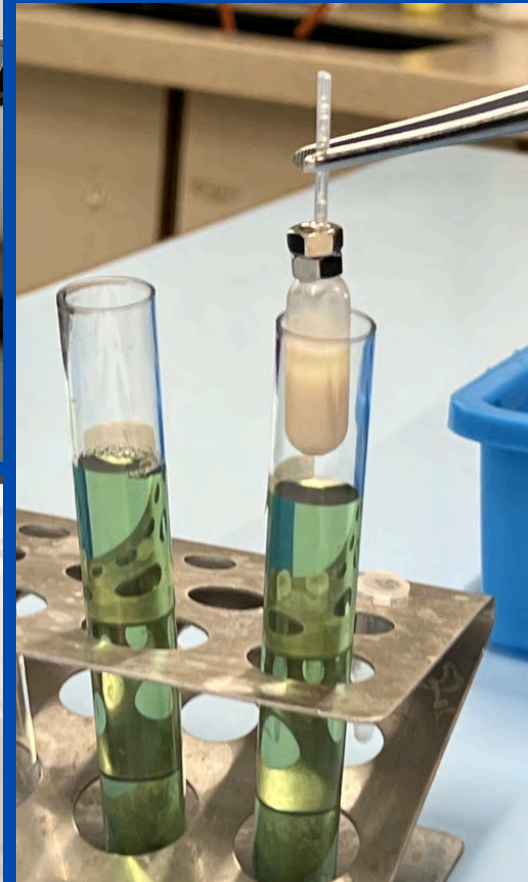
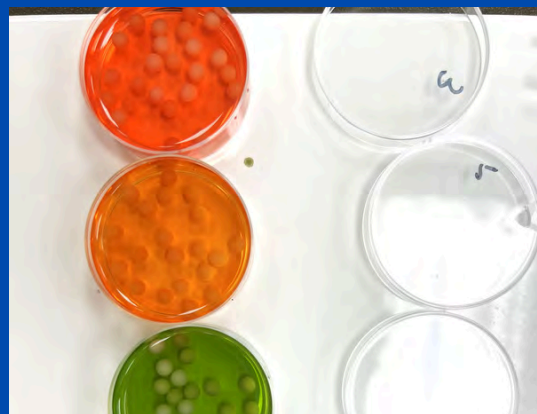
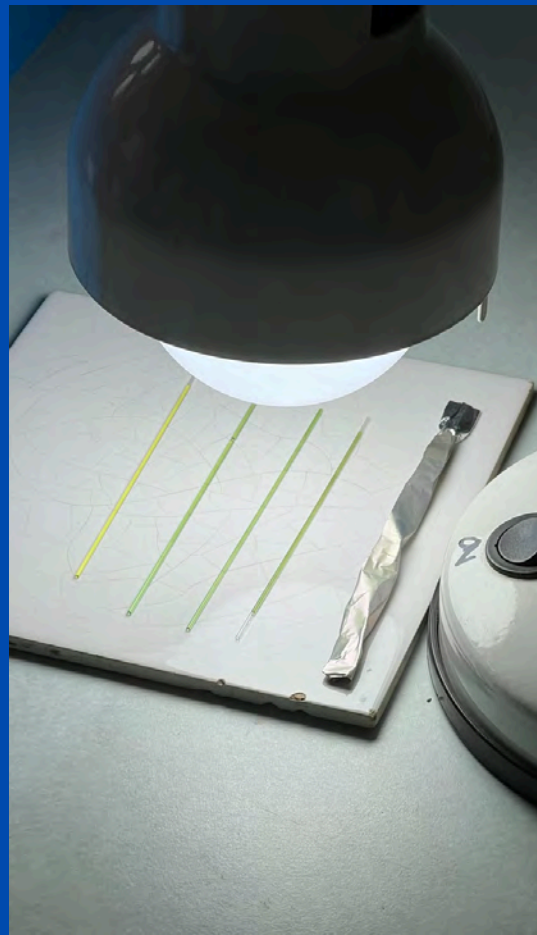
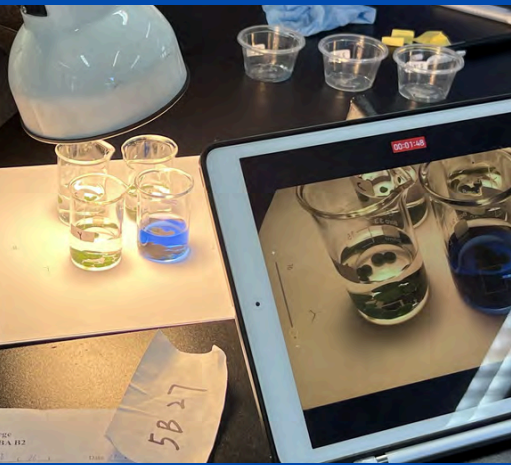




# Innovations in Biology Investigations



## **PROJECT TEAM**

Dr CHAN Kam Ho, The University of Hong Kong  
Dr CHENG Ka Lok, The University of Hong Kong  
Mr HO Tik Shun, The Chinese Foundation Secondary School  
Mr TSUI Chi Shing, Hong Kong Examinations and  
Assessment Authority  
Mr LAU Siu Pan, The University of Hong Kong

## **ART and DESIGN**

Ms CHENG Hiu Yeung  
Ms LAU Cheuk Yan



Faculty of **Education**  
The University of Hong Kong

Room 313, Runme Shaw Building  
Pok Fu Lam Road  
The University of Hong Kong  
Email: [kennedyckh@hku.hk](mailto:kennedyckh@hku.hk)



# Table of Contents

## Table of Contents

	<b>Page</b>
<b>1 Background Information</b>	<b>1-5</b>
• 1.1 Overview	1
• 1.2 Background Information	2-5
<b>2 Investigative Practical Work</b>	<b>6-239</b>
• 2.1 Microscale Amylase Investigation	1-25
• 2.2 Yeast Bead Invertase Investigation	1-25
• 2.3 Yeast Bead Catalase Investigation	1-29
• 2.4 Banana Ripening Investigation	1-17
• 2.5 Lipase Inhibitor Investigation	1-27
• 2.6 Photosynthesis Inhibitor Investigation	1-23
• 2.7 Cat Grass Investigation	1-27
• 2.8 Yeast Respirometer Investigation	1-25
• 2.9 Brine Shrimp Investigation	1-27
<b>3 Concluding Remarks</b>	<b>240-243</b>
• 3.1 Summary	1-2
• 3.2 Resources	1



# Acknowledgement

## Acknowledgement

- The project team would like to acknowledge the participation and contribution of the following biology teachers in workshops and/or trial implementations of the tasks, as well as their schools:

Name	School*
Dr AU Siu Chung	Chinese YMCA College
Mr CHAN Pat Chun	Queen Elizabeth School Old Students' Association Secondary School
Ms CHEUNG Hoi Yan	Wa Ying College
Mr CHOI Ka Shing	Wa Ying College
Mr FAN Kei Chu	Po Leung Kuk Celine Ho Yam Tong College
Mr FEE Wai Chun	St. Paul's School (Lam Tin)
Ms HO Pui Shuen	The Chinese Foundation Secondary School
Mr LAU Ho Ngai	Pui Kiu College
Mr LAU Yiu Chung	Immaculate Heart of Mary College
Mr LEE Yat Shing	Wah Yan College (Kowloon)
Mr LEUNG Tsz Chung	Salesian English School
Mr LI Ho Pan	Buddhist Ho Nam Kam College
Mr LO Wai Kit	Po Leung Kuk Wu Chung College
Mr NG Sze Hon	Yan Oi Tong Tin Ka Ping Secondary School
Mr NG Yu Hin	Po Leung Kuk Wu Chung College
Mr NGAI Wing Sing	Po Leung Kuk Laws Foundation College
Mr TAM Long Hei	Hong Kong Management Association David Li Kwok Po College
Mr TO Ching Yuet	Po Leung Kuk Laws Foundation College
Ms YEUNG Shuk Laam	The Chinese Foundation Secondary School

- Their participation in the project is critical to the fruition of this educative curriculum material package.
- The project team would also like to thank the following biology educators for reviewing the initial drafts of some of the investigative practical work tasks: Mr CHAN Ka Loi (Po Leung Kuk Ngan Po Ling College\*), Mr CHENG Yuen Shan (Jockey Club Ti-I College), Mr CHUI Hing Wah (Tin Ka Ping Secondary School\*), Mr LAM Lok Hei (Pui Kiu College\*), Ms LAU Wai Yin (Pope Paul VI College), Dr LAU Sui Yee (Tsuen Wan Public Ho Chuen Yiu Memorial College), Ms LEUNG Miu Fun (Belilios Public School), Mr LEUNG Wing Cheuk (Po Leung Kuk Mrs. Ma Kam Ming-Cheung Fook Sien College), and Dr SUEN Ka Chun (Po Leung Kuk Laws Foundation College). Their comments have greatly improved the quality of the tasks. However, the project team is responsible for and accountable for ensuring the final quality of the task.
- The project team would like to thank Mr TSANG Yau Shing for his technical support.
- The project team also acknowledges the funding support from Quality Education Fund (QEF). Promoting effective use of practical work in senior secondary Biology classrooms through cognitively demanding practical work and video analysis of teaching practices, Quality Education Fund (Project No.: 2019/0283).

*Note:* \*denotes the secondary school where the teacher/educator was serving at the time of the project.



# Background Information

---



# Overview

## Overview

- This educative curriculum material package was designed and developed by secondary school biology educators in Hong Kong to enhance the teaching and learning of scientific inquiry skills in secondary biology classrooms.

## Aims of the Package

- To provide teachers with ideas for designing and implementing effective investigative practical work related to biology that effectively enhances students' scientific inquiry skills.
- To share classroom-proven, robust and reliable laboratory protocols and instructional materials.

## Organisation of the Package

- This educative curriculum material package consists of three major components: (1) *Background Information*; (2) *Investigative Practical Work*; (3) *Concluding Remarks*.
- The *Background Information* section provides an overview of the package and a succinct description of the research that underpins the investigative practical work.
- Each of the nine sets of *Investigative Practical Work* adheres to the following common structure:
  - (1) Introduction
    - This section includes a brief description of the investigative practical work, including the scientific inquiry skills it targets, a suggested teaching plan, the key features of the investigation and important notes related to the investigation when applicable.
  - (2) Instructional materials
    - This section includes student worksheets, teacher notes, laboratory manuals and student work samples. Both Chinese and English versions are included when applicable.
  - (3) Supplementary resources
    - This section provides ideas for possible adaptations and modifications of the investigative practical work activities. Technician notes are also included. Major references and further resources related to the investigation are also listed.
- The *Concluding Remarks* section synthesises the design principles and implementation strategies for investigative practical work in biology and provides a list of resources.

