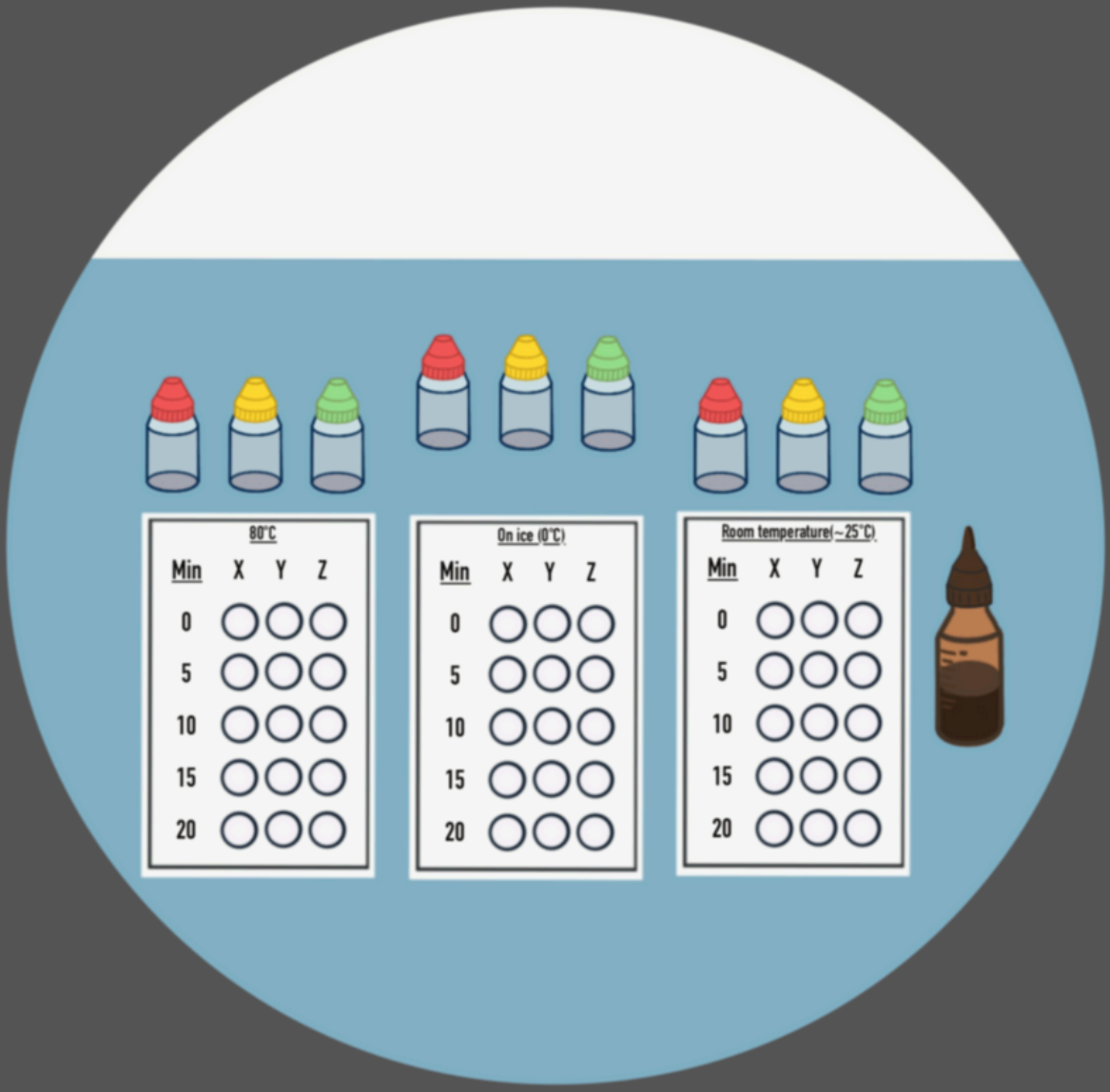
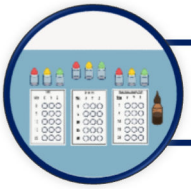
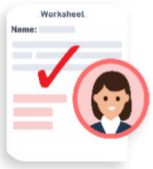


Microscale Amylase Investigation



Microscale Amylase Investigation

Table of Content



Notes for teachers

- Scan the QR code to get the electronic files.
- Teachers are strongly encouraged to adapt and modify these resources as necessary.

Innovations in Biology Investigations

Microscale Amylase Investigation

Overview

- The *Microscale Amylase Investigation* is a decision-making task in which students use data to determine the type of amylase that should be used for dishwashing and washing clothes.
- Students are given the opportunity to design and carry out an experiment in which they work with multivariate data to discern trends and patterns.
- Students assess their data sets to identify any anomalous data.

Teaching Plan & Key Features

Prerequisite knowledge (scientific ideas)

- The action of amylase on starch

Prerequisite manipulative skills

- Using an autopipette to transfer a small volume of solution

Lesson	Lesson sequence	Duration (mins)	Resources
Stage ❶ Preparing for the investigation • The investigation is set in a decision-making context (**Decision-making Task**). • It is situated in an authentic context related to the daily-life application of enzymes (**Contextualisation**).			
1	• The teacher discusses the investigation context with students. • The teacher distributes *Worksheet 1*.	40	*Worksheet 1*
Stage ❷ Designing the investigation • Students use a template to design their own experimental set-ups (*Investigation Planning Template*). • Students have the chance to evaluate their own and their peers' experimental set-ups (*Self & Peer Evaluation*).			
2	• The teacher provides feedback on students' experimental designs in *Worksheet 1*.	40	Student Samples 1
3	• The teacher discusses with the students some questions related to the experimental design. • The teacher provides students with laboratory manual for preparation at home.	40	Teacher Notes 1
Stage ❸ Carrying out the investigation • Students use microscale instrumentation that reduces the time of the experiments (**Microscale Instrumentation**). • Students collect more complex data sets by setting up replicates (**Complex Data Set**). • Students collect data using a template (*Data Collection Sheet*).			
4	• The teacher asks questions to help students connect their lab experience and related ideas/scientific inquiry skills. • Students carry out the investigation.	40	Laboratory manual
Stage ❹ Explaining and evaluating data • Students assess the quality of the data collected, including the presence of anomalous data. • Students use the data to guide their decision as to which type of amylase to use for specific applications.			
Before Lesson 5	• Students complete data reporting and analysis at home. • The teacher collects and marks student responses.		Teacher Notes 2
5	• The teacher provides feedback on students' performance related to data reporting and analysis.	40	Teacher Notes 2

Important Notes

- Students are *not* required to explain why the three types of enzymes show different temperature profiles. Rather, they are expected to use the data to determine the differential effect of temperature on the three types of amylases.
- Students should avoid direct skin contact with enzyme solutions.

