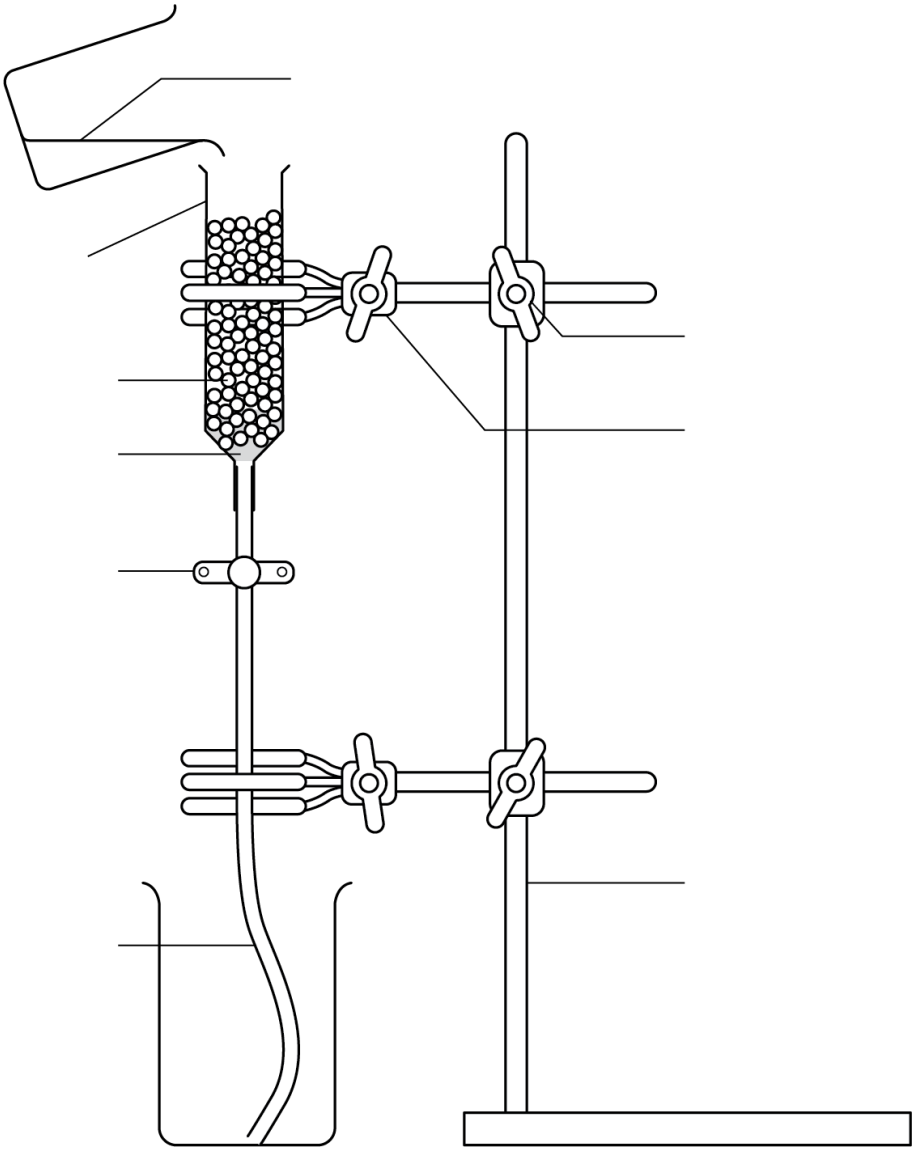
- 3% sodium alginate solution (5 cm^3)
- lactase solution (5 cm^3)
- 1.5% calcium chloride solution (100 cm^3)
- milk (50 cm^3)

Here is some typical laboratory apparatus available to you. Note that you will **not** need to use all items.

						
distilled water	plastic cup	250 cm^3 beaker	100 cm^3 beaker	test-tube	retort stand, clamp & boss	evaporating dish
						
spatula	glass rod	measuring cylinder	mortar & pestle	syringe (10 cm^3)	syringe (25 cm^3)	syringe (100 cm^3)
						
dropping pipette	water bath / hot plate	thermometer	rubber tubing	Hoffman clip	sieve	nylon gauze
						
syringe barrel (50 cm^3)	funnel	burette with tap	timer	glucose test strips	Benedict's solution	iodine solution

Worksheet D: Immobilised enzyme reaction vessel

The diagram shows the arrangement of equipment that you will construct and use in the practical lesson. Complete the missing labels. Reflect on your own design by answering the questions below. Write down your answers.



1. How is the arrangement of this apparatus **similar** to your design?

2. How is the arrangement of this apparatus **different** to your design?

3. How would arranging the apparatus in this way improve the yield of product, compared to your design?

4. Can you identify any other advantages and disadvantages of this arrangement when compared with your design?

Worksheet E: The method

Note that lactase will have already been mixed with sodium alginate to produce an enzyme–alginate mixture before the start of the practical lesson.

Step	Rationale
1. Fill a 10 cm^3 syringe with the enzyme–alginate mixture. Add 100 cm^3 of calcium chloride solution to a 250 cm^3 beaker.	
	This allows drops of enzyme–alginate mixture to make contact with the calcium chloride solution, so that they form a semi-solid bead.
3. Leave the beads for 10 minutes in the calcium chloride solution.	
	This removes any calcium chloride solution from the surface of the beads.
5. Remove the plunger from a clean 10 cm^3 syringe.	
	This prevents beads from blocking the outlet and enables the substrate to run through.
7. Clamp the syringe barrel to the top of a retort stand. Transfer the beads from the sieve to the barrel using a spatula and then use the plunger to gently 'squash' the contents.	
	This will enable the milk to pass through the barrel, due to gravity.