## How to Use this Package

- Readers are encouraged to read the *Background Information* section before using any of the sets of investigative practical work, as well as refer to the *Concluding Remarks* section.
- For each set of investigative practical work, teachers are advised to first read the *Introduction* section to familiarise themselves with the scientific inquiry skills, the suggested teaching and learning sequence, and the key features of the investigation.
- Teachers should also read the *Notes for Teachers* boxes, which include reminders and tips for classroom implementation.



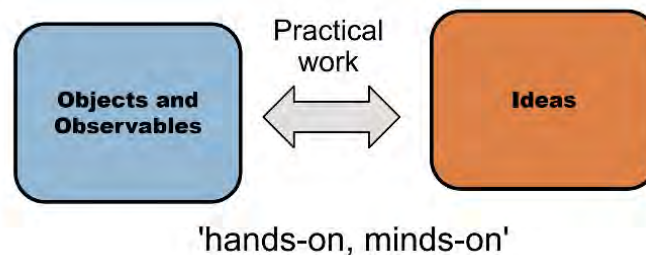
# Background Information

## Background of the Package

- According to the Science Education Key Learning Area Curriculum Guide (Primary 1–Secondary 6) 2017, it is essential for students to gain personal experience of science through hands-on practical work activities, as science subjects are practical subjects (Curriculum Development Council, 2017).
- Practical work refers to “any teaching and learning activity which at some point involves the students in observing or manipulating the objects and materials they are studying” (Millar, 2004, p. 2).
- Generally speaking, practical work aims to help students:
  - develop their knowledge and understanding of scientific ideas and concepts (i.e., scientific understanding)
  - learn how to use scientific apparatuses and follow procedures (i.e., practical skills)
  - understand the scientific approach to inquiry (i.e., scientific inquiry skills, e.g., designing experiments, analysing data).



- One important task for teachers is to help students make connections between what they are doing or observing (i.e., the domain of observables) and the “thinking behind the doing” (i.e., the domain of ideas) to ensure that practical work activities are not only “hands-on” but also “minds-on” (Abrahams & Millar, 2008).



- Scientific investigation (or called investigative practical work) is an example of practical work in which students find answers to a scientific question. They often collect data and construct claims/explanations based on this data to answer the scientific question.
- The process of a scientific investigation comprises the following stages: (1) preparing for the investigation; (2) planning and designing the investigation; (3) carrying out the investigation; (4) analysing, interpreting, evaluating and explaining data (Pedaste et al., 2015; Rönnebeck et al., 2016).



- Although the process of scientific investigation is delineated into stages above, it should be emphasised that the actual process is non-linear, dynamic, and iterative rather than strictly following a linear sequence (UC Museum of Paleontology Understanding Science, 2024). The delineation into stages is intended to facilitate teachers' planning on how to implement scientific investigations.
- Students need not only practical skills but also scientific understanding and scientific inquiry skills to engage in scientific investigations successfully.
- When teachers engage students in investigative practical work activity, an important task for teachers is to help students think about scientific inquiry skills (e.g., procedural understanding, understanding about the nature of science and process of scientific inquiry) in all the stages.
- Readers are encouraged to explore the references cited above to gain a deeper understanding of the research foundation concerning the effective design and implementation of scientific investigations.

## References

- Abrahams, I., & Millar, R. (2008). Does practical work really work? A study of the effectiveness of practical work as a teaching and learning method in school science. *International Journal of Science Education*, 30(14), 1945–1969.
- Curriculum Development Council. (2017). *Science Education Key Learning Area Curriculum Guide (Primary 1 – Secondary 6)*. Hong Kong SAR Government.
- Rönnebeck, S., Bernholt, S., & Ropohl, M. (2016). Searching for a common ground—A literature review of empirical research on scientific inquiry activities. *Studies in Science Education*, 52(2), 161–197.
- Pedaste, M., Mäeots, M., Siiman, L. A., De Jong, T., Van Riesen, S. A., Kamp, E. T., ... & Tsourlidaki, E. (2015). Phases of inquiry-based learning: Definitions and the inquiry cycle. *Educational Research Review*, 14, 47–61.
- UC Museum of Paleontology Understanding Science. (2024) The Understanding Science Flowchart. <https://undsci.berkeley.edu/the-understanding-science-flowchart-text-description/>

## Scientific Inquiry Skills

- The table below lists some scientific inquiry skills required for *Stages 1, 2 and 4*.

<b><i>Preparing for, planning and designing investigations</i></b>
<ol style="list-style-type: none"><li>1. Ask scientific questions.</li><li>2. Use the appropriate design for a given scientific question (e.g., experimental, field-based, fair testing, classifying, pattern-seeking).</li><li>3. Formulate the hypothesis for testing.</li><li>4. Elaborate how the predicted results do/do not support the hypothesis.</li><li>5. Identify issues related to the sampling method(s) and sample size.</li><li>6. Suggest and explain ways to reduce sampling errors and average out the effect of variations within a sample.</li><li>7. Identify the independent variable(s) and dependent variable(s) in the investigation and connect the variables to manipulation/method(s) of measurement.</li><li>8. Identify multiple independent variables and dependent variables.</li><li>9. Identify important control variables and explain why they are important to control.</li><li>10. Explain the limitations related to the manipulation/measurement method(s)/ instrument(s) for the variable(s).</li><li>11. Discuss the strengths and limitations of the alternative measurement method(s).</li><li>12. Explain why multiple control set-ups are needed.</li><li>13. Explain why some procedures can reduce measurement errors.</li><li>14. Identify the significant assumptions of the design.</li><li>15. Explain why a specific step is conducted and its impact on the validity and reliability of the experimental design.</li><li>16. Evaluate the overall validity and reliability of the experimental design.</li><li>17. Discuss the limitations and strengths of the alternative designs.</li><li>18. Apply biology principles to the experimental designs.</li></ol>
<b><i>Analysing, interpreting, evaluating and explaining data</i></b>
<ol style="list-style-type: none"><li>1. Record qualitative data using clear descriptions and record quantitative data properly.</li><li>2. Construct and use appropriate representations (e.g., tables, graphs and/or diagrams) to organise and display data.</li><li>3. Apply basic statistical concepts (e.g., range, variance, standard deviation, error bar) to compare and explain datasets.</li><li>4. Identify anomalous data and suggest possible explanations or ways to confirm if the data are anomalous.</li><li>5. Interpret the results in the control(s) to evaluate the success of the experiment or the influence of the experimental manipulation.</li><li>6. Describe and explain the relationships/trends and patterns in both simple and more complex datasets (e.g., with multiple variables) using scientific ideas and principles to relate to the investigative problem.</li><li>7. Evaluate whether the hypothesis is supported, refuted or remains undetermined according to the data.</li><li>8. Discuss alternative hypotheses.</li><li>9. Construct and evaluate evidence-based claims/explanations.</li><li>10. Explain the impact of measurement error on the validity and reliability of the data or conclusions.</li><li>11. Suggest and explain valid improvements or further data collection to address the limitations of the experimental design in relation to the investigative problem.</li><li>12. Make informed decisions about the problem using valid reasoning.</li><li>13. Discuss how to modify or extend the investigation to answer a new investigation question.</li><li>14. Discuss the generalisability of the results and conclusions.</li><li>15. Evaluate the overall validity and reliability of the methods and how they influence the validity and reliability of the data/evidence.</li><li>16. Assess the appropriateness and adequacy of the experimental design based on the data.</li></ol>

- Although students primarily use their practical skills in *Stage 3*, they may also need some of the scientific inquiry skills described above during this stage, particularly if they need to modify or troubleshoot the procedures.
- Teachers are encouraged to be intentional and purposeful in teaching and assessing the above scientific inquiry skills in secondary school biology classes.

## Overview of All Investigations

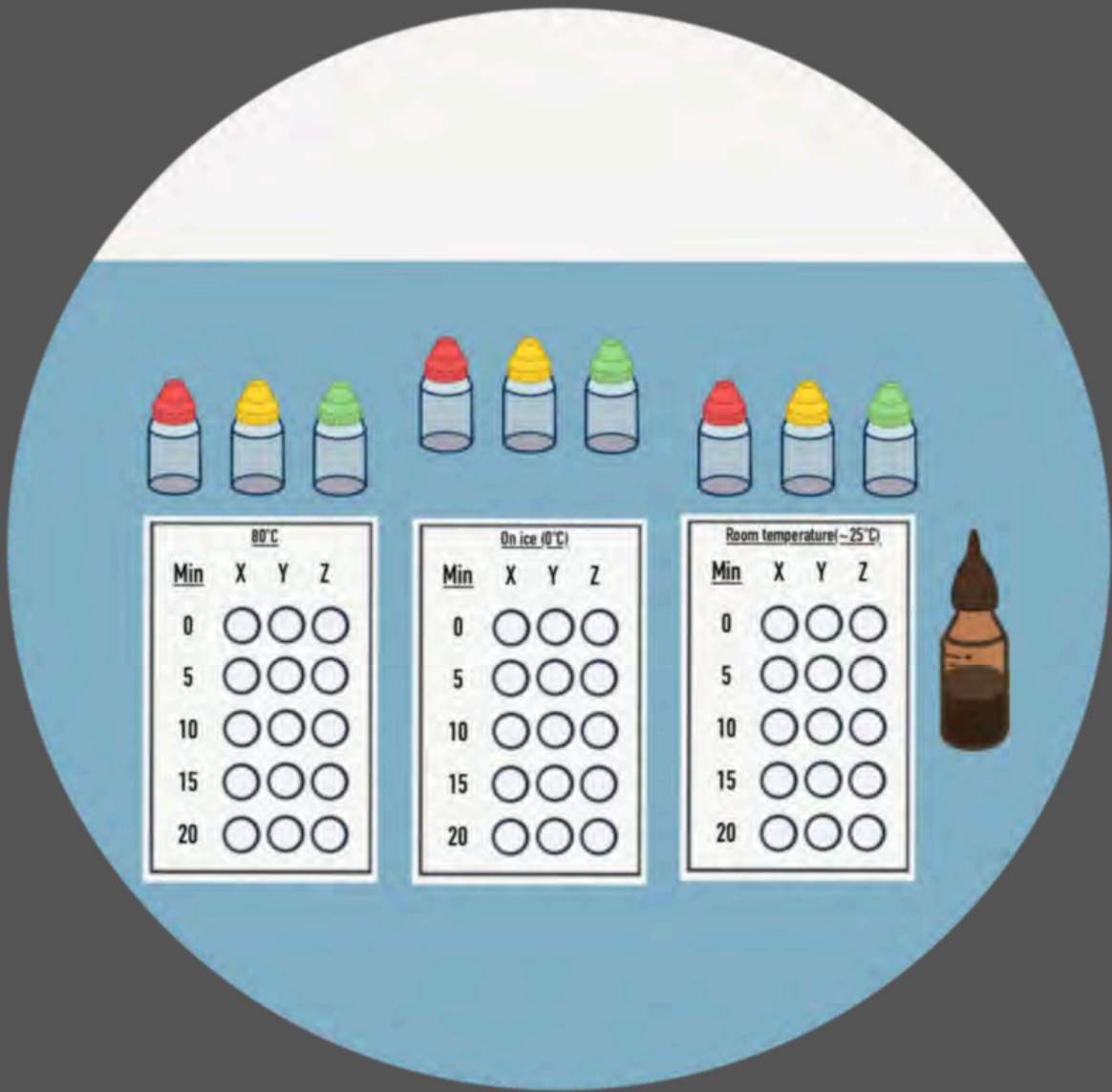
- In the nine sets of investigative practical work, students gain hands-on experience, experience science as a process and develop a deeper understanding of scientific understanding and inquiry skills.
- Some suggestions for guiding questions/student worksheets are included to help teachers focus on teaching and assessing the scientific inquiry skills related to each investigation:

Investigation	Related topics in the curriculum	Scientific inquiry skills addressed	
		Designing investigations	Data analysis and interpretation
❶ Microscale Amylase Investigation	Enzyme	8, 9, 17, 18	1, 4, 11, 12
❷ Yeast Bead Invertase Investigation	Enzyme	5, 6, 7, 16	4, 6, 11, 13
❸ Yeast Bead Catalase Investigation	Enzyme	10, 14, 15	2, 6
❹ Banana Ripening Investigation	Nutrition in humans	3, 4, 17	7, 11
❺ Lipase Inhibitor Investigation	Enzyme, Nutrition in humans	7, 11, 12	2, 13, 14
❻ Photosynthesis Inhibitor Investigation	Photosynthesis	6, 7, 11, 18	1, 2, 3, 9, 10, 11
❼ Cat Grass Investigation	Photosynthesis	9, 12, 13	9, 13, 14
❽ Yeast Respirometer Investigation	Respiration	7, 14, 17	12, 13, 16
❾ Brine Shrimp Investigation	Applied ecology	1, 2, 12, 13, 17	1, 5, 9

- Teachers are strongly encouraged to adapt and modify these resources as necessary. They should exercise their professional judgement about which scientific inquiry skills to include in the lessons based on the interests and abilities of their students.
- Teachers should *not* introduce these scientific inquiry skills as content or authoritative statements to memorise. Rather, they should focus on helping students develop the ability to apply these skills in various contexts.

## Risk Assessment

- Most practical activities presented in this package are microscale experiments. One benefit is that the risk has been reduced by using smaller quantities of chemicals.
- However, teachers should perform a risk assessment in line with safety guidelines.
- Teachers should also rehearse the procedures before carrying out any of these practical activities.
- Whenever necessary, students should wear laboratory coats, safety gloves and goggles when conducting the practical work.



# Microscale Amylase Investigation

---



# 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
<b>Stage 1 Preparing for the investigation</b>			
<ul style="list-style-type: none"> <li>• The investigation is set in a decision-making context (<b>Decision-making Task</b>).</li> <li>• It is situated in an authentic context related to the daily-life application of enzymes (<b>Contextualisation</b>).</li> </ul>			
1	<ul style="list-style-type: none"> <li>• The teacher discusses the investigation context with students.</li> <li>• The teacher distributes <i>Worksheet 1</i>.</li> </ul>	40	<i>Worksheet 1</i>
<b>Stage 2 Designing the investigation</b>			
<ul style="list-style-type: none"> <li>• Students use a template to design their own experimental set-ups (<i>Investigation Planning Template</i>).</li> <li>• Students have the chance to evaluate their own and their peers' experimental set-ups (<i>Self &amp; Peer Evaluation</i>).</li> </ul>			
2	<ul style="list-style-type: none"> <li>• The teacher provides feedback on students' experimental designs in <i>Worksheet 1</i>.</li> </ul>	40	Student Samples 1
3	<ul style="list-style-type: none"> <li>• The teacher discusses with the students some questions related to the experimental design.</li> <li>• The teacher provides students with laboratory manual for preparation at home.</li> </ul>	40	Teacher Notes 1
<b>Stage 3 Carrying out the investigation</b>			
<ul style="list-style-type: none"> <li>• Students use microscale instrumentation that reduces the time of the experiments (<b>Microscale Instrumentation</b>).</li> <li>• Students collect more complex data sets by setting up replicates (<b>Complex Data Set</b>).</li> <li>• Students collect data using a template (<i>Data Collection Sheet</i>).</li> </ul>			
4	<ul style="list-style-type: none"> <li>• The teacher asks questions to help students connect their lab experience and related ideas/scientific inquiry skills.</li> <li>• Students carry out the investigation.</li> </ul>	40	Laboratory manual
<b>Stage 4 Explaining and evaluating data</b>			
<ul style="list-style-type: none"> <li>• Students assess the quality of the data collected, including the presence of anomalous data.</li> <li>• Students use the data to guide their decision as to which type of amylase to use for specific applications.</li> </ul>			
Before Lesson 5	<ul style="list-style-type: none"> <li>• Students complete data reporting and analysis at home.</li> <li>• The teacher collects and marks student responses.</li> </ul>		Teacher Notes 2
5	<ul style="list-style-type: none"> <li>• The teacher provides feedback on students' performance related to data reporting and analysis.</li> </ul>	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:

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

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*.



**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

To investigate the effect of temperature on the enzymatic activity of the three types of amylase

1 cm<sup>3</sup> potato cube (A method)

dropper  
amylase X/Y/Z solution from different temperature water baths (ice bath / water bath (80°C) / boiling water bath (100°C))  
timer

10 min later

dropper  
iodine solution  
1 cm<sup>3</sup> potato cube with amylase X/Y/Z solution from different temperature water baths

Then observe the iodine solution colour of

(10 min water bath)

thermometer  
Ice bath  
5 cm<sup>3</sup> of amylase X solution (Test tube A)  
5 cm<sup>3</sup> of amylase Y solution (Test tube B)  
5 cm<sup>3</sup> of amylase Z solution (Test tube C)  
beaker  
Timer

(10 min water bath)

thermometer  
water bath (80°C)  
5 cm<sup>3</sup> amylase X solution (test tube D)  
5 cm<sup>3</sup> amylase Z solution (test tube F)  
5 cm<sup>3</sup> amylase Y solution (test tube E)  
beaker  
Timer

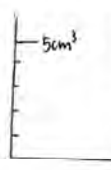
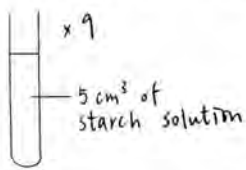
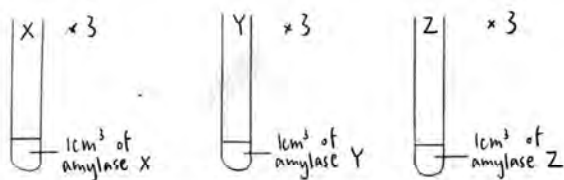
(10 min water bath)

thermometer  
boiling water bath (>100°C)  
5 cm<sup>3</sup> amylase solution (tube G)  
5 cm<sup>3</sup> amylase Y solution (tube H)  
5 cm<sup>3</sup> amylase Z solution (tube I)  
beaker  
Timer

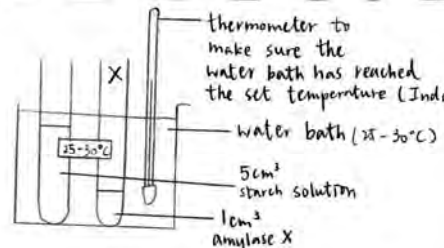
Possible questions

- 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?

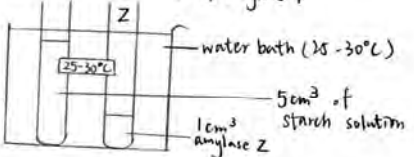
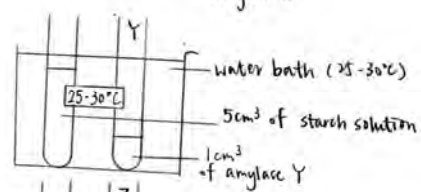
**Sample 2**



Beaker is used to measure the volume of starch solution and amylase used (controlled variables)  
 It is because amount of starch will affect the time taken to break down all the starch, thus affecting the estimated amylase activity. Also, the amount of amylase used will affect the rate of reaction

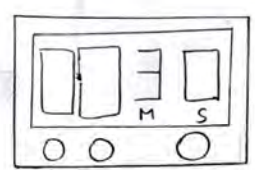
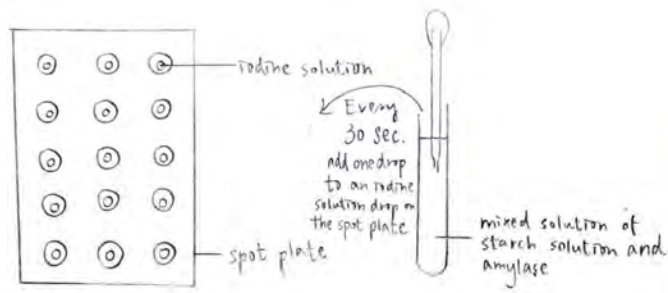
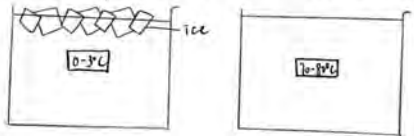


Put the test tubes added with different brands of amylase (X, Y, Z) into the water bath. (Independent variable)



Arrange the same set-up but replace the water bath with a different set temperature for the next trial.

Test tubes are put into water bath that has different set temperatures (0-5°C, 25-30°C, 70-80°C). (Independent variable)



A timer is used to count the time for each 30-second interval.

**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 it

### 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 there 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 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 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~~ activity of all 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.

## 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. 水浴槽的水量	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箇優點。
- (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。

**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 °C

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*.