Instructional Materials

Stage 1 Preparing for the investigation

Student Worksheet 1

Notes for teachers

- Teachers can distribute *Worksheet 1* and instruct students to design the investigation.
- Student work can be collected.
- Alternatively, this task can also be done as a take-home assignment.

Task 1

- Read the following information and source materials in the data file.
- Answer the questions that follow.

Scenario

Amylase is an enzyme that catalyses the breakdown of starch into maltose. It is used in many industrial applications such as the production of detergents.

Andrew found three brands of amylase (*Amylase X*, *Amylase Y*, and *Amylase Z*) in the laboratory. His biology teacher asked him for advice on which brands of amylase can be used and which brand is the most efficient (i.e., that with the highest enzyme activity) for the following purposes:

	Description
Dishwashing	• Washing at 70–80°C in the dishwasher
Washing clothes	• Washing at 25–30°C in the washing machine

His teacher also asked him to check whether the three brands of amylase remain active when stored on ice.

To achieve the aim, Andrew would like to investigate the effect of temperature on the enzymatic activities of the three types of amylase. He found the following materials and apparatuses in the laboratory:

Amylase *X* solution	Ice bath	Glucose test strips
Amylase *Y* solution	Water bath (80°C)	Glucose solution
Amylase *Z* solution	Boiling water bath (>100°C)	DCPIP solution
Distilled water	Timer	Starch solution
Spotting plates	Test tubes	Iodine solution
Beakers	Thermometers	Potato

Hint: It is not necessary to use all the materials listed.

Some materials not relevant to the investigation are given such that students need to decide which materials are suitable.

You will use your biological knowledge of enzymes and how to design valid and reliable experiments to complete this investigation.

(a) Complete the following investigation planning template:

Independent variable(s) (IV[s]) (What is/are the IV[s]? How to change and manipulate the IV[s]?)	**Dependent variable(s) (DV[s])** (What is/are the DV[s]? What parameter to measure? How to measure the DV[s]?)	**Control variables** (Anything else that likely affects the DV[s]? Why are these variables important to control?)
Controls (Do you need a control? Why?)	**Precautionary steps** (Steps to be taken to ensure that the data collected are valid.)	**Other considerations**

This *Investigation Planning Template* provides students with scaffolds to design experiments.

(b) Use an *annotated diagram* (a labelled diagram with short explanatory notes) to explain how you would use the materials and apparatuses to achieve the aim.
Notes: Your diagram should include the following:

- independent variable(s)
- how you will manipulate the independent variable(s)
- the dependent variable(s)
- how you will measure the dependent variable(s)
- at least *two* important control variables, with a brief explanation of why controlling for these variables is important
- any design decisions to ensure that the data collected are accurate and reliable

(c) Briefly explain how you will manipulate and analyse the data to identify the effects of temperature on the enzymatic activities of the three types of amylase.
(You can use diagrams and/or written descriptions to express your ideas.)

Students are allowed to use alternative ways other than words to express their design decisions.

Scan the QR code to get a copy of the *Google Form*.

學生工作紙（一）

任務 1

- 閱讀以下資訊和資料檔案中的資料。
- 回答隨後的問題。

情境

澱粉酶是一種能夠將澱粉分解成麥芽糖的酶。這種酶在工業生產中被廣泛應用,例如在洗滌劑的製造過程中。

小明在實驗室中找到了三種不同品牌的澱粉酶: 澱粉酶 *X*、澱粉酶 *Y* 和澱粉酶 *Z*。他的生物老師要求他提供建議,包括哪些品牌的澱粉酶可以用於以下用途,以及哪種澱粉酶品牌的效率最高(即最高的酶活性):

	描述
洗碗	• 洗滌過程在洗碗機中進行,溫度為 70–80°C。
洗衣	• 洗滌過程在洗衣機中進行,溫度為 25–30°C。

老師還要求他檢查這三種澱粉酶品牌在冰浴中仍具有活性。

為了達成上述目標, 小明想探究不同溫度對這三種澱粉酶活性的影響。他在實驗室裡找到了以下材料和儀器:

澱粉酶 *X* 溶液	冰浴	葡萄糖試紙
澱粉酶 *Y* 溶液	水浴 (80°C)	葡萄糖溶液
澱粉酶 *Z* 溶液	沸水浴 (>100°C)	DCPIP 溶液
蒸餾水	計時器	澱粉溶液
滴試板	試管	碘液
燒杯	溫度計	馬鈴薯

*提示:*你可能不需要使用所有的材料。

你將運用關於酶的生物知識以及如何設計有效可靠的實驗來完成這個探究。

(a) 完成以下探究實驗策劃模板

自變量 (自變量是什麼?如何改變和處理自變量？)	**因變量** (因變量是什麼?如何量度因變量？)	**控制變量** (有沒有其他可能影響因變量的因素？為什麼需要控制這些因素?)
對照裝置/組 (你是否需要對照？為什麼？)	**預防措施** (為確保所收集的數據有效而採取的步驟)	**其他考慮**

(b) 運用註釋圖(具標注及簡要說明之繪圖)解釋如何使用上述材料和儀器來實現實驗目標。

註釋: 你的圖表應該包括:

- 自變量;
- 如何操縱自變量;
- 因變量;
- 如何測量因變量;
- 至少*兩個*重要的控制變量,並簡要解釋為什麼有必要控制它們;
- 任何旨在確保收集的數據準確且可靠的設計決策。