Step	Rationale
9. Firmly attach a piece of rubber tubing to the nozzle of the syringe. Secure loosely in place near the bottom of the retort stand, above a 100 cm^3 beaker.	
10. Place an adjustable Hoffman clip just under the nozzle of the syringe and fully tighten it to close the rubber tubing.	
11. Warm 50 cm^3 of milk in a 100 cm^3 beaker to 40°C using a hot plate.	
	This gives time for the enzyme to act on its substrate.
13. Loosen the Hoffman clip.	
	This tests for the presence of glucose, one of the products of lactose digestion.
15. Place a glucose test stick into a sample of **un**treated milk.	
16. Compare the colours of the test sticks with the manufacturer's colour chart.	

Worksheet F: Free versus immobilised enzymes

A student investigated the relationship between temperature and the rate of reaction by a digestive enzyme. He used two types of enzyme, free and immobilised. His results are shown below.

Temperature / °C	10	20	30	40	50	60	70	80
Rate of reaction of free enzyme / arbitrary units	100	100	100	100	96	90	62	22
Rate of reaction of immobilised enzyme / arbitrary units	100	100	100	100	100	98	92	70

1. Draw a graph of the data for both enzymes.

2. Describe the relationship between temperature and the activity of **free** enzyme.

3. Explain the difference in the activity of free and immobilised enzyme as the temperature increases.

4. The student conducted further investigations with the two enzymes and calculated the value of the Michaelis–Menten constant (K_m). He found that the value of K_m for the immobilised enzyme was greater than the value for free enzyme. Suggest an explanation for this finding.

Worksheet G: Planning an investigation

Your group's task is to plan an investigation that would allow you to accept or reject a given null hypothesis.

First, discuss if you think the null hypothesis would be accepted or rejected, and why.

Then, use the following prompts to write a clear plan, including a method that someone else could follow. Your finished method should be written on a piece of A3 paper, in the form of a poster, for other groups to evaluate later.

1. Identify the **independent variable** (the factor that you will change). How can you change this variable?

2. For the independent variable, state: the **range** of values to use, how many values you will use, and what **intervals** you will have between the values, and how you will measure these. Why did you make these decisions?

3. Identify the **dependent variable** (the factor that you will measure) and explain how you will measure it precisely, and how frequently, to increase the accuracy of collected data.

4. Describe some of the **sources of error** in measuring the dependent variable and how they could be minimised. Do you need to standardise the method? If so, how?

5. List any variables in your investigation that should be **controlled** to increase experimental validity, and explain how and why.

6. Describe a **control experiment** to remove the effect of the independent variable. Why is this important?

7. Decide whether to **repeat measurements**. If you do, decide how many repeats and explain what you would do with them.

8. Construct a **table** in which you will record your data. Remember that your table must:
    - include headings for the columns, including units
    - be fully ruled with no units in the body of the table
    - contain an appropriate number of columns and rows (think about what variable is being changed and what data is being collected; the independent variable should be in the leftmost column).

9. Assuming the null hypothesis is rejected, sketch a **graph** to predict the relationship between the independent and dependent variables. Make sure you can explain the relationship using your knowledge of enzyme activity.

10. Suggest how you could illustrate the **reliability** of your data on your graph.

11. Suggest what **statistical test** you might use to analyse data in order to accept or reject the null hypothesis. Ensure you explain the relevance of $P < 0.05$.

12. Identify hazards in your investigation and describe one **safety precaution** that you would incorporate to minimise risk.

Worksheet H: Evaluating methods

This checklist provides a model plan for investigating the following null hypothesis:

Read the plan written by your classmates and use this checklist to evaluate their work.

	Step in plan	Step completed? (Tick the correct box) Yes	No	Do you agree with their suggestions? Can you think of any improvements?
1	Identify the independent variable and how to vary it.			
2	For the independent variable, state the range of values to use, how many values to use, and what intervals to have between the values, and how to measure these.			
3	Identify the dependent variable and how to measure it precisely, and how frequently.			
4	Describe some of the sources of error in measuring the dependent variable and how they could be minimised.			
5	List the variables that should be controlled.			

Step in plan		Step completed? (Tick the correct box) Yes	No	Do you agree with their suggestions? Can you think of any improvements?
6	Describe a control experiment to remove the effect of the independent variable.			
7	Decide if it is necessary to repeat measurements, how many repeats and what to do with them.			
8	Construct a table in which: (tick each one included) (i) the independent variable is in the left column and the dependent variable is in the right column (ii) the correct units only present in the table headings.	(i) (ii)	(i) (ii)	
9	Sketch a graph in which: (tick each one included) (i) the independent variable is on the *x*-axis and the dependent variable is on the *y*-axis (ii) units are labelled on axes (iii) a line shows a relationship between the variables.	(i) (ii) (iii)	(i) (ii) (iii)	
10	State how to indicate reliability of the data on the graph.			
11	Identify if a statistical test could be used to determine whether any differences are significant. Explain the relevance of $P < 0.05$.			
12	Describe one safety precaution to minimise risk.			

Evaluation List three aspects of the other groups' work that you would now like to incorporate into your own experimental design.