<u>On Ice (0°C)</u>			
<u>Min</u>	<u>X</u>	<u>Y</u>	<u>Z</u>
0	○	○	○
5	○	○	○
10	○	○	○
15	○	○	○
20	○	○	○

<u>Room Temperature (~25°C)</u>			
<u>Min</u>	<u>X</u>	<u>Y</u>	<u>Z</u>
0	○	○	○
5	○	○	○
10	○	○	○
15	○	○	○
20	○	○	○

<u>80°C</u>			
<u>Min</u>	<u>X</u>	<u>Y</u>	<u>Z</u>
0	○	○	○
5	○	○	○
10	○	○	○
15	○	○	○
20	○	○	○

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

### 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 anomaly? 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			

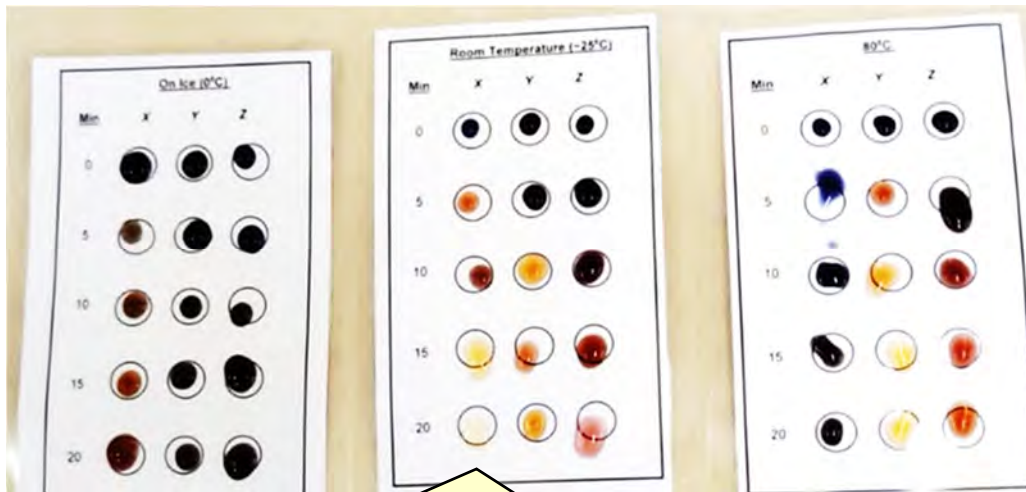
4. You noticed the differences in the sizes of the droplets (i.e. the reaction mixtures) taken from the reaction mixtures at different time points.
  - (a) Explain why this can affect the experimental results.
  - (b) Suggest and explain *one* way of reducing this error.



**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 anomaly? Why do you think so?

My data didn't show anomaly because the data didn't show anything wrong such as the colour suddenly become 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 turn 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 temperature of the dishwashing takes place 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 turn 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. The colour change of iodine solution is more obvious in X compare to other, which means amylase X breakdown more starch per minutes time.

### Sample 4

- (e) Chris made the following claim:

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

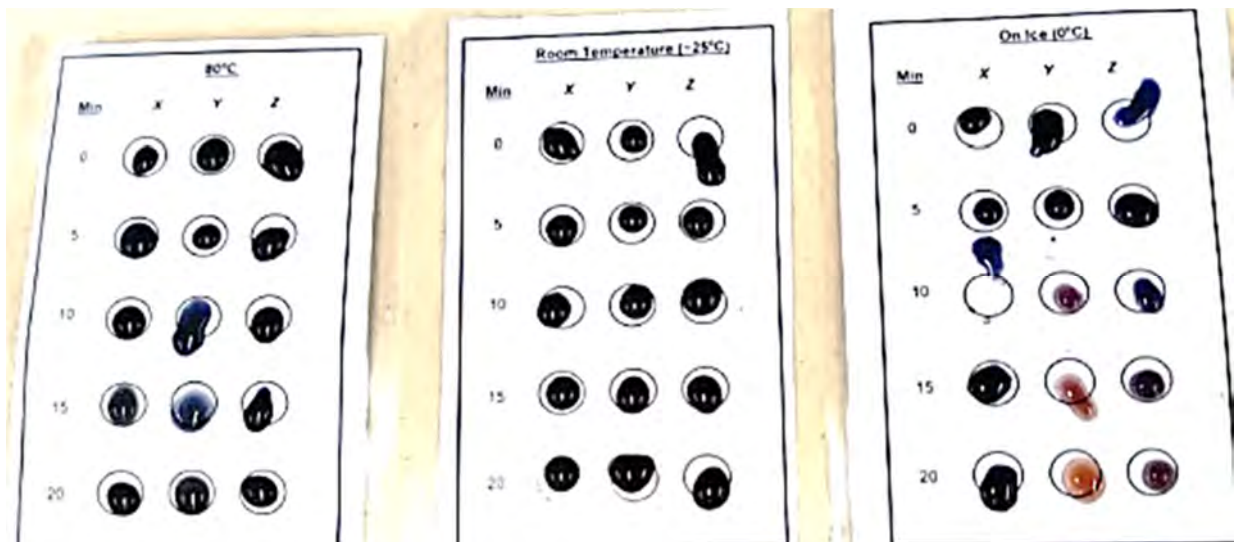
- (1) Explain whether your data support or reject this claim. U  B  G

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 anomaly? Why do you think so?

Yes, <sup>unrelated</sup> the result of the iodine solution turn to blue-black colour as we add too ~~many~~ <sup>Almost all</sup> 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 anomaly? Why do you think so?

Yes, <sup>unrelated</sup> 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 <i>Based on (1) and (2)</i>
Dishwashing	<i>Not match with your own results</i> Y, Z <del>X</del>	Y <del>X</del>	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°C.
Washing clothes	X, Y, Z <del>X</del>	X <del>X</del>	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 X? (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).

**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<sub>2</sub> 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<sub>2</sub> 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.

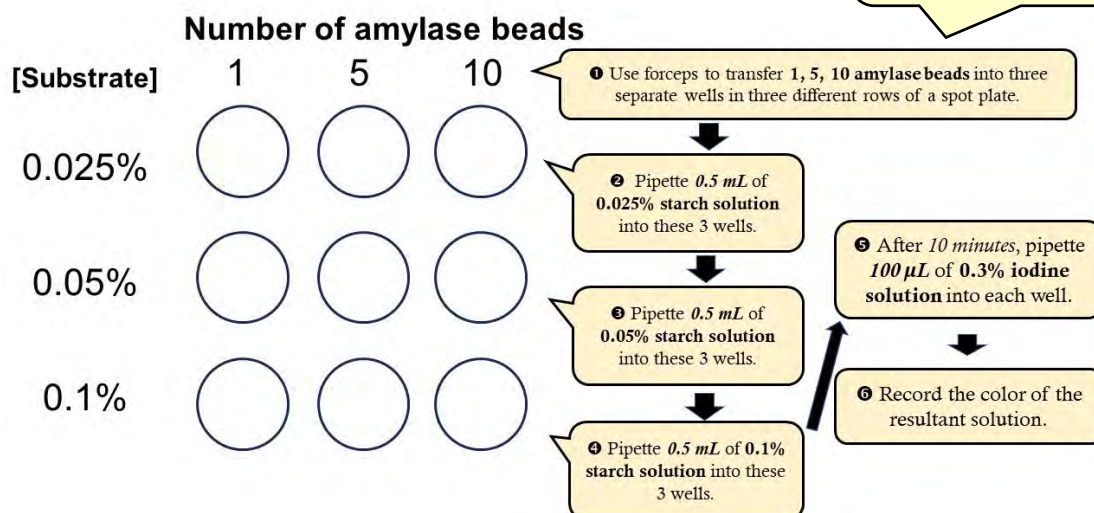


## Effect of substrate concentration on amylase activity

### 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  $\mu\text{L}$  of 0.3% iodine solution into each well.
6. Record the colour of the resultant solutions.

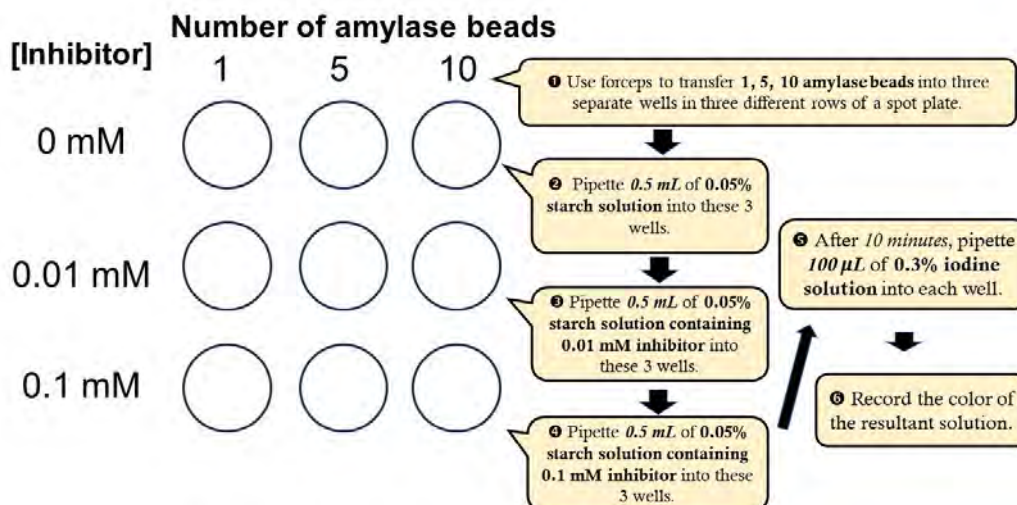
*Integrated Instruction Sheet facilitates students understanding of the procedures.*



## 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  $\mu\text{L}$  of 0.3% iodine solution into each well.
6. Record the colour of the resultant solutions.



### Materials for Task 3

#### *Chemicals to be prepared*

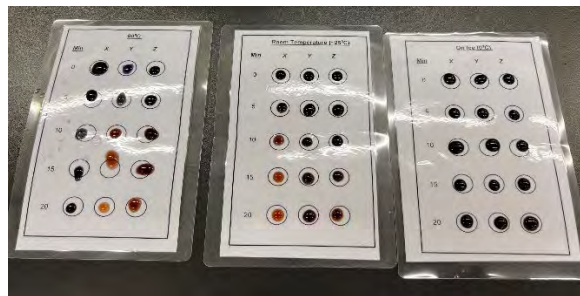
- Amylase X 0.05% (0.05 g in 100 mL)
- Amylase Y 0.05% (0.05 g in 100 mL)
- Amylase Z 0.05% (0.05 g in 100 mL) (or replaced with a lower % of Amylase Y)
- 0.5% starch (0.5 g in 100 mL) (Stored at 4<sup>o</sup>C)
- \* Amylase Y and Z are heat-resistant amylases.