(c) 簡要解釋如何操縱和分析數據,以識別溫度對三種澱粉酶活性的影響。

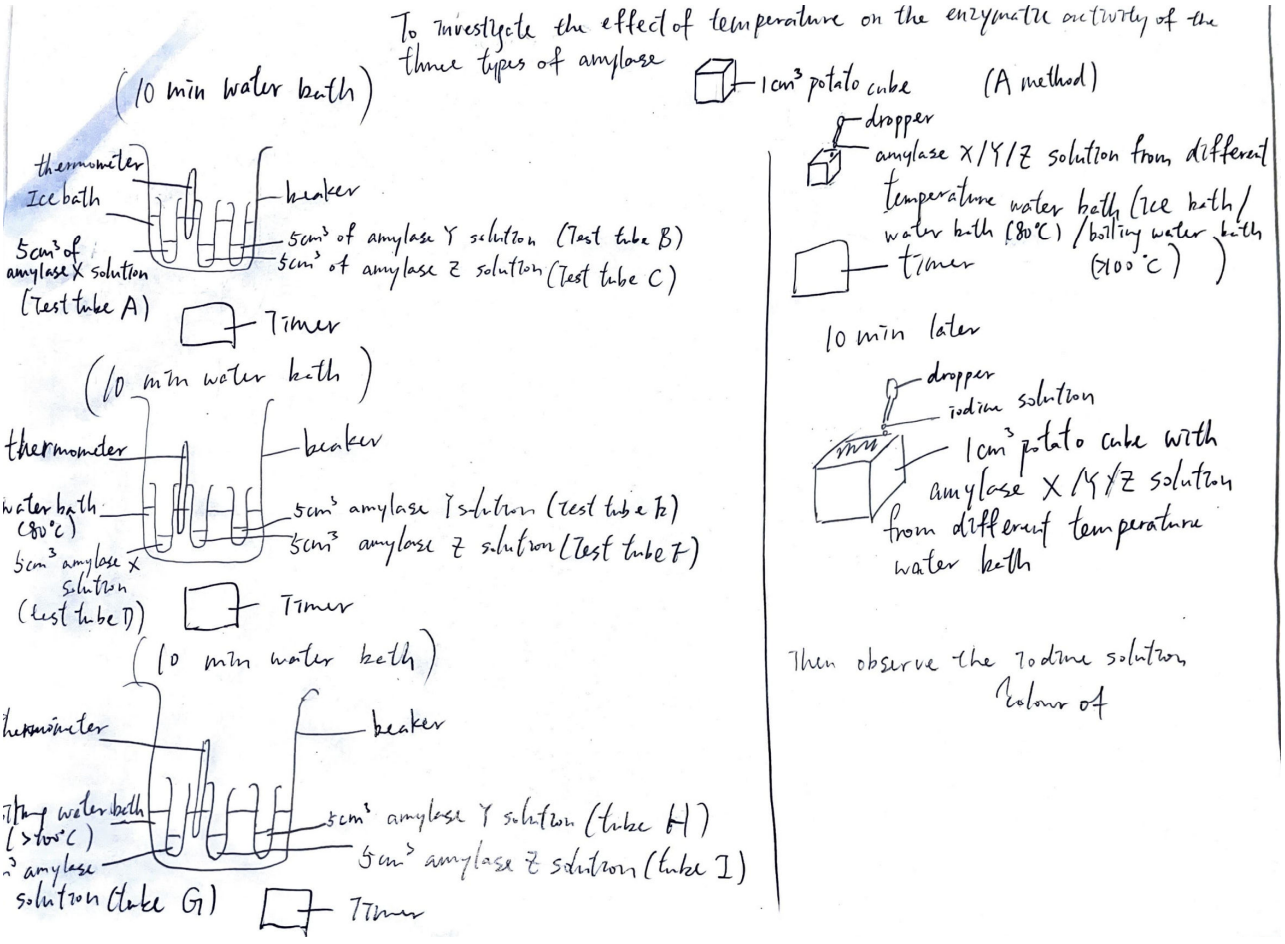
(你可以使用圖表和/或書面描述來表達你的想法。)

掃描二維碼以獲取 *Google Form* 的副本。

Notes for teachers

- Teachers can select student drawings (anonymised) for discussion.
- For example, Sample 1 can be used to stimulate a discussion on the number of independent variables being investigated and the appropriate method to measure the dependent variable.
- Students may be asked to further revise Sample 2. Concepts such as the importance of precautionary steps and the limitation of the instrument used (i.e., beaker) can be discussed.

Sample 1



Possible questions

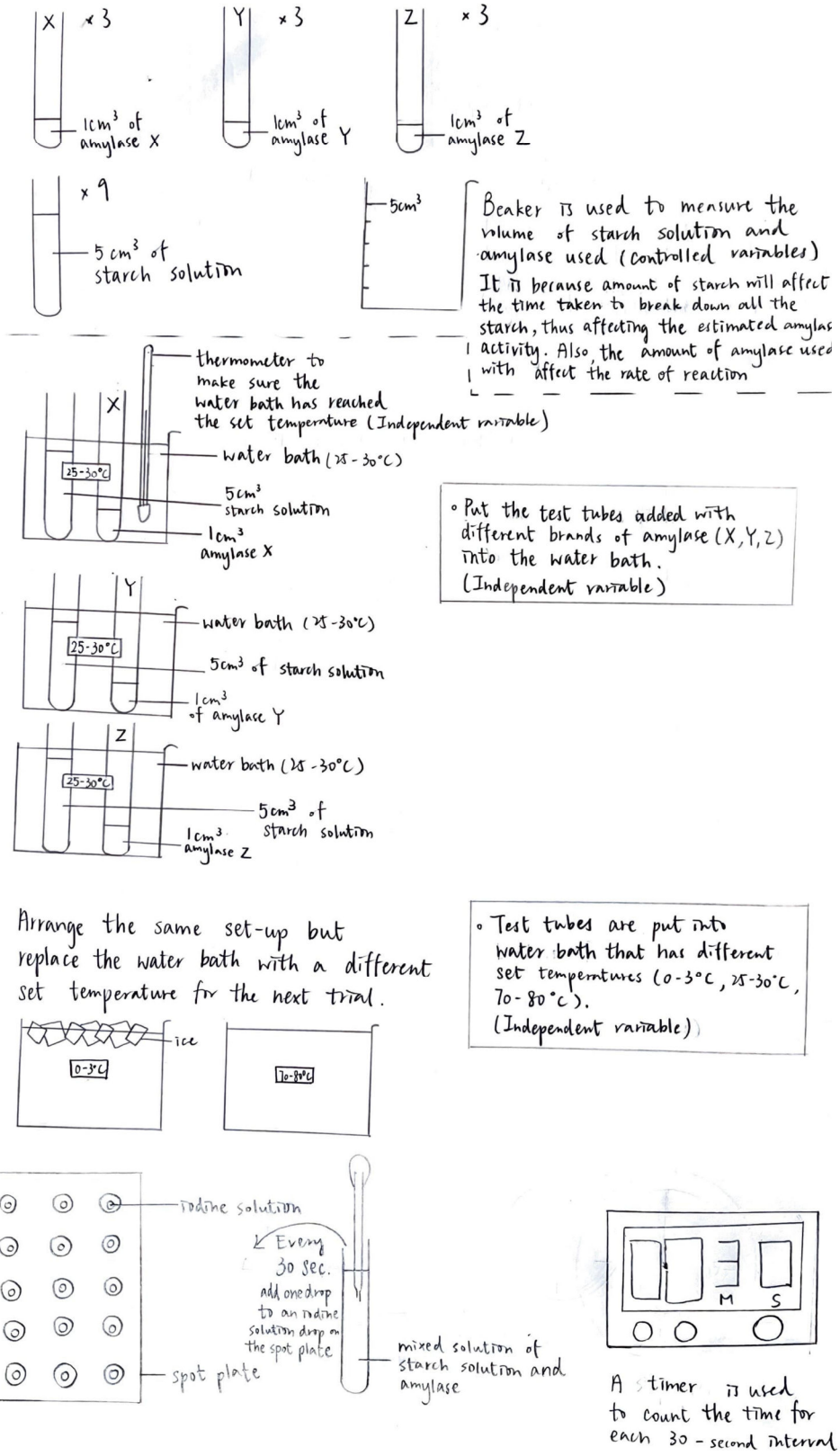
- With reference to the aim of the investigation, answer the following questions:

(1) How many independent variables are being studied? Why do you think so?