Step	Hypothesis: There is no significant difference between the concentration of glucose (mmol dm^{-3}) in lactase-treated milk produced using …		
	… spherical enzyme–alginate beads with different diameters	… reaction columns with different rates of milk flow	… milk of different pH values
1	The independent variable is the diameter of the bead. This can be varied by using syringes with attachments with different bore diameters; or adjusting the force applied to the plunger to form drops more quickly/slowly.	The independent variable is the rate of flow of milk through the reaction vessel. This can be modified by adjusting the tightness of the Hoffman clip on the rubber tubing under the outlet of the syringe barrel. The tighter it is, the slower the milk will flow.	The independent variable is the pH of the milk. This can be modified by adjusting the acidity or alkalinity by adding equal volumes of appropriate buffer solutions covering a range of pH values.
2	Beads of at least three different diameters are prepared, e.g. 4 mm, 8 mm and 12 mm. These are large enough to be rinsed on the sieve, but small enough to fit inside the syringe barrel. These are measured with a ruler with millimetre intervals, or Vernier callipers.	Milk is allowed to flow through the vessel at three different rates (at least). The differences in rate will be quantified by measuring the number of drops of milk (e.g. 5, 10 and 15) that flow through the vessel in 30 seconds. This is measured by counting and by using a stop clock.	Milk of at least three different pH values are prepared, e.g. 5, 7 and 9. (Dissolved substances in milk are likely to precipitate at values of pH lower than 5 or higher than 9.) The pH is measured using an electronic pH probe.
3	The dependent variable is the concentration of glucose in a specific volume of milk (e.g. 50 cm^3) that has passed through the reaction vessel. This will be measured either semi-quantitatively (comparing the colour of a glucose test strip with the manufacturer's colour chart) or quantitatively using a glucose biosensor.		
4	If the concentration of glucose is determined using glucose test strips, the possibility of random errors can be minimised by ensuring that the same person judges the colour comparison each time. However, this data is disposed to systematic errors based on the judgement being subjective (with the naked eye). If the concentration of glucose is measured using a biosensor, the possibility of random errors can be minimised by taking three separate readings of glucose concentration and then calculating a mean. To minimise the possibility of systematic error, the biosensor should be calibrated using solutions of known glucose concentration before the collected milk is analysed.		
5	The following factors should be standardised in this investigation, to improve the validity of the collected data: pH of milk; volume of milk; temperature of milk; concentration of enzyme in the enzyme–alginate mixture; total volume of the beads; temperature of reaction; time for incubation of the milk with the beads (flow rate) and the time that the glucose test strip is immersed in the product.	The following factors should be standardised in this investigation, to improve the validity of the collected data: pH of milk; volume of milk; temperature of milk; concentration of enzyme in the enzyme–alginate mixture; total volume of the beads; diameter of the beads; number of beads; and temperature of the reaction and the time that the glucose test strip is immersed in the product.	The following factors should be standardised in this investigation, to improve the validity of the collected data: volume of milk; temperature of milk; concentration of enzyme in the enzyme–alginate mixture; total volume of the beads; diameter of beads; number of beads; temperature of reaction; time for incubation of the milk with the beads (flow rate) and the time that the glucose test strip is immersed in the product.

Step	Hypothesis: There is no significant difference between the concentration of glucose (mmol dm^{-3}) in lactase-treated milk produced using …		
	… spherical enzyme–alginate beads with different diameters	… reaction columns with different rates of milk flow	… milk of different pH values
6	An appropriate control would be to use a reaction vessel containing a mixture of beads of all diameters (of equal mass) used in the investigation. This removes the effect of the independent variable. The expected outcome would be a concentration of glucose that is approximately an average of that obtained in all three reaction vessels.	An appropriate control would be to incubate three identical volumes of milk in three reaction vessels for time periods equal to the time it takes for the milk to flow through each of the vessels used in the investigation. This removes the effect of the independent variable as the passage of milk through the syringe is avoided.	An appropriate control would be to use the same volume of distilled water instead of pH buffer in an identical reaction column. This removes the effect of the independent variable and accounts for the change in concentration of the milk caused by the addition of the pH buffer.
7	It is good experimental practice to repeat the investigation, at least two more times, in order to obtain triplicate readings that should highlight anomalous data. In addition, a mean can be calculated from these three data values, which enhances the reliability of the results and conclusions drawn from them.		
8	Table drawn using a ruler. The independent variable, bead diameter, is in the left-hand column, and the concentration of glucose is in the right-hand column. Units (mm and mmol dm^{-3} respectively) are provided in the headings only.	Table drawn using a ruler. The independent variable, milk flow rate, is in the left-hand column, and the concentration of glucose is in the right-hand column. Units (drops per second and mmol dm^{-3} respectively) are provided in the headings only.	Table drawn using a ruler. The independent variable, milk pH, is in the left-hand column, and the concentration of glucose is in the right-hand column. Units (N/A and mmol dm^{-3} respectively) are provided in the headings only.
9	Assuming that the null hypothesis is rejected, a graph is sketched that has bead diameter / mm on the *x*-axis and concentration of glucose / mmol dm^{-3} on the *y*-axis. The sketch shows that as bead diameter increases, the concentration of glucose decreases (a negative correlation). This is because the surface area of smaller beads would be collectively greater.	Assuming that the null hypothesis is rejected, a graph is drawn that has milk flow rate / drops per second on the *x*-axis and concentration of glucose / mmol dm^{-3} on the *y*-axis. The sketch shows that as milk flow rate increases, the concentration of glucose decreases (a negative correlation).	Assuming that the null hypothesis is rejected, a graph is drawn that has substrate pH (no units) on the *x*-axis and concentration of glucose / mmol dm^{-3} on the *y*-axis. The sketch shows that as pH increases from 5 to 9, the concentration of glucose increases, reaches a peak at around 6, and then falls.
10	Error bars could be shown on each plotted data point in order to show the spread of data around the mean. More reliable values will have smaller error bars. If any error bars for different data points overlap, then the significance of any difference between these data points could be questionable.		