#### *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		

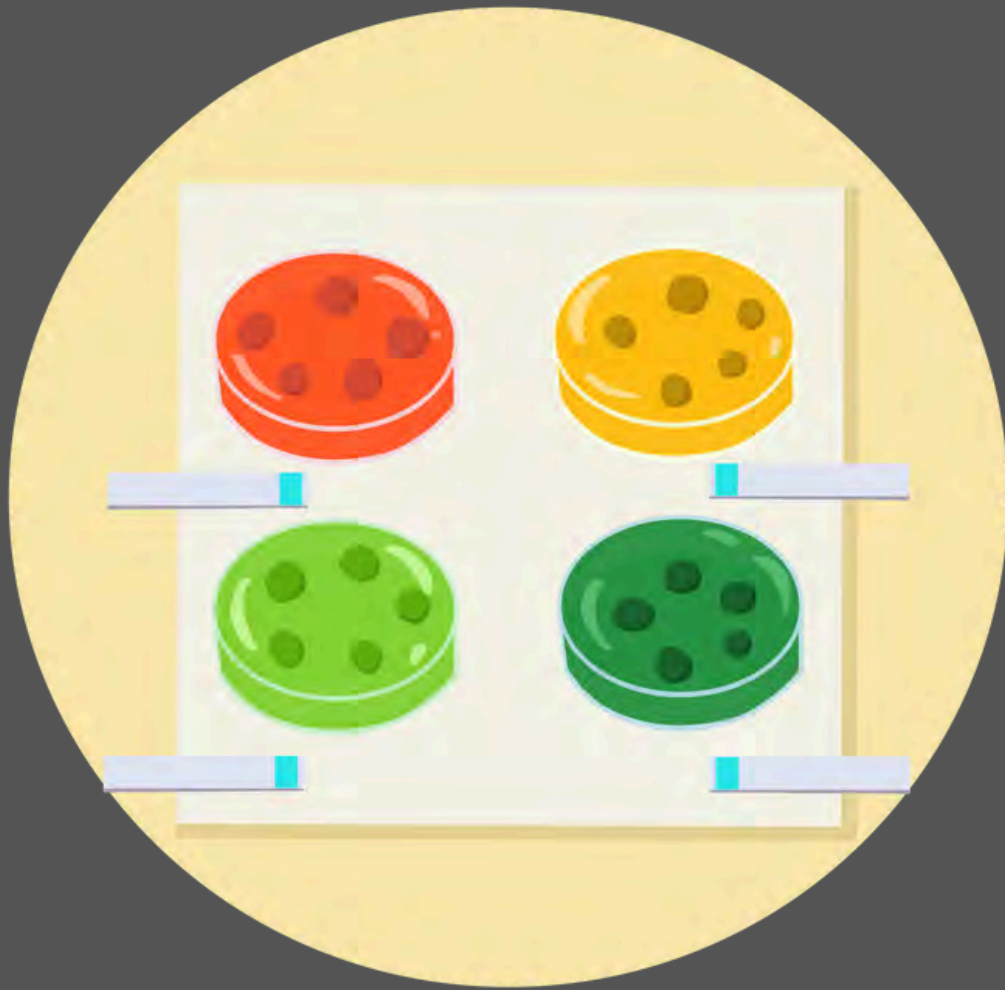
\* Dropper bottles can be replaced with glass vials and plastic droppers.

\* Do *not* use marker pen for labelling.



## References

- Chan, K. K. H., Ho, D. T. S., & Lau, D. S. P. (2024). Using amylase beads to investigate factors affecting enzyme activity. *The American Biology Teacher*, 86(3), 153–160.
- 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.



# **Yeast Bead Invertase Investigation**

---



# Yeast Bead Invertase Investigation

## Overview

- The *Yeast Bead Invertase Investigation* is about the industrial application of immobilised yeasts for the production of invert syrup.
- Immobilised yeasts, known as yeast beads, contain invertase (Bryer, 2016).
- Students investigate the effect of pH on the activity of yeast bead invertase.
- Students collect semi-quantitative data (i.e. the colour intensity of glucose test strips) to determine the invertase activity.
- Students are given the opportunity to design and carry out an experiment and assess the accuracy of the measurement tool, and the reliability of the data.

## Teaching Plan

*Prerequisite knowledge (scientific ideas)*

- Properties, actions, and roles of enzymes
- Factors that affect the actions of enzymes

Lesson	Lesson sequence	Duration (mins)	Resources
<b>Stage ① Preparing for the investigation</b>			
<ul style="list-style-type: none"> <li>• It is situated in an authentic context related to the industrial application of invertase in chocolate making (<b>Contextualisation</b>).</li> <li>• Students read information to familiarise themselves with the background of the investigation (<i>Reading Materials</i>).</li> </ul>			
Before Lesson 1	<ul style="list-style-type: none"> <li>• The teacher distributes <i>Worksheet 1</i> for students to complete at home so that they can be familiar with the background of the investigation.</li> </ul>		<i>Worksheet 1</i>
1	<ul style="list-style-type: none"> <li>• The teacher discusses the investigation context with students.</li> <li>• The teacher provides feedback on students' responses in <i>Worksheet 1</i>.</li> </ul>	40	Student Samples 1
<b>Stage ② Designing the investigation</b>			
<ul style="list-style-type: none"> <li>• Students interact with a virtual laboratory to familiarise themselves with the materials and apparatuses they use in the investigation (<i>Virtual Laboratory</i>).</li> <li>• Students use a template to design their own experimental set-ups (<i>Investigation Planning Template</i>).</li> <li>• Students have the chance to evaluate their own and their peers' experimental set-ups (<i>Self &amp; Peer Evaluation</i>).</li> </ul>			
2	<ul style="list-style-type: none"> <li>• The teacher presents the main investigation context and discusses with students questions related to their experimental designs.</li> <li>• The teacher provides students with the laboratory manual for preparation at home.</li> </ul>	40	Teacher Notes 1
<b>Stage ③ Carrying out the investigation</b>			
<ul style="list-style-type: none"> <li>• Students collect data using a template (<i>Data Collection Sheet</i>).</li> </ul>			
3	<ul style="list-style-type: none"> <li>• Teacher asks questions to help students connect their lab experience and related ideas/scientific inquiry skills.</li> <li>• Students carry out the investigation.</li> </ul>	40	Laboratory Manual
<b>Stage ④ Explaining and evaluating data</b>			
<ul style="list-style-type: none"> <li>• Students assess the limitations of the data collected in answering the investigation question.</li> </ul>			
Before Lesson 4	<ul style="list-style-type: none"> <li>• Students complete data reporting and analysis at home.</li> <li>• The teacher collects and marks student responses.</li> </ul>		Teacher Notes 2
4	<ul style="list-style-type: none"> <li>• The teacher provides feedback on students' performance related to data reporting and analysis.</li> </ul>	40	Teacher Notes 2

## Important Notes

- Students should avoid direct skin contact with buffer solutions.



# Instructional Materials

## Stage 1 Preparing for the investigation

### Student Worksheet 1



#### Notes for teachers

- Teachers can distribute *Worksheet 1* and ask students to read the background information related to the investigation and design their experimental set-ups at home.
- Teachers can collect students' drawing using a *Google Form*.
- Scan the QR code to get a copy of the *Google Form*.



#### Task 1

- Read the scenario and answer the questions that follow:

#### Scenario

Invertase is an enzyme that catalyses the breakdown of sucrose into fructose and glucose. It is widely used in the food industry to produce creams, jams, and artificial honey.

Yeast (*Saccharomyces cerevisiae*) is a rich source of invertase. Yeast cells are immobilised to form yeast beads, which can be easily removed from the sucrose solution and reused.

In this investigation, you will study yeast bead invertase. Read the information in the *Data File* to familiarise yourself with the background of this investigation. You will use your biological knowledge of enzymes and the design of valid and reliable experiments to complete this investigation.

#### Question

1. You have found that you can control the time for making invert sugar syrup by changing the temperature during the making of invert sugar syrup. Therefore, you want to investigate the effects of temperature on the activity of yeast bead invertase.

- You have been provided with the following materials:

20% Sucrose solution	Water bath	Plastic dropper
Yeast beads	Glucose test strips	Thermometer
Timer	Vials	Forceps
Measuring cylinder	Spotting plate	Colour chart
White tile	Spoon	

- You may also make use of other common apparatuses in the laboratory.

The virtual laboratory provides students with opportunities to get familiar with materials used in the investigation.



Scan the QR code to see the materials

- (a) Briefly describe how you would use the above materials to plan an experiment to achieve the aim of the investigation.

- You can draw your experimental design.
- Write down any important experimental design decisions
- The *Investigation Planning Chart* (scan the QR code) can help you with this.

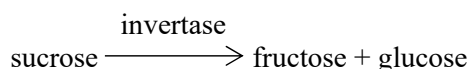


## Data File

Your biology teacher asks you to read the following source materials to prepare for planning a scientific investigation on yeast bead invertase:

### Source 1: Industrial application of invertase

Invertase is an enzyme that catalyses the breakdown of sucrose into fructose and glucose.



Invertase is commonly used in the food industry to produce 'invert syrup' from sucrose solutions. Invert syrup contains a mixture of fructose and glucose from sucrose treated with invertase. The products formed in the enzyme reaction (i.e., fructose and glucose) have a higher solubility than the substrate (i.e., sucrose).



A well-known use of invertase is in the production of chocolates with a soft centre, such as *Lindt Lindor* milk chocolate truffles. One way to make this type of chocolate is to add a small amount of invertase to the solid sugar filling, which consists of table sugar (sucrose). The mixture of fructose and glucose from the sucrose treated with invertase becomes liquid in the chocolate shell.



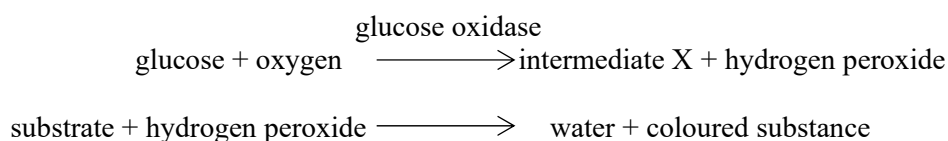
Scan the QR code to learn more about how invertase is used in the food industry.



### Source 2: Testing for glucose concentration in urine samples

Glucose is found in urine samples from patients with diabetes. A convenient way to detect the presence of glucose in urine is to use a glucose paper test strip.