(2) (a) What method do you propose to measure the dependent variable?

(b) Will you choose to use this method to measure the dependent variable? Why do you think so?

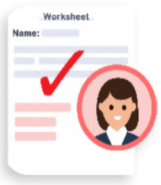
Sample 2



Possible question

- Evaluate the experimental design. How would you improve the design? What are the reasons for your suggested improvements?

Teacher Notes 1



Notes for teachers

- After receiving feedback on their experimental designs, the following shows questions that teachers may use to guide students in thinking about or assessing the scientific inquiry skills related to their experimental designs.
- Some student work samples are shown below to illustrate possible student thinking to some questions.

Task 2

Possible questions

1. Andrew is discussing with his peers David and Vincent to brainstorm variables related to the investigation. The variables are as follows:

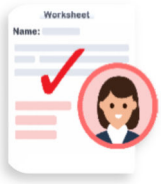
A.	Volume of starch solution	B.	Brand of amylase	C.	Size of the test tube	D.	Size of the spotting plate
E.	Temperature	F.	Volume of water in the water bath	G.	Amylase activity	H.	Concentration of the amylase solution

(Use the letters corresponding to the answers for (a), (b), and (c) (1).)

(a) Which variable(s) should Andrew change in this investigation?
(b) Which variable(s) should Andrew measure in this investigation?
(c) (1) Which variable(s) are important to be controlled in this investigation?
(2) Explain why one of the variables you chose in (c) (1) must be controlled?

2. David advises Andrew to transfer the reaction mixtures for the iodine test every 1 minute instead of every 2 minutes.
(a) Identify *one* strength of David's proposed modification.
(b) Vincent expressed concerns about the possibility of errors when liquid is repeatedly collected from the reaction mixtures using a dropper. Explain why this may cause errors.

3. Andrew observes that when he adds the reaction mixtures collected at 1 minute (i.e. $t = 1$ min) from all three brands of amylase to the iodine solution, the solution remains brown.
(a) How can he modify the procedure to determine which brand of amylase is more active at room temperature?
(b) Explain your answer in (3) (a) based on your biological knowledge of enzymes.



Notes for teachers

- Q.1 assesses students' understanding of variables, particularly to identify multiple variables and to identify and explain important control variables.
- Q.2 assesses students' ability to discuss the limitations and strengths of alternative designs.
- Q.3 assesses students' ability to apply biological principles to improve the validity of the experimental design.

The following are examples of students' responses to Q.2(a):

Sample 1

(1) Identify *one* strength of this modification proposed by David. U☐B☑G☐E☐

The accuracy of the result can be enhanced.

How?

Sample 2

(1) Identify *one* strength of this modification proposed by David. U☐B☐G☑E☐

The time-interval will be shorter which is more accurate to measure the amylase activity.

by i.e.

Sample 3

(1) Identify *one* strength of this modification proposed by David. U☐B☐G☐E☐

The interval between values can be narrowed so that the colour change pattern in the data can be identified more exactly.

About the samples

- The samples show varying sophistication in terms of identifying the strength of the alternative design.
- Sample 1 simply mentioned the term accuracy while Samples 2 and 3 related to the idea of time intervals. Sample 3 further connected to the idea of data pattern.

The following are some examples of students' responses to Q.2(b):

Sample 1

(2) Vincent has expressed concerns about the possibility of errors when liquid is repeatedly collected from the reaction mixtures using a dropper. Explain why this may cause errors. U☐B☐G☑E☐

Because everytime liquid is collected, the amount of amylase in the mixture may be reduced. The rate of completing the enzymatic reaction may lower as they is less amylase for the reaction.

How about compare with ~~other~~ trials with other samples?

About the sample

- The sample identified the effect of removing different volumes of solution from the reaction mixtures using a dropper, which is an imprecise instrument.
- Some more ideas should be discussed:
  - The volume of reaction mixture withdrawn from the test tubes would be different (because of the use of a dropper by squeezing the bulb using different amounts of force).
  - Repeatedly collecting liquid from the tubes using a dropper can introduce variability in the changes in volume across the test tubes.
  - More frequent collection of liquid from the tubes using a dropper may lead to a higher variability in the changes in volume across the test tubes.

The following are some examples of students' responses to Q.3:

Sample 1

(e) Andrew observes that when he adds the reaction mixtures collected at a time of 1 minute (i.e., $t = 1$ minute) from all three brands of amylase to the iodine solution, the solution remains brown.

(1) How can he modify the procedures to determine which brand of amylase is more active at room temperature? U ☐ B ☐ G ☑

add the reaction mixtures collected at a time earlier so that the starch haven't been breakdown.

(2) Explain your answer in (e) (1) based on your biological knowledge about enzymes. U ☑ B ☐ G ☐

The solution remains brown because the starch has already been breakdown so we need to add mixture more earlier (fully) to see which brand of amylase most active at room temperature.

not relate to

(relate to design only)

Sample 2

(e) Andrew observes that when he adds the reaction mixtures collected at a time of 1 minute (i.e., $t = 1$ minute) from all three brands of amylase to the iodine solution, the solution remains brown.

(1) How can he modify the procedures to determine which brand of amylase is more active at room temperature? U ☐ B ☐ G ☐

Increase the volume of starch solution adding to the amylase.

(2) Explain your answer in (e) (1) based on your biological knowledge about enzymes. U ☐ B ☐ G ☐

When the concentration of the starch solution which is increase, the time taken for enzymatic reaction will be longer which allow us to discover the difference more easier.

Sample 3

(e) Andrew observes that when he adds the reaction mixtures collected at a time of 1 minute (i.e., $t = 1$ minute) from all three brands of amylase to the iodine solution, the solution remains brown.

(1) How can he modify the procedures to determine which brand of amylase is more active at room temperature? U ☐ B ☐ G ☐

Use less ~~amount of~~ amylase solution to react with starch solution.

(2) Explain your answer in (e) (1) based on your biological knowledge about enzymes. U ☐ B ☐ G ☐

Because enzyme is reusable but cannot break down two or more starch at the same time, if the amount of amylase is reduced, the time for starch break down is increase, therefore, the ~~result~~ amylase activity of three brands can be compared to find out which amylase is more active at room temperature.

About the samples

- The correct modifications were identified in all the three samples. However, the modification suggested in Sample 1 is not related to biological knowledge about the enzyme.
- In Samples 2 and 3, biological knowledge (i.e., the effect of increasing substrate concentration or decreasing enzyme concentration on enzyme activity) was used to explain the modifications.

教師筆記（一）

任務 2

參考問題

1. 小明與他的同學大衛和小美討論,以列出與這項實驗相關的變量。這些變量包括:

A.	澱粉溶液的體積	B.	澱粉酶的品牌	C.	試管的大小	D.	滴試板的大小
E.	溫度	F.	水浴糟的水量	G.	澱粉酶的活性	H.	澱粉酶溶液的濃度

使用上述字母來回答 (a)、(b) 和 (c)(1) 的問題。

(a) 小明應該改變哪項 / 些變量?