Step	Hypothesis: There is no significant difference between the concentration of glucose (mmol dm^{-3}) in lactase-treated milk produced using …		
	… spherical enzyme–alginate beads with different diameters	… reaction columns with different rates of milk flow	… milk of different pH values
11	A student's *t*-test should be used to determine whether there is a significant difference between the mean values of glucose concentration produced by any pair of bead diameters. If the calculated value falls below the critical value for $P < 0.05$, then the difference between them is statistically significant. This means that the probability that the observed difference arose by chance is less than 0.05.	A student's *t*-test should be used to determine whether there is a significant difference between the mean values of glucose concentration produced by any pair of milk flow rates. If the calculated value falls below the critical value for $P < 0.05$, then the difference between them is statistically significant. This means that the probability that the observed difference arose by chance is less than 0.05.	A student's *t*-test should be used to determine whether there is a significant difference between the mean values of glucose concentration produced by any pair of substrate pH values. If the calculated value falls below the critical value for $P < 0.05$, then the difference between them is statistically significant. This means that the probability that the observed difference arose by chance is less than 0.05.
12	A safety precaution is to wear eye protection and gloves as lactase powder, sodium alginate and calcium chloride are irritants and may be allergens.		

Teacher Instructions 1: Multiple choice questions

Here are three example multiple choice questions about lactase for use in the *Briefing Lesson* starter. The correct answers are underlined. Consider displaying them at the front of the class, large enough for everyone to see. Learners will need to discuss their thoughts with a partner for 30 seconds before submitting their answer.

QUESTION 1

Which statements about lactase are true?
(I) It is a globular protein.
(II) It breaks down a disaccharide into two monosaccharides.
(III) It is absent in people who are lactose tolerant.
(IV) It acts on its substrate inside cells.

a. I only
b. II only
c. <u>I and II only</u>
d. I, II, III and IV.

QUESTION 2

The Michaelis–Menten constant (K_m) of lactase:

a. <u>Is determined by identifying the concentration of substrate that gives a rate of reaction exactly half of the maximum</u>
b. Is proportional to the affinity for lactose
c. Has units of $dmmol^{-1}$
d. Is lower at a pH of 1.

QUESTION 3

Lactase, which has an optimum pH of 6 and optimum temperature of 37°C, will denature:

a. When its active site becomes more complementary to the shape of the substrate
b. <u>When hydrogen bonds in the tertiary structure are broken</u>
c. More rapidly at 50°C than at 60°C
d. Irreversibly, when placed into a solution of high alkalinity.

Teacher Instructions 2: Teacher demo and Model 1

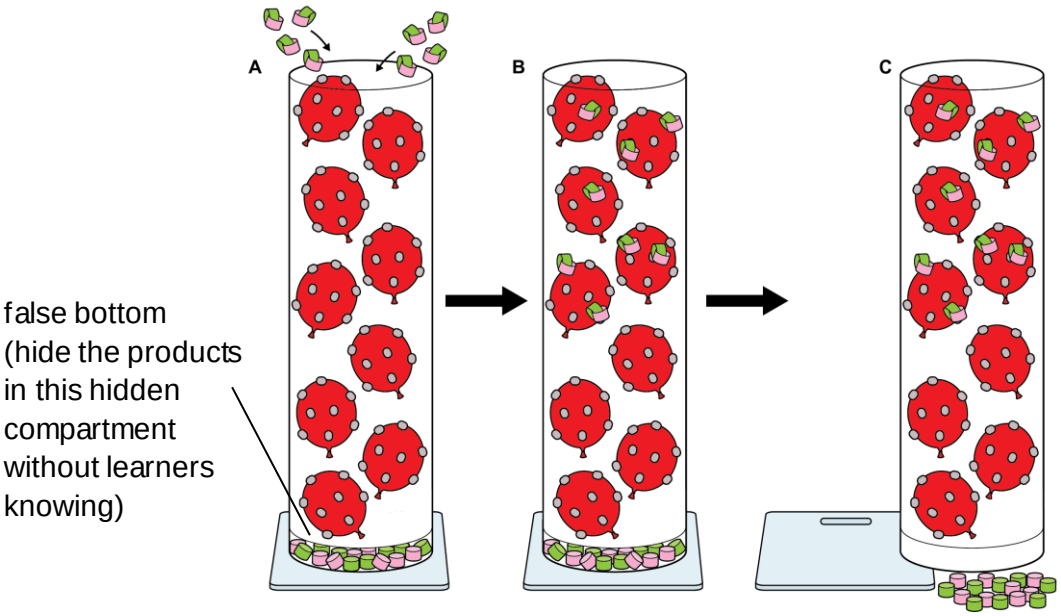
Teacher demo – overview

Before the start of the lesson, partially fill a bucket or similar container with several balls of scrunched-up sticky tape and paper representations of glucose and galactose (see *Making the paper molecules* below). Mix the contents thoroughly. Note that the balls of sticky tape should still have some sticky surfaces so that they will stick to the paper.