A glucose test strip contains *glucose oxidase*. This enzyme catalyses the conversion of oxygen into hydrogen peroxide. The hydrogen peroxide produced reacts with the substrate on the test strip to form a coloured substance. The simplified reactions are as follows:



After dipping in the urine sample, the paper test strips change colour if glucose is present. The resulting colour indicates the concentration of glucose semi-quantitatively.

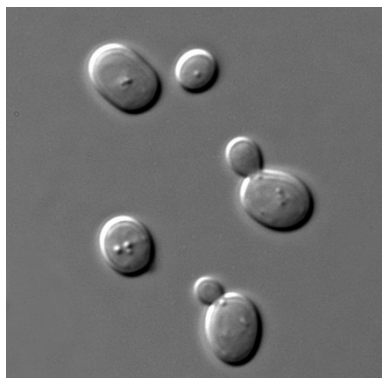


Scan the QR code to learn how to use a glucose test strip.

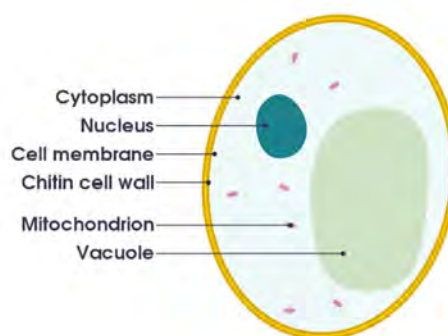


**Source 3:** What are yeast and yeast beads?

Yeast (*Saccharomyces cerevisiae*) is a eukaryotic organism. A eukaryotic cell has a true nucleus and membrane-bound organelle. Although yeast cells have cell walls, the chemical composition of their cell walls is different from that of plant cells. The following diagrams show yeast cells under a light microscope and a drawing of a yeast cell, respectively.



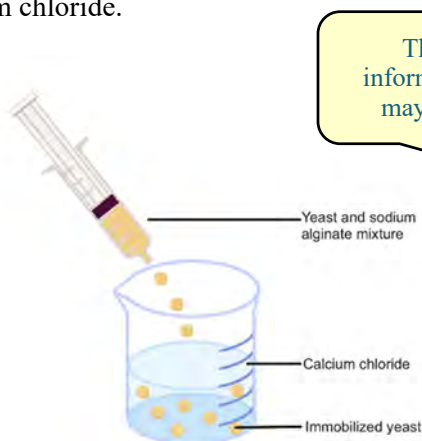
Yeast cells under a microscope



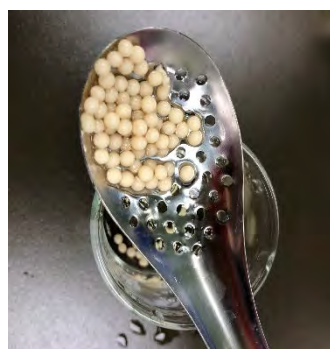
Drawing of a yeast cell

Yeast cells contain invertase, which catalyses the conversion of sucrose to fructose and glucose.

Scientists use yeast for many industrial applications, including brewing beer and making bread. In some applications, scientists immobilise whole yeast cells to form yeast beads using sodium alginate and calcium chloride.



The reading materials provide information about yeast beads, which may be unfamiliar to the students.



Yeast in alginate solution + Calcium chloride  $\longrightarrow$  Yeast beads

*(Yeast immobilised in insoluble calcium alginate)*

Immobilised yeasts are also active. The yeast beads can be collected and reused after the reaction.



Scan the QR code to learn how to make yeast beads.



### 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

轉化酶 (Invertase) 是一種可以催化蔗糖分解為果糖和葡萄糖的酶。它在食品工業中被廣泛用於生產奶油、果醬和人工蜂蜜。

酵母(*Saccharomyces cerevisiae*)是一種豐富的轉化酶來源。酵母細胞被固定化以形成酵母凝膠珠，可輕易地從蔗糖溶液中取出並重複使用。

在這次探究，你將研究酵母凝膠珠轉化酶。閱讀資料檔中的資訊，了解這次探究的背景。你將利用你對酶的生物知識以及透過設計有效且可靠的實驗來完成這次探究。

### 問題

你發現在製造反轉糖漿的過程中，通過改變 pH 值可以控制製造反轉糖漿的時間。因此，你想研究 pH 值對酵母凝膠珠轉化酶活性的影響。

- 你收到以下材料和儀器：

20%的蔗糖溶液	水浴
酵母凝膠珠	葡萄糖試紙
計時器	小瓶
量筒	點滴板
白色瓷磚	勺子
溫度計	塑料滴管
鑷子	顏色圖

你也可以運用實驗室常用的物料及儀器。



掃描此二維碼以  
查看實驗材料

(a) 簡要描述你將如何使用上述材料來設計探究，以達到上述目標。

- 你可以畫出你的實驗設計。
- 請寫下有關實驗的重要設計決定。
- 你可參考實驗策劃模板(掃描二維碼)。

掃描二維碼以獲取  
實驗策劃模板



掃描二維碼以獲取  
Google Form 的副本



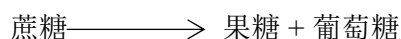
## 資料檔案

你的生物老師要求你閱讀下面的資料，為設計一個與酵母凝膠珠轉化酶有關的科學探究做準備。

### 資料 1: 轉化酶的工業應用

轉化酶 (Invertase) 是一種可以催化蔗糖分解為果糖和葡萄糖的酶。

#### 轉化酶



轉化酶在食品工業中被廣泛用於從蔗糖溶液生產“反轉糖漿”。反轉糖漿含有經轉化酶處理後蔗糖所產生的果糖和葡萄糖混合物。酶反應形成的產物(即果糖和葡萄糖)比起起始物(蔗糖)具有更高的溶解度。



轉化酶在生產軟心巧克力(如瑞士蓮牛奶朱古力夾心)中也有著名的用途。製作這類朱古力的一種方法是在堅硬的糖餡料(即蔗糖)中添加少量轉化酶。經轉化酶處理的蔗糖不再成為固體，而是成為朱古力外殼內的液體餡料。



掃描二維碼，可了解更多關於轉化酶在食品工業中的應用。



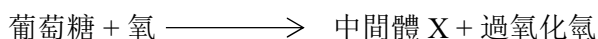
## 資料 2: 尿液中葡萄糖濃度的測試

糖尿病患者的尿液中存在葡萄糖。使用葡萄糖試紙是一種檢測尿液中葡萄糖的便捷方法。

葡萄糖試紙含有葡萄糖氧化酶。這種酶可以催化氧氣轉化為過氧化氫。產生的過氧化氫與試紙上的底物發生反應，形成一種著色物質。這一過程可以概括如下：



葡萄糖氧化酶



將試紙浸入尿液後，如果尿液中含有葡萄糖，試紙會發生顏色變化。最終的顏色可以半定量地反映葡萄糖的濃度。

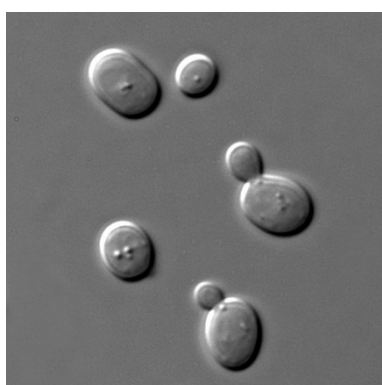


掃描二維碼，了解如何使用葡萄糖試紙。

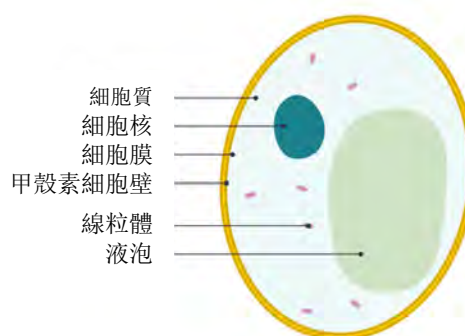


## 資料 3: 什麼是酵母和酵母凝膠珠?

酵母(*Saccharomyces cerevisiae*)是一種真核生物。真核細胞具有真正的細胞核和多種具有膜結構的細胞器。雖然酵母細胞有細胞壁，但其細胞壁的化學組成成分與植物細胞的不同。下圖分別為顯微鏡下的酵母細胞圖和酵母細胞繪圖。



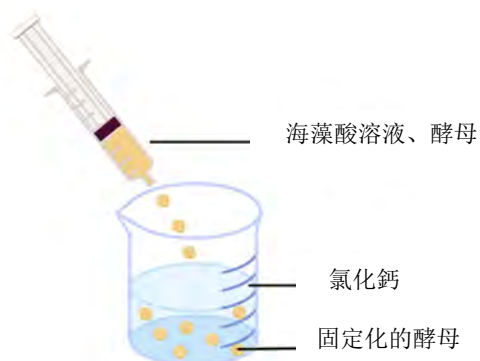
顯微鏡下的酵母細胞圖



酵母細胞繪圖


酵母細胞含轉化酶。轉化酶能催化蔗糖分解為果糖和葡萄糖

科學家利用酵母進行許多工業應用，包括釀造啤酒、製作麵包等。在某些應用中，科學家使用海藻酸鈉和氯化鈣使整個酵母細胞固定，以形成酵母凝膠珠。



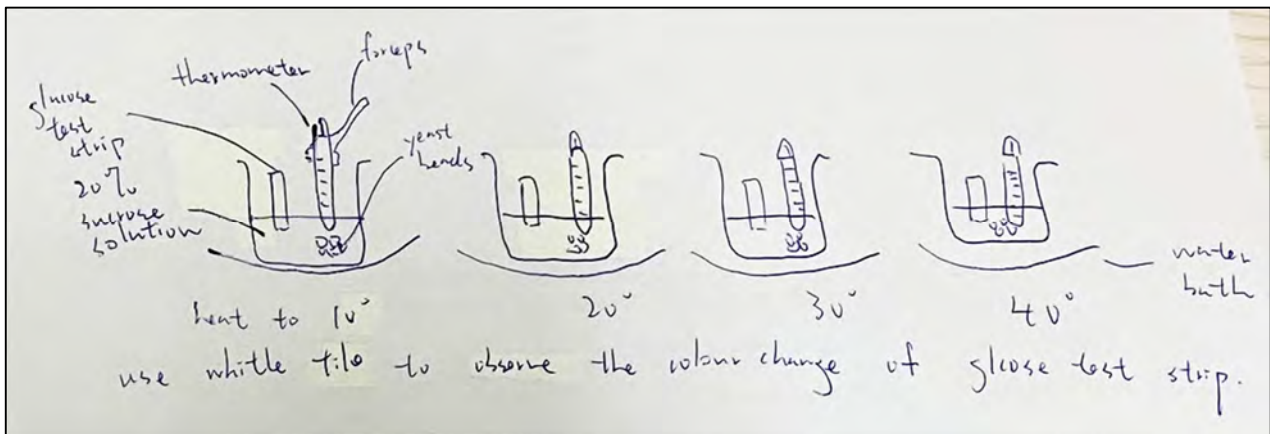
海藻酸溶液中的酵母 + 氯化鈣  $\longrightarrow$  酵母凝膠珠  
(酵母在非溶性的海藻酸鈣中被固定)