(b) 在這項探究中, 小明應該測量哪項 / 些變量?

(c) (1) 在這項探究中,哪項 / 些變量是重要的控制變量?

(2) 解釋為什麼你在(c)(1)中選擇的其中一個變量需要被控制?

2. 大衛建議小明應該每 1 分鐘而不是每 2 分鐘轉移反應混合物進行碘液試驗。

(a) 識別大衛這一項修改的*一個*優點。

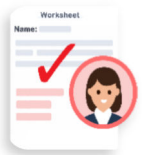
(b) 小美對重複使用滴管從反應混合物中收集液體可能造成誤差表示擔憂。解釋這可能導致誤差的原因。

3. 小明發現當他把在 1 分鐘時間點 (t = 1 分鐘) 收集的三種澱粉酶品牌的反應物加入碘溶液時,溶液仍保持棕色。

(a) 他應如何修改程序來確定哪個澱粉酶品牌在室溫下活性更高?

(b) 根據你對酶的生物知識,解釋你在 3(a) 中的答案。

Laboratory Manual



Notes for teachers

- Teachers can distribute the manual for students to read and prepare before the investigation.
- Teachers can ask questions to check if students fully understand the procedures and the precautions (e.g., the reasons for incubating the samples to reach the desired temperature).
- The *Supplementary Resource* section contains the list of materials.
- Teachers can remind students to take photos of the spotting plate and submit the photos.
- Scan the QR code to view the process of the experiment.

Task 3

- Read the following procedures to carry out the investigation.

Safety reminders

- *Be aware of the hot water in the water bath.*
- *Be aware of the pressure built up in the dropper bottle.*
- *Avoid direct skin contact with enzyme solutions.*

Procedure

1. Place the glass vials containing the starch solution and the 2-mL tube containing three types of amylase solution in the ice bath for *at least* 5 minutes.
2. Place the glass vials containing the starch solution and the three types of amylase solution in the 80°C water bath for *at least* 5 minutes.

Reminder: Place the glass vials on the rack.

Room temperature

1. Add one drop of iodine solution to each well of the spotting plate.
2. Add 2 mL of 0.5% starch solution to each labelled dropper bottle using an autopipette.
3. Add 1 mL of 0.05% amylase solutions *X*, *Y*, and *Z* to each labelled dropper bottle using an autopipette.
4. Gently swirl the dropper bottle to mix the solution well.
5. After 0, 5, 10, 15, and 20 minutes, add one drop of reaction mixture from each dropper bottle.

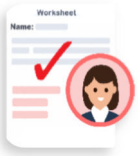
Reminder: There is no need to use the caps of the dropper bottles because a smaller cap can be used to close the dropping bottles.

On ice

1. Add one drop of iodine solution to each well of the spotting plate.
2. Add 2 mL of 0.5% starch solution (at 0°C) to each labelled dropper bottle using an autopipette.
3. Add 1 mL of 0.05% amylase solutions *X*, *Y*, and *Z* (at 0°C) to each labelled dropper bottle using an autopipette.
4. Gently swirl the dropper bottle to mix the solution well.
5. After 0, 5, 10, 15, and 20 minutes, add one drop of reaction mixture from each dropper bottle.

At 80℃

1. Place the dropper bottle rack in the water bath.
2. Add one drop of iodine solution to each well of the spotting plate.
3. Add 2 mL of 0.5% starch solution to each labelled dropper bottle using an autopipette.
4. Add 1 mL of 0.05% amylase solutions *X*, *Y*, and *Z* to each labelled dropper bottle using an autopipette.
5. Gently swirl the dropper bottle to mix the solution well.
6. After time 0, 5, 10, 15, and 20 minutes, add one drop of reaction mixture from each dropper bottle.



Notes for teachers

- Two of the enzymes (*Y* and *Z*) are heat-resistant enzymes.
- Remind the technician to adjust the relative concentration of the enzyme and starch as different brands of enzymes have different activities.
- A milk warmer can be used as a mini water bath. If each group has one mini water bath, students can stay at their own bench when carrying out the experiment.
- Squeeze dropper bottles are commonly used in microscale activities. Teachers may replace the dropper bottles with vials and plastic droppers. See Owen (2019) for an example.
- Laminated data collection sheets are also commonly used in microscale activities.
- Scan the QR code for copy of the *Data Collection Sheet*.

On Ice (0°C)

Min	X	Y	Z
0			
5			
10			
15			
20			

Room Temperature (~25°C)

Min	X	Y	Z
0			
5			
10			
15			
20			

80°C

Min	X	Y	Z
0			
5			
10			
15			
20			

The *Data Collection Sheet* provides guidance for students to collect data.

實驗指南

任務 3

- 閱讀以下實驗步驟以進行探究:

注意

- 注意水浴的高溫
- 注意反應瓶內的壓力積聚

實驗步驟

1. 將含有 0.5% 澱粉溶液的玻璃瓶和分別含有 0.05% 澱粉酶 *X*、*Y*、*Z* 溶液的 2 mL 試管放入 0°C 冰浴中至少 5 分鐘。
2. 將含有 0.5% 澱粉溶液的玻璃瓶和 0.05% 澱粉酶 *X*、*Y*、*Z* 溶液放入 80°C 水浴中至少 5 分鐘。

注意: 將玻璃瓶置於架子上,以便於取用和操作

室溫

1. 在滴試板的每個孔中加入一滴碘液。
2. 使用自動移液器向每個標記的反應瓶中加入 2 mL 0.5% 澱粉溶液。
3. 使用自動移液器向每個標記的反應瓶中加入 1 mL 0.05% 澱粉酶 *X*、*Y*、*Z* 溶液。
4. 輕輕旋轉反應瓶以充分混合溶液。
5. 在 0、5、10、15 和 20 分鐘時,從每個反應瓶中取出一滴反應物加入到滴試板相應位置

冰

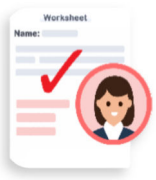
1. 在滴試板的每個孔中加入一滴碘液。
2. 使用自動移液器向每個標記的反應瓶中加入 2 mL 0.5% 澱粉溶液(0°C)。
3. 使用自動移液器向每個標記的反應瓶中加入 1 mL 0.05% 澱粉酶 *X*、*Y*、*Z* 溶液(0°C)。
4. 輕輕旋轉反應瓶以充分混合溶液。
5. 在 0、5、10、15 和 20 分鐘時,從每個反應瓶中取出一滴反應物加入到滴試板相應位置。

80°C

1. 將裝有反應瓶的架子放入 80°C 水浴中,以維持反應溫度。
2. 在滴試板的每個孔中加入一滴碘液。
3. 使用自動移液器向每個標記的反應瓶中加入 2 mL 0.5% 澱粉溶液。
4. 使用自動移液器向每個標記的反應瓶中加入 1 mL 0.05% 澱粉酶 *X*、*Y*、*Z* 溶液。
5. 輕輕旋轉反應瓶以充分混合溶液。
6. 在 0、5、10、15 和 20 分鐘時,從每個反應瓶中取出一滴反應物加入到滴試板相應位置。

掃描二維碼以獲取數據收集表

Teacher Notes 2



Notes for teachers

- The following are some possible questions that teachers can use to guide students in identifying or assessing their scientific inquiry skills related to data analysis and interpretation.
- Some student work samples are shown below to illustrate possible student thinking to some questions.