In front of the learners, add lactose molecules (see *Making the paper molecules*) to the bucket and mix the contents thoroughly. Turn the bucket upside down so that the contents are on a flat surface, clearly visible to the whole class. Make sure they know what each component is. The learners should see that the substrate (lactose), products (glucose and galactose) and enzyme (lactase) are all mixed up together. Ask them what this shows: elicit the idea that it is difficult to obtain a 'pure product', and that the enzyme is not recoverable and hence could be wasted. Explain that this model demonstrates that at the end of an enzyme-catalysed reaction, it would be useful to have a step to separate the enzyme from the product.

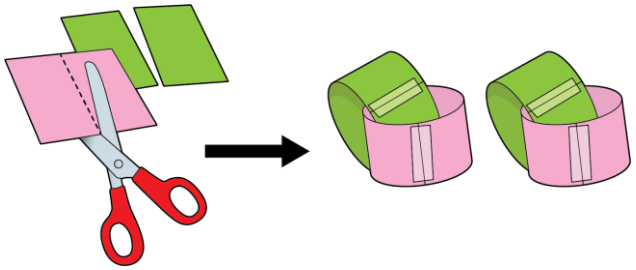
Model 1 – overview

Give pairs of learners a balloon and some sticky tape. Ask them to **half** inflate the balloon and cover it with balls of scrunched-up sticky tape; explain that there should be gaps between the balls of tape so that you can still see some of the balloon. Demonstrate using 2–3 balls of tape and one balloon if required. Ask them to add their completed balloon to a whole-class 'reaction vessel' **(A)** (see *Making the reaction vessel* below). When the whole class has done this, ask learners to place their lactose paper chains on top (some learners might need to re-make these if they tore them earlier in the lesson) and give the vessel a very gentle shake to allow the chains to fall inside and stick to the balloons **(B)**. Carefully remove the base of the vessel and empty the contents of the hidden compartment (glucose and galactose) onto a flat surface that the whole class can see **(C)**. Ask them what has happened and what the model demonstrates: elicit that the enzymes have been held in place, making it possible to separate them from the product so they can be used again and the product can be collected easily. **Note:** It is important that you remember to place the glucose and galactose molecules inside the hidden compartment at the base of the vessel **before** the lesson.



Making the paper molecules

To create a model of the disaccharide lactose, cut two sticky notes in half and create two loops that interlink.

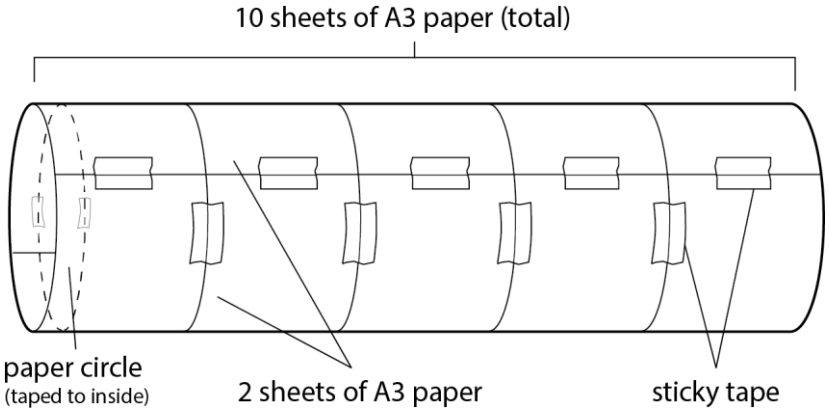


For the teacher demo, make as many as seems appropriate for your container. For Model 1, you will need one set for each pair of learners.

To create the products galactose and galactose, just use two halves of a sticky note (like the sticky notes used to make the lactose). For the teacher demo, use as many as required to appropriately match the number of lactose used. For Model 1, provide two halves of a sticky note for each pair.

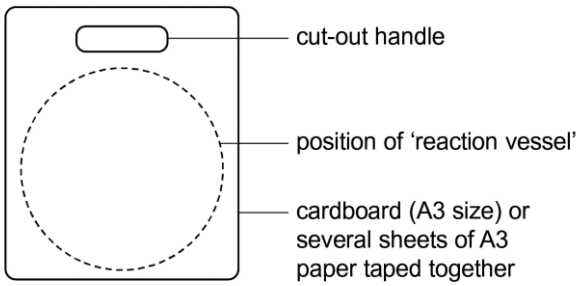
Making the reaction vessel

To make the reaction vessel for Model 1, fix together 10 sheets of A3 paper to make a long tube with a diameter capable of holding 3–4 half-inflated balloons. The total number of balloons inside the tube should be around 15–20 (depends somewhat on class size). Cut out a paper circle with a diameter that matches the vessel and stick this inside the vessel near the bottom, to create a 'false' bottom to the vessel. When the vessel is placed upright on top of the pull-out base, this false bottom creates a small hidden compartment under the vessel. Hide the paper glucose and galactose molecules in this hidden compartment so that when the pull-out base is removed and the vessel lifted, the 'products' of the reaction are released from the vessel.



Making the pull-out base for the reaction vessel

The base of the reaction vessel should be constructed from a sheet of card or a number of sheets of A3 paper fixed together with sticky tape to make a rigid structure. It should completely cover the opening in the bottom of the reaction vessel. A 'handle' may be added such that it can be easily removed by sliding out from under the bottom of the reaction vessel during the demonstration, i.e.