固定化的酵母仍然有活性。酵母凝膠珠在反應後可被回收及重用。

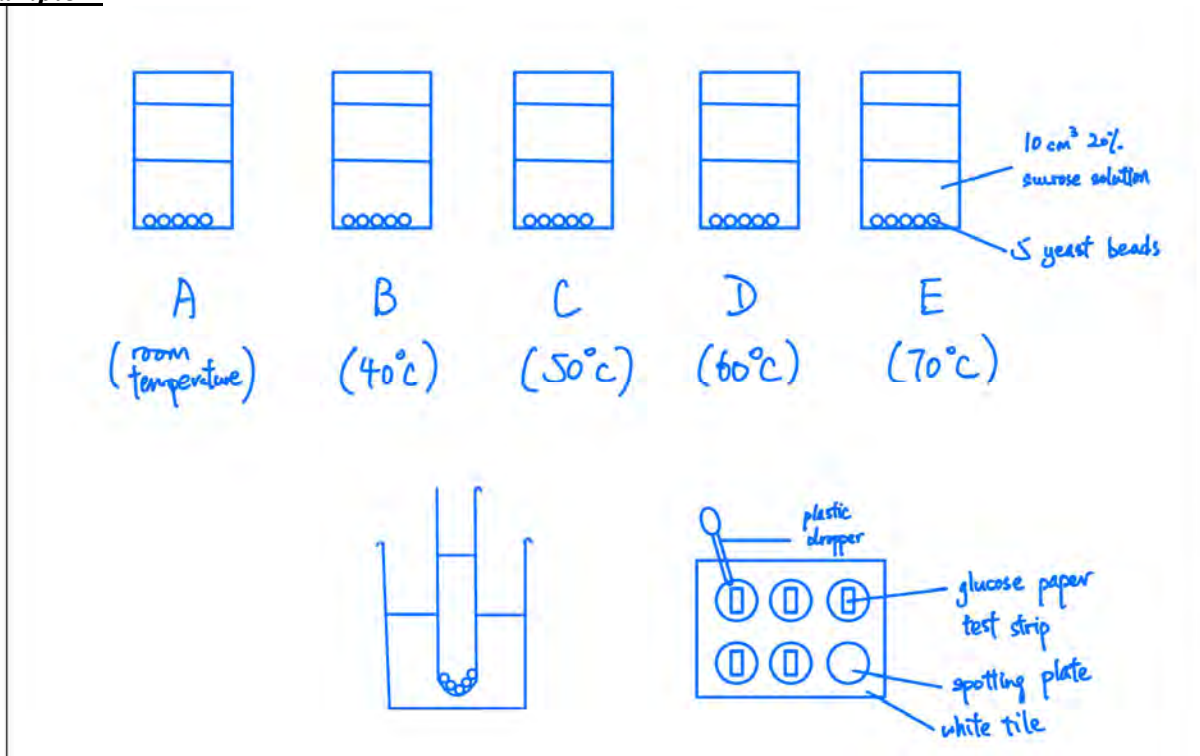
 掃描二維碼以查看如何製作酵母凝膠珠。



Sample 1



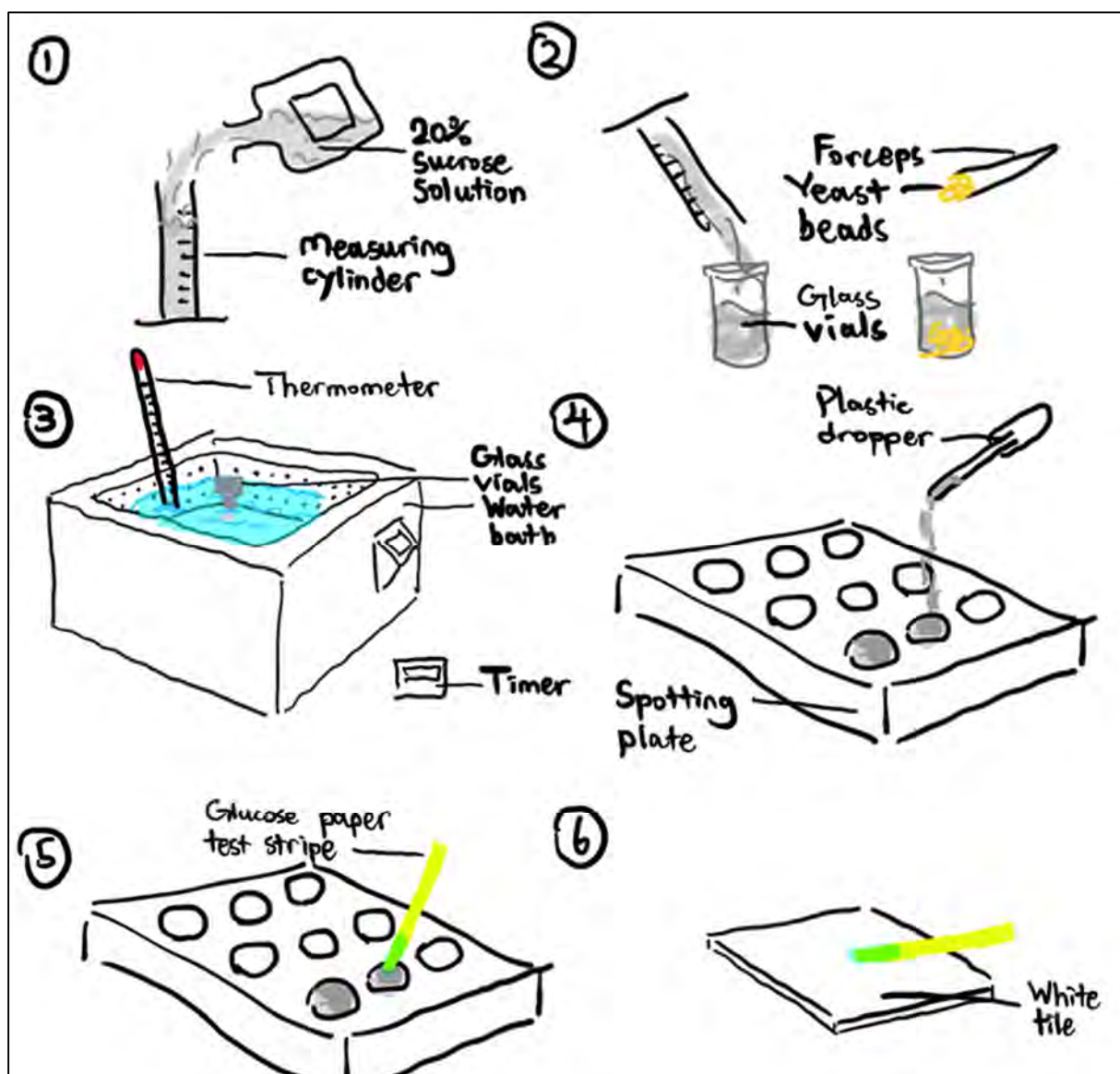
Sample 2



Brief explanation of my design:

Firstly, make some yeast beads by using a plastic dropper. Next, measure 10 cm<sup>3</sup> of 20% of sucrose solution by using a measuring cylinder, then transfer the solution into a glass vial and repeat this step for four times. After that, use a water bath and put a thermometer to seek the respective wanted temperatures of the solution, also set a timer for 12 mins. Then, transfer five yeast beads into each glass vial and start the timer once the beads are ur into the vial by a spoon. Afterwards, place a spotting plate on a white tile and add five glucose paper test strips on the plate by a forcecep while waiting the experiment to complete. After 12 minutes, use a plastic dropper to add two drops of each of the mixture to the plate. At last, observe the colour change of the test paper to deduce how much sucrose has been converted into glucose.

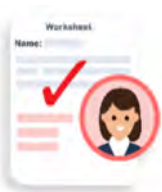
### Sample 3



#### Notes for teachers

- Teachers can select student drawings (anonymised) for discussion.
- Teachers can discuss the following scientific inquiry skills: (1) range and interval of independent variable; (2) measurement of dependent variable; (3) significant assumptions; (4) important precautionary steps; (5) number of yeast beads used.





**Notes for teachers**

- The following shows the main investigation context that requires students to design an investigation with another independent variable.
- There are some questions that teachers may use to guide students in thinking about or assessing the scientific inquiry skills related to experimental designs.
- Some student work samples are shown below to illustrate possible student thinking.

**Task 2**

**Scenario**

Invertase is an enzyme that catalyses the breakdown of sucrose into fructose and glucose. Invertase is often used in the food industry for the production of invert syrup from sucrose.

Yeast (*Saccharomyces cerevisiae*) is a rich source of invertase. Yeast cells are immobilised and form yeast beads that can be easily removed from the sucrose solution and reused.

Since sucrose solutions with different food additives have different pH values, the efficiency of the yeast beads in converting sucrose into fructose and glucose may be different. In this study, you would like to investigate the effect of pH on the invertase activity of the yeast beads.

**Design of the investigation**

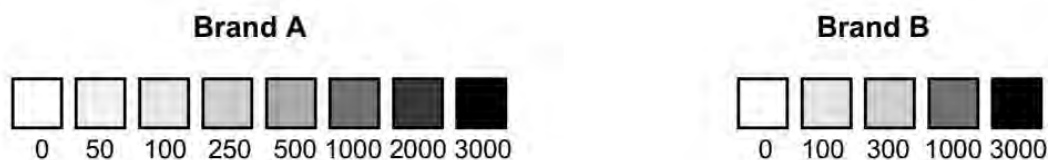
Your teacher has given you the following materials:

5% Sucrose solution at pH 3, 5, 7, 9	Distilled water	Timer
Buffer solution* (pH = 3, 5, 7, 9)	Glucose test strip	Forceps
Yeast bead	Petri dish	Spoon
Plastic vial	Measuring cylinder	White tile
Spotting plate	Plastic dropper	Colour chart

\* Buffer solutions are used to maintain the pH of the solution mixture.

**Possible questions**

1. You have found two different brands of glucose paper test strips in the laboratory. Below you can see the colour charts of the two brands, which you can use to determine the concentration of glucose.