Task 4

Possible questions

1. Take a photograph of the spotting plates.

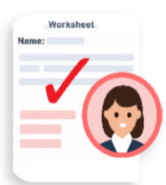
2. Anomalous data (i.e. outliers [experimental data that do not fit within a pattern]) may be obtained in experiments.

   Do your data show anomality? Why do you think so?

3. (1) Based on your results, which enzyme brand(s) can you use for the following purposes?
   (2) Which enzyme brand is the most efficient (i.e., that with the highest enzyme activity) when used for the following purposes?
   (3) Explain your answers.

	(1) Enzyme brand(s) that can be used	(2) Most efficient enzyme brand	(3) Explanation
Dishwashing			
Washing clothes			

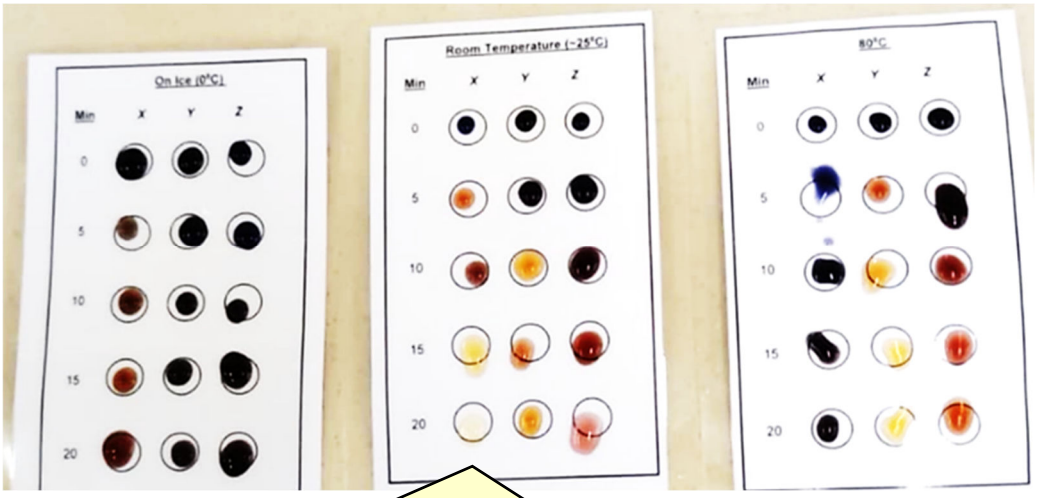
4. You noticed the differences in the sizes of the droplets (i.e. the reaction mixtures) taken from the reaction mixtures at different time points.
   (a) Explain why this can affect the experimental results.
   (b) Suggest and explain *one* way of reducing this error.



Notes for teachers

- Q.2 assesses students' ability to identify anomalous data within their own data set.
- Q.3 assesses students' ability to use their data to inform decision-making.
- Q.4 assesses students' ability to explain the impact of errors common among students in this experiment and explain ways to mitigate the errors.

The following are some examples of students' responses to Q.2, Q.3 and Q.4:



Anomalous data present in the dataset but not identified in the response below.

Sample 1

(c) Anomalous data (i.e., outliers [the experimental data that do not fit within a pattern]) may be obtained in experiments. U ☑ B ☐ G ☐

Do your data show anomality? Why do you think so?

My data didn't show anomality. Because the data didn't show anything wrong such as the colour suddenly became darker than the result test before.

Sample 2

Yes, for 25°C, the trend of iodine solution colour of X should be from blue-black to dark brown to pure brown, the purity should be in decreasing trend. However, at 10 minutes, it's even darker than at 5 minutes. The darkness of colour may be affected by the colour of iodine solution. It's because we drop the iodine solution at different time and the colour of iodine solution become darker after few minutes, therefore, the colour observed is affected.

Sample 3

(d) (1) Based on your results, which enzyme brand(s) can you use for the following purposes? U ☐ B ☐ G ☑

(2) Which enzyme brand is the most efficient (i.e. has the highest enzyme activity) when used for the following purposes. U ☐ B ☐ G ☑

(3) Explain your answers. U ☐ B ☐ G ☑

	(1) Enzyme brand(s) that can be used	(2) Most efficient enzyme brand	(3) Explanation
Dishwashing	Y, Z	Y	Both amylase Y and Z can turns iodine solution from blue to brown in 80°C, which means both amylase Y and Z can breakdown starch in 80°C, it match the temperture of the dishwashing takesplace at. The colour change of iodine solution is more obvious in Y compare to other, which means amylase Y breakdown more starch per minutes time
Washing clothes	X, Y, Z	X	Both amylase X, Y, Z can turns iodine solution from blue-black to brown in 25°C, which means they can breakdown the starch where the washing clothes process takes place at. water The colour change of iodine solution is more obvious in X compare to other, which means amylase X breakdown more starch per minute time

shorter time for colour change

Sample 4

(e) Chris made the following claim:

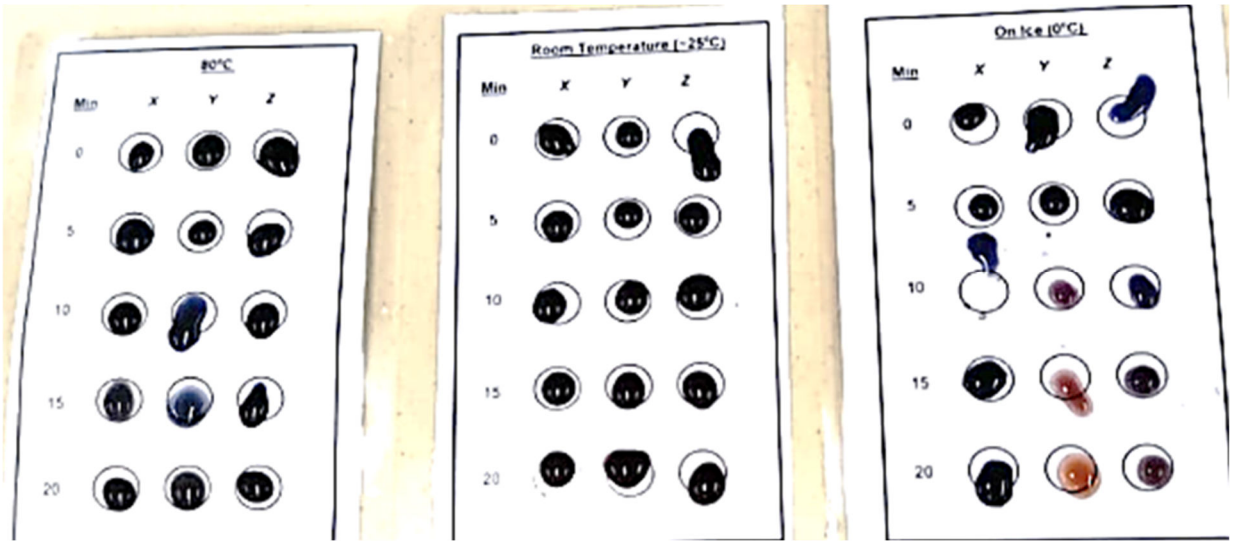
"All the three amylase brands are denatured when stored on ice."