Teacher Instructions 3: Model 2

This activity might not be suitable in some parts of the world, so use at your discretion. You might also need to move learners to a more open area outside the classroom.

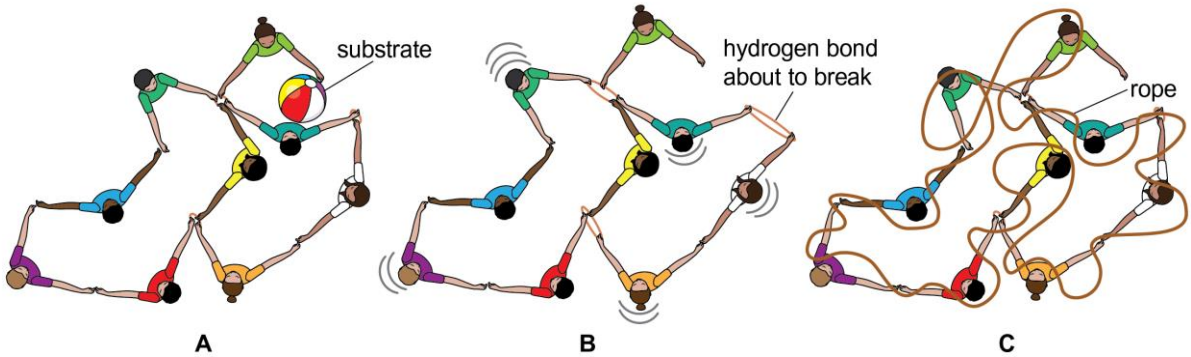
Overview
Learners model individual amino acids arranged into the tertiary structure of a digestive enzyme (see image below and Worksheet B).

In the model:

- Peptide bonds between adjacent amino acids in a particular sequence (primary structure) are represented by learners holding hands with an adjacent classmate (the linkage of hands represent the bonds)
- Hydrogen bonds between non-adjacent amino acids (secondary structure) are represented by elastic bands, looped around the fingers of non-adjacent residues (learners) in the chain
- You need to prepare an 'active site' by creating a region of the chain capable of collectively holding a substrate
- The substrate should be modelled by something that can be held in the 'active site,' ideally by more than one learner, such as a cushion or inflatable beach ball.

The model
The diagram below shows a top-down view of nine learners arranged in the tertiary structure of an enzyme **(A)**. Once learners are set up, explain that you will call out 'heat' and they should wiggle their arms up and down whilst trying to stay holding hands and keeping the elastic bands in place. Explain that each time you say 'heat' again, they need to wiggle their arms more vigorously. Repeat this until eventually, they should be wiggling so much that the elastic bands will ping off and the substrate falls out of place **(B)**. It is important that the learners try to retain the grip of each other's hands, as peptide bonds rarely break at temperatures used in the laboratory.

Ask how this relates to what happens to the enzyme as you increase the temperature of an enzyme-catalysed reaction: elicit that the heat increases the kinetic energy of the molecule; as the energy increases, the hydrogen bonds within the enzyme become less and less stable. Beyond a certain temperature, the bonds break, distorting the shape of the active site so that the substrate can no longer bind, denaturing the enzyme. Ask them what they think would happen if they repeated this but they were tied together in some way, for example with rope (do **not** actually do this). They should suggest that they would not be able to move or wiggle their arms as much and thus the bonds would become more stable under conditions of increasing temperature **(C)**.



Teacher Instructions 4: Practical questions

Below are a number of questions you could ask the learners in quiet periods during the practical lesson in order to elicit discussions. These could be displayed on the board and learners given a whiteboard marker to contribute when they have thoughts during, or after, the activity. The answers should be discussed at the end of the lesson.

1. Manufacturers use lactase to produce lactose-free milk.

    a. How could a quantitative measure of glucose concentration be obtained? **(Low demand)**

    b. How do manufacturers of lactose-free milk use measurements of glucose concentration to increase their profit? **(Intermediate demand)**

    Example answers

    a. A glucose biosensor

    b. Manufacturers of lactose-free milk could use this information to help them determine the rate of the hydrolysis reaction. They could use this data to modify variables such as the concentration of lactase used, the temperature of the milk, and so on, in order to improve the yield of product.

2. Galactose has a similar tertiary structure to lactose and is an inhibitor of lactase.

    a. What type of inhibitor is galactose, and why? What impact will this have on the rate of hydrolysis of lactose? **(Low demand)**

    b. The rate at which the untreated milk enters the vessel and the treated milk leaves the vessel is called the 'flow rate'. How might the inhibition of galactose influence the flow rate of the milk that a manufacturer would choose? (**Intermediate demand)**

    Example answers

    a. As galactose has a similar shape to lactose, it will compete with lactose for access to the active site of the enzyme and act as a competitive inhibitor, limiting the number of enzyme–substrate complexes that are formed. The rate of hydrolysis of lactose will hence decrease.

    b. If the flow rate is too slow, then the treated milk will be slower to leave the vessel containing the beads and there will be a higher concentration of product galactose in the reaction vessel. This will inhibit the lactase, reducing the efficiency of further lactose digestion. If the flow rate is too fast, then the probability of lactose and lactase successfully colliding in the column is reduced which would limit the rate of this enzyme-catalysed reaction.

3. Suggest other possible uses, both industrial and medical, of immobilised enzymes or other molecules. (**High demand; this may require learners to access their textbooks and/or the internet to undertake research)**

   Example answers
   - Immobilised urease in an artificial kidney.
   - Immobilised dehydrogenases to reduce water pollution.
   - Immobilised glucose isomerase to increase profitability in sugar manufacture.

   In addition, immobilised monoclonal antibodies could be used to extract specific proteins from cell preparations, which would have a range of potential applications in medicine and biotechnology.

4. An alternative to immobilising lactase is to immobilise *E.coli* cells that are expressing lactase. Evaluate the advantages and disadvantages of this method. (**High demand)**

   Example answers

   One advantage of immobilising enzymes is that they do not need a supply of nutrients, which reduces maintenance costs. An advantage of immobilising cells is that, unlike enzymes, they are self-replenishing and over time do not need to be replaced if damaged. A disadvantage of immobilising cells is that they could contaminate and disrupt the product (either themselves or by releasing other substances) and make it unsuitable for human consumption. They are also more susceptible to changes in temperature and pH.

Worksheet C: Answers

1. When the solution of sodium alginate is added drop by drop to the calcium chloride solution, spherical beads of calcium alginate form. Calcium ions cross link the alginate strands. The effect of this is that the reacting solutions become semi-solid in nature.

   Beads containing an enzyme could be made by mixing solutions of enzyme and sodium alginate together first. This mixture can be drawn up into a syringe and gently dropped into a beaker containing a solution of calcium chloride. This would result in the production of small spheres containing enzyme molecules mixed with the insoluble calcium alginate, which means that the enzyme is encapsulated throughout the bead. If the enzyme solution was mixed with the calcium chloride solution, much of the enzyme would be wasted as the majority of the calcium chloride is discarded. In addition, the beads would not be as effective in encapsulating the enzyme. Instead of the enzyme being spread throughout the bead, it would likely be found mainly on the surface layer of the bead.

2. Learners' own suggestions and discussions should be considered. Good general laboratory practice should be highlighted. Ensure that the following considerations are discussed:
   - The advantages of using a **syringe barrel** as a reaction vessel include the fact that it has a diameter wide enough to hold a substantial number of beads, and that a funnel is not required to pour in the milk from its reaction vessel. Although the burette does have the advantage of a built-in tap to regulate the rate of flow of the milk, burettes are usually made of glass and have a substantial vertical height, which present added safety hazards. The narrow bore of a burette can also limit the number and size of beads that can be used.
   - A **10 cm^3 syringe** should be used to make the beads and minimise wastage of reagents, as this holds a volume sufficient to produce 30–40 beads. The **50 cm^3 syringe barrel** should be used as the reaction vessel, which provides enough volume to hold both the beads and the milk substrate.
   - The **rubber tubing** and a **Hoffman clip** allow the controlled transfer of the milk from the bottom of the reaction vessel and its collection in the beaker for analysis.
   - The **100 cm^3 beaker** is preferred for collecting the milk after it has run through the reaction column, rather than a **250 cm^3 beaker** (too large), a **test tube** (too small) or a **plastic cup** (cannot provide an indication of the volume of milk collected).
   - The **retort stand, boss and clamp** should be used to hold the reaction column above the beaker, which allows the milk to flow through the apparatus due to gravity.
   - While a **spatula** should be used to transfer the beads into the reaction vessel, a blunt-ended **glass rod** or the syringe **plunger** should be used to compress them. Using a spatula to do this may damage the beads.
   - The **water bath/hot plate** should be used to warm the milk to a value around the optimum temperature of the enzyme lactase. In practice, the temperature should be slightly more than this value, in light of the fact that the substrate will cool slightly when applied to the beads at room temperature. A **thermometer** should be used to assess when the temperature of the milk reaches 40°C.

- The advantage of the **glucose test strips** as a means to semi-quantitatively measure glucose concentration in the collected product is that glucose is specifically detected. The **Benedict's reagent** would detect lactose (another reducing sugar), so providing a false-positive result. The **iodine solution** is used to detect starch, which is not a reactant or product in this task.
- The **nylon gauze** should be positioned at the bottom of the empty syringe barrel before the beads are inserted. This prevents the blockage of the nozzle and allows the milk to flow out.
- The **sieve** provides a quick means by which to separate the beads from the calcium chloride solution (rather than the slower method using **filter funnel and filter paper**), which also facilitates the washing of the beads over a sink with **distilled water** from a **wash bottle**.
- The **timer** should be used to ensure that at least 5 minutes elapses between applying the milk to the beads and opening the Hoffman clip, in order to allow for the hydrolysis of lactose to occur.

Worksheet D shows the apparatus arrangement that will be used in the practical lesson.