(1) Explain whether your data support or reject this claim. U ☐ B ☑ G ☐

Reject. the colour of iodine solution didn't change did not means that the amylase are denatured. Since at low temperature, the kinetic energy of substrate and enzyme molecules is low, it cause the enzymes are inactive, which means the rate of enzymatic reaction is low.

About the samples

- Sample 1 erroneously concluded that there were no anomalous data present while Sample 2 correctly identified the anomalous data and provided an appropriate explanation.
- Sample 3 accurately identified the enzyme brands suitable for the purposes based on the data collected.
- Sample 4 did not use the collected data to refute the claim though the data collected show that Enzyme *X* still shows enzyme activity when stored on ice.



Sample 5

(c) Anomalous data (i.e., outliers [the experimental data that do not fit within a pattern]) may be obtained in experiments. U☐ B☐ G☐

Do your data show anomality? Why do you think so?

unrelated

Yes, the result of the iodine solution turn to blue-black colour as we add too ~~Almost all~~ many drops of mixture to iodine solution, So that all the iodine solution turn to ~~which~~ contain more amount of starch blue-black colour.

Sample 6

(c) Anomalous data (i.e., outliers [the experimental data that do not fit within a pattern]) may be obtained in experiments. U☑ B☐ G☐

Do your data show anomality? Why do you think so?

unrelated

~~Yes~~ amylase Z does not breakdown the starch at 20 minutes. ~~The brand of the amylase solution does not breakdown the starch in 20 minutes.~~

Sample 7

(d) (1) Based on your results, which enzyme brand(s) can you use for the following purposes? U☑B☐G☐

(2) Which enzyme brand is the most efficient (i.e. has the highest enzyme activity) when used for the following purposes. U☑B☐G☐

(3) Explain your answers. U☐B☐G☑

	(1) Enzyme brand(s) that can be used	(2) Most efficient enzyme brand	(3) Explanation
Dishwashing	Not match with your own results Y, Z	Y	Based on (1) and (2) At 80°C, iodine solution with amylase Y show the most brown colour which indicate it has the lowest amount of starch in the mixture. This means amylase Y the most reactive to catalyze breakdown of starch at 80
Washing clothes	X, Y, Z	X	At room temperature, iodine solution with amylase X show the most brown colour which indicate it has the lowest amount of starch in mixture. This means amylase X is the most reactive to catalyze breakdown of starch at room temperature.

Sample 8

(e) Chris made the following claim:

"All the three amylase brands are denatured when stored on ice."

(1) Explain whether your data support or reject this claim. U☐B☑G☐

Reject. At the end of the iodine test, mixture with amylase X show brown colour of iodine solution which indicate the absence of starch. Amylase X is not denatured when stored on ice and catalyze the breakdown of starch, so that the iodine solution remain brown.

should be Y? (based on your result)

About the samples

- The reasons cited in Samples 5 and 6 for the anomalous data do not correlate with the obtained results.
- The enzyme brands identified in Sample 7 do not match the obtained results.
- The explanation provided in Sample 8 did not correspond with the results that were obtained (i.e., Enzyme *Y* still shows enzyme activity when stored on ice).

教師筆記（二)

任務 4

參考問題

1. 請拍攝滴試板的照片。

2. 實驗中可能會出現異常數據(即離群值)。你的數據中是否顯示異常情況?你認為原因是什麼?

3. (a) 根據你的實驗結果,哪些酶品牌可用於以下用途?
   (b) 在以下用途中,哪種酶品牌的效率(即酶活性)最高?
   (c) 解釋你的答案。

	(1) 可用的酶品牌	**(2) 最高效率的酶品牌**	**(3) 解釋**
洗碗			
洗衣			

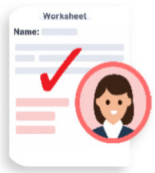
4. 你注意到在不同時間點取樣的液滴(即反應混合物)大小是不同的。
   (a) 解釋這如何影響實驗結果。
   (b) 提出並解釋一*種*減少這種誤差的方法。

Supplementary Resources

Possible Modifications

1. Using immobilised amylase beads to investigate factors that affect amylase activity

- Amylase can be immobilised using sodium alginate solution. Immobilised amylase beads can be used to investigate factors that affect amylase activities.
- The following shows the procedures for preparing immobilised amylase beads and for investigating the effects of substrate concentration and competitive inhibitors on amylase activities.



Notes for teachers

- Teachers can use the following procedures. See Chan et al. (2024) for a detailed description.
- Read the *Technician Notes* section for the materials required for this experiment.
- Note that even though the effects of substrate concentration and competitive inhibitors on enzymatic activities are not within the scope of the curriculum, teachers can still ask students to investigate these effects. The focus should be on how students use their data to construct claims about the effects based on their data.
- It is suggested that teachers can use the *integrated instruction sheets* (Paterson, 2019), which combine diagrams and textual instructions about the experimental procedures to help students better understand the procedures.

Preparation of immobilised amylase beads

Procedure

1. Add 10 mL of 0.1% amylase solution to 10 mL of 3% sodium alginate solution in a 50-mL tube (amylase–sodium alginate solution).
2. Mix the solution gently by inverting the 50-mL tube to create an amylase–sodium alginate solution.
3. Add a few drops of food colouring.
4. Let the mixture sit for 10 minutes to avoid bubbles.
5. Hold the plastic dropper (without a cap) with a stand and clamp.
6. Pour 200 mL of 2% $CaCl_2$ into a 500-mL beaker.
7. Prepare the experimental set-up shown in *Figure 1*.
8. Add the amylase–alginate solution to the plastic dropper. Beads should form when the drop comes into contact with the $CaCl_2$ solution and falls to the bottom of the beaker.
9. Wait 5 minutes for the beads to harden.
10. Collect the amylase beads with a sieve (*Figure 2*).
11. Wash the amylase beads several times with distilled water from a wash bottle.
12. Store the amylase beads at 4°C in a zipper bag.