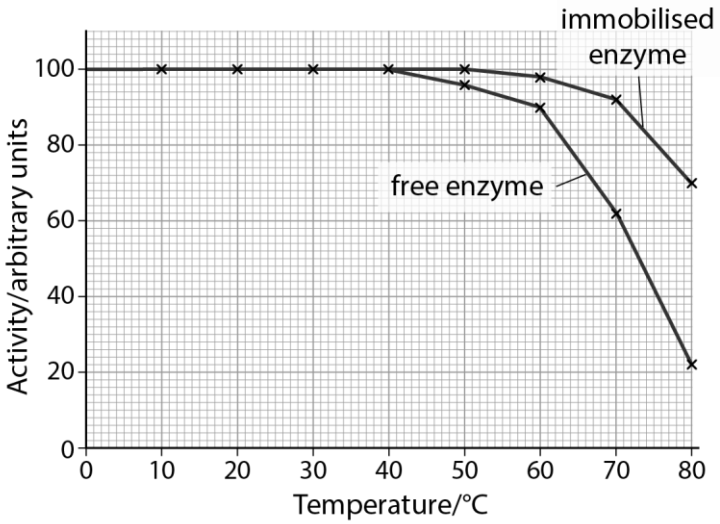
Worksheet E: Answers

Step	Rationale
1. Fill a 10 cm^3 syringe with the enzyme–alginate mixture. Add 100 cm^3 of calcium chloride solution to a 250 cm^3 beaker.	The use of a syringe makes it possible to control the formation of beads as the solution is dropped into the calcium chloride. This volume of enzyme–alginate mixture will produce around 30–40 beads.
2. Force down the plunger of the syringe, adding the mixture drop by drop into the calcium chloride solution.	This allows drops of enzyme–alginate mixture to make contact with the calcium chloride solution, so that they form a semi-solid bead.
3. Leave the beads for 10 minutes in the calcium chloride solution.	This allows the beads to fully harden.
4. Rinse the beads using distilled water. Do this through a sieve over a sink.	This removes any calcium chloride solution from the surface of the beads.
5. Remove the plunger from a clean 10 cm^3 syringe.	This empty syringe barrel will act as the enzyme column. This creates a vessel that is big enough to tightly pack the beads in, whilst still containing a reasonable volume of milk.
6. Place a piece of nylon gauze in the bottom of the syringe barrel so that it sits just above the outlet of the syringe.	This prevents beads from blocking the outlet and enables the substrate to run through.
7. Clamp the syringe barrel to the top of a retort stand. Transfer the beads from the sieve to the barrel using a spatula and then use the plunger to gently 'squash' the contents.	This reduces the space between the beads to maximise the number of beads present in the barrel but not so tightly that the milk cannot flow past and between the beads.
8. Clamp the barrel so that it sits at the very top of a retort stand.	This will enable the milk to pass through the barrel, due to gravity.
9. Firmly attach a piece of rubber tubing to the nozzle of the syringe. Secure loosely in place near the bottom of the retort stand, above a 100 cm^3 beaker.	This will allow the milk to run out of the barrel after passing through the beads.
10. Place an adjustable Hoffman clip just under the nozzle of the syringe and fully tighten it to close the rubber tubing.	This will make it possible to keep the substrate in the syringe barrel for a period of time to allow for substrate hydrolysis, and to prevent the milk from flowing through the column too quickly.
11. Warm 50 cm^3 of milk in a 100 cm^3 beaker to 40°C using a hot plate.	This is to ensure that the lactase operates at a temperature close to its optimum.
12. Pour milk slowly into the reaction vessel so that it covers the beads, and leave to incubate for 5 minutes.	This gives times for the enzyme to act on its substrate.

Step	Rationale
13. Loosen the Hoffman clip.	This allows the milk to leave the syringe barrel and drop slowly through the rubber tubing into the beaker at the bottom of the retort stand.
14. Place a glucose test stick into the collected milk.	This tests for the presence of glucose, one of the products of lactose digestion.
15. Place a glucose test stick into a sample of **un**treated milk.	This confirms that the presence of any glucose in the other sample is due to the activity of lactase and not because glucose was present in the original substrate, or entered the product from another source.
16. Compare the colours of the test sticks with the manufacturer's colour chart.	This provides a semi-quantitative measure of the concentration of glucose in the collected milk.

Worksheet F: Answers

1. The graph might look something like that shown below.



- The graph should be drawn with a sharp pencil and any straight lines are drawn with a ruler.
- The independent variable (temperature) should be on the *x*-axis and the dependent variable (enzyme activity) on the *y*-axis, with units as appropriate.
- The points should be joined with straight lines or a smooth line of best fit that goes through the majority of the points.
- The two lines should be labelled or a key provided to illustrate which is the data for the free lactase and which is the data for the immobilised lactase.

2. The activity of free lactase remains at 100 arbitrary units up to 40°C but then begins to fall gradually as the temperature increases to 60°C. Then the rate of decrease increases between 60°C and 80°C until it reaches 22 arbitrary units.

3. The activity of both enzymes decreases at temperatures of 60°C or more because the kinetic energy of the molecules has increased to such a point that hydrogen bonding in the tertiary structure of the enzyme is disrupted and there is a change in the tertiary structure and therefore shape of the active site. However, the rate of reaction of the immobilised enzyme is less affected by high temperatures than the free enzyme, suggesting that it is more resistant to higher temperatures than the free enzyme. This is because the material that was used to immobilise the enzyme acts to stabilise its tertiary structure. This means that the enzyme is able to bind to its substrate at higher temperatures compared to when it is free in solution.

4. The value of K_m is a measure of the affinity of an enzyme for its substrate. The higher value of K_m for the immobilised enzyme indicates that it has a lower affinity for the substrate. This is because the material that was used to immobilise the enzyme may prevent the substrate from binding to the active site of the enzyme.

Cambridge Assessment International Education
The Triangle Building, Shaftesbury Road, Cambridge, CB2 8EA, United Kingdom
t: +44 1223 553554 f: +44 1223 553558
e: info@cambridgeinternational.org www.cambridgeinternational.org