Figure 1

Figure 2

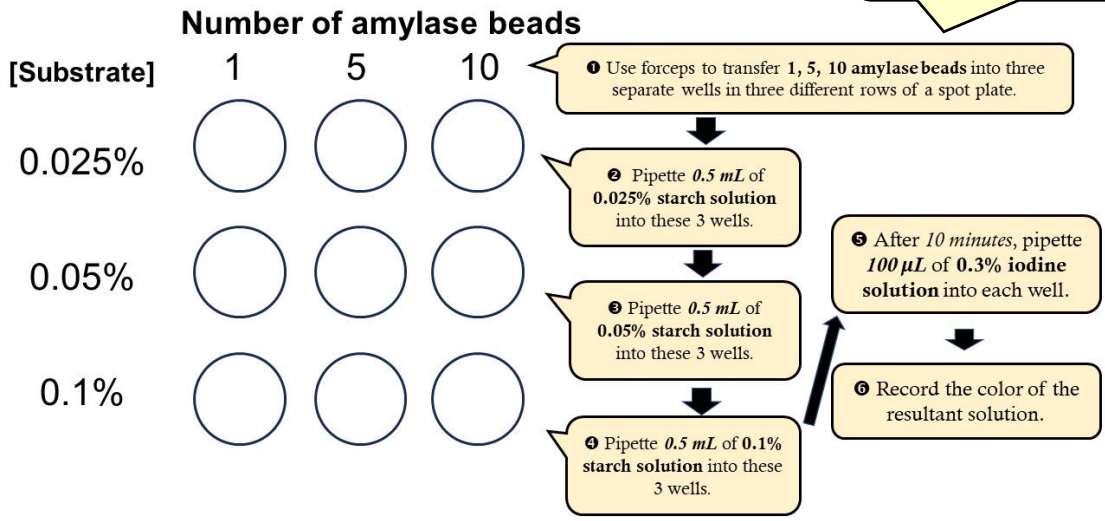
Scan the QR code to view how to make amylase beads.

Effect of substrate concentration on amylase activity

Procedure

1. Use forceps to transfer sets of 1, 5, and 10 beads to three separate wells in three different rows of a spotting plate.
2. Pipette 0.5 mL of 0.025% starch solution into the three wells.
3. Pipette 0.5 mL of 0.05% starch solution into the three wells.
4. Pipette 0.5 mL of 0.1% starch solution into the three wells.
5. After 10 minutes, pipette 100 µL of 0.3% iodine solution into each well.
6. Record the colour of the resultant solutions.

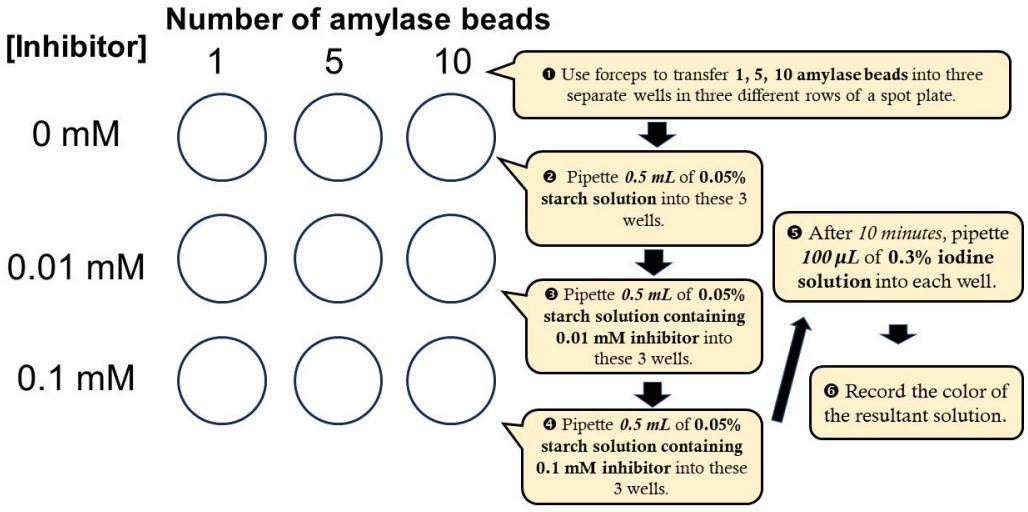
Integrated Instruction Sheet facilitates students understanding of the procedures.



Effect of inhibitors concentration on enzyme activity

Procedure

1. Use forceps to transfer sets of 1, 5, and 10 beads to three separate wells in three different rows of a spotting plate.
2. Pipette 0.5 mL of 0.05% starch solution into the three wells.
3. Pipette 0.5 mL of 0.05% starch solution containing 0.01 mM inhibitor into the three wells.
4. Pipette 0.5 mL of 0.05% starch solution containing 0.1 mM inhibitor into the three wells.
5. After 10 minutes, pipette 100 µL of 0.3% iodine solution into each well.
6. Record the colour of the resultant solutions.



Technician Notes

Materials for Task 3

Chemicals to be prepared

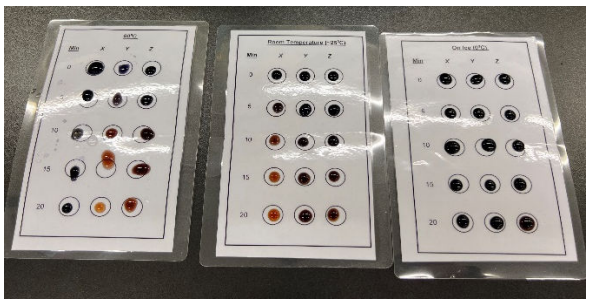
- Amylase *X* 0.05% (0.05 g in 100 mL)
- Amylase *Y* 0.05% (0.05 g in 100 mL)
- Amylase *Z* 0.05% (0.05 g in 100 mL) (or replaced with a lower % of Amylase *Y*)
- 0.5% starch (0.5 g in 100 mL) (Stored at 4^0C)

* Amylase *Y* and *Z* are heat-resistant amylases.

Materials for each group

• Mini water bath	• 2 mL 0.05% Amylase solution *X*, *Y*, *Z* in glass vials X 3	• Dropper bottle rack
• Thermometer	• 7 mL 0.5% Starch solution in glass vials X 3	• 5 mL Dropper bottle X 9 (3 different colours)
• Ice bath	• Laminated spotting plate	• Rubbish bin
• Autopipette (P-1000)	• Autopipette tips (P-100)	• Labels
• *Pen		

* Dropper bottles can be replaced with glass vials and plastic droppers.
* Do *not* use marker pen for labelling.



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Owen, M. (2019). Amylase activity: A microscale approach in biology. *African Journal of Chemical Education*, *9*(3), 64–74.

Paterson, D. J. (2019). Design and evaluation of integrated instructions in secondary-level chemistry practical work. *Journal of Chemical Education*, *96*(11), 2510–2517.

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