Which brand, A or B, will you use in this investigation? Why?

2. State *one* significant assumption in this investigation.  
(An assumption is something we think it is true, though we cannot be sure. A significant assumption is the one that the experiment cannot make any conclusion without assuming it to be true).

3. Your teacher has also given you the following reminders:

Terms are defined using student-friendly language.

*Reminders*

- Place the yeast beads in the petri dish containing the buffer solution for at least 5 minutes before mixing with the sucrose solution.
- Gently shake the plastic vials with the yeast beads and the sucrose solution from time to time.

Suppose you have overlooked your teacher’s reminders and

- (a) forgot to put the yeast beads into the buffer solution before adding them to the sucrose solution.
- (b) shook the plastic vials too vigorously and spilled half of the sucrose solution out of the plastic vials.

Explain how each of these mistakes would impact the experimental results.

*Hints:* Be sure to include the following parts in your answers:

- the effect on the data being collected
- explanation for the effect

The checklist serves to scaffold students’ responses.

	Impact on experimental results
(a)	
(b)	



**Notes for teachers:**

- Q.1 assesses students’ ability to reduce measurement errors by choosing the glucose test strip brand that is more sensitive.
- Q.2 assesses students’ ability to identify the significant assumption.
- Q.3 assesses students’ ability to explain how specific steps can impact on the validity of the data.

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

Sample 1

We assume that every yeast bead works the same

Sample 2

That all yeast beads are equally efficient at breaking down sucrose if placed in the same conditions.



**About the samples**

- Neither sample mentioned the significant assumption concerning the relationship between the measurement and the dependent variable.

The following examples demonstrate varying levels of sophistication in quality:

**Unattained**

- Environmental conditions are the same.
- Yeast beads have the same size and shape.

**Basic**

- All yeast beads work the same.

**Good**

- Amount of invertase in each yeast bead is the same.

**Excellent**

- Glucose is only contributed by the activity of invertase in the yeast beads.

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

### Sample 1

Impact on experimental results	
(1)	The pH value of sucrose solution will be affected, because if yeast beads didn't put into buffer solution, the pH value of yeast beads and sucrose solution will be different. So the result won't be the pH you want.

### Sample 2

Impact on experimental results	
(1)	The amount of glucose may be higher initially for the set-ups with lower pH. The buffer solution ensures that the yeast beads are already at the pH of the sucrose solution. In this case, the yeast beads will only change its pH once in contact with the sucrose solution, meaning that it will not be denatured beforehand and more of the enzymes will be able to break down sucrose into glucose.

### Sample 3

Impact on experimental results	
(1)	Since <del>it is not</del> at yeast beads were not added into their respective buffer solutions, pH of yeast beads are not the same <del>as</del> with sucrose solutions, that the pH of solution obtained after the experiment is different from the initial pH of the sucrose solution, resulting in either higher or lower glucose concentration <del>is</del> in the solution obtained.



#### About the samples

- Sample 1 did not clearly explain how omitting the precautionary step would impact the yeast beads' ability to reach the desired pH at the start of the experiment.
- Both Samples 2 and 3 described the effect of this missing step. Sample 3 specifically stated that the effect could result in either a higher or lower glucose concentration, depending on the pH profile of the invertase enzyme. In contrast, Sample 2 assumed a lower pH would lead to higher invertase activity, despite lacking experimental evidence to support that claim.

## 任務 2

### 情境

轉化酶是一種催化蔗糖分解為果糖和葡萄糖的酶。轉化酶通常用於食品工業，將蔗糖中生產轉化糖漿。

酵母(*Saccharomyces cerevisiae*)是轉化酶的一個豐富來源。酵母細胞被固定形成酵母凝膠珠，可以很容易地從蔗糖溶液中移除並重新使用。

由於含有不同食品添加劑的蔗糖溶液具有不同的 pH 值，酵母凝膠珠將蔗糖轉化為果糖和葡萄糖的效率可能不同。在這項研究中，你想研究 pH 值對酵母凝膠珠中的轉化酶活性的影響。

### 實驗設計

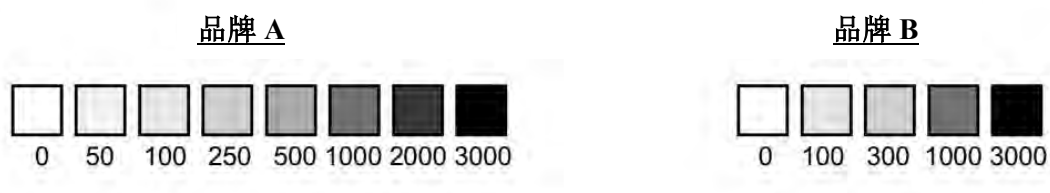
你的老師給了你提供了以下物料。

pH 值分別為 3、5、7、9 的 5% 的蔗糖溶液	蒸餾水	計時器
緩衝溶液* (pH = 3, 5, 7, 9)	葡萄糖試紙	鑷子
酵母凝膠珠	培養皿	勺子
塑料小瓶	量筒	白色瓷磚
點滴板	塑料滴管	顏色圖

\* 緩衝溶液是用來維持溶液混合物的 pH 值的。

### 參考問題

- 你在實驗室找出了兩種不同品牌的葡萄糖試紙。下面你可以看到這兩個品牌的顏色圖，你可以用它來測定葡萄糖的濃度。



在這次實驗中，你將使用哪個品牌，A 或 B？為什麼？

2. 說明這項探究中的一個重要假設。(假設是我們認為是真實的東西，儘管我們不能確定。一個重要的假設是，如果不假設它是真的，實驗就不能得出任何結論)。
3. 你的老師也給了你以下提示。

提示

- 在與蔗糖溶液混合之前，將酵母凝膠珠放在含有緩衝溶液的培養皿中至少 5 分鐘。
- 不時輕輕搖動裝有酵母凝膠珠和蔗糖溶液的塑料小瓶。

假設你忽略了老師的提示，並且

- (a) 忘了在加入蔗糖溶液前將酵母凝膠珠放入緩衝溶液中。
- (b) 過於激烈地搖晃塑料小瓶，從塑料小瓶中溢出一半的蔗糖溶液。

解釋一下這些錯誤會如何影響實驗結果。

提示：請確保在你的答案中包括以下部分。

- 對正在收集的數據的影響
- 對該影響的解釋

	對於實驗結果的影響
(a)	
(b)	

**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 (e.g., how many glucose test strips do you need for this experiment? Why?).
- The *Supplementary Resource* section contains the list of materials.
- Teachers can print the *Data Collection Sheet* and laminate the printouts for use in class.
- Scan the QR code to view the process of the experiment.

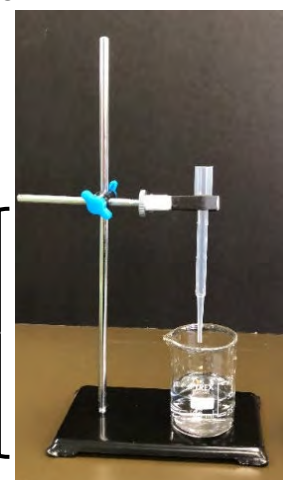
**Task 3:**

- Read the following procedures to carry out the investigation.

**Procedure****Preparation of yeast beads**

1. Add 10 mL of 10% yeast (in a vial) to 10 mL of 2% sodium alginate solution in a 50 mL-tube.
2. Mix the solution well by inverting the 50 mL-tube to make a yeast–sodium alginate solution.
3. Hold the plastic dropper (without cap) with a stand and clamp.
4. Pour 50 mL 2%  $\text{CaCl}_2$  (calcium chloride) into a plastic cup/100 mL-beaker.
5. Assemble the set up shown in *Figure 1*.
6. Add the yeast sodium alginate solution to the plastic dropper (a bead should form when the drop comes into contact with the  $\text{CaCl}_2$  solution and falls to the bottom of the beaker).
7. Wait 5 minutes until the beads have hardened.
8. Discard any floating yeast beads with a plastic spoon.
9. Collect the beads with a sieve.
10. Wash the beads several times with distilled water from a wash bottle over a plastic cup.

&gt; 17 cm

*Figure 1***Incubation of the yeast beads in buffer solution**

1. Add 5 mL buffer solution (pH = 3.0, 5.0, 7.0, 9.0) to 4 different petri dishes.
2. Use a spoon to gently move at least 15 yeast beads into each buffer solution (pH = 3.0, 5.0, 7.0, 9.0).
3. Wait at least 5 minutes.

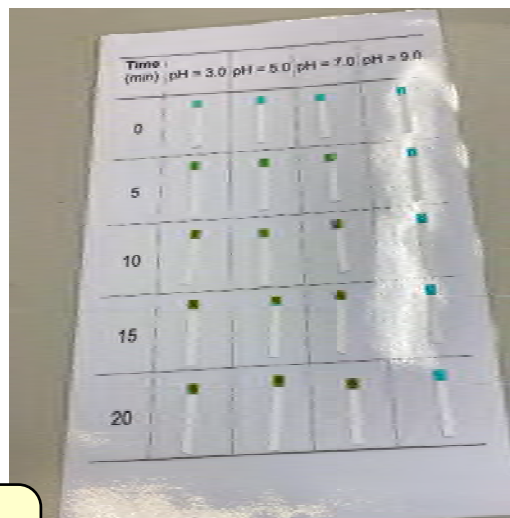
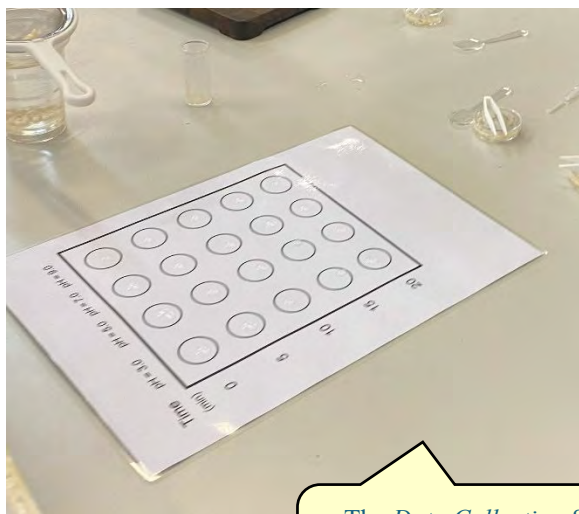
### Testing the invertase activity

1. Transfer 5 mL of the sucrose solution with a pH of 3.0 into a plastic vial.
2. Repeat *Step 1* with the sucrose solution with different pH values (5.0, 7.0 and 9.0).
3. Transfer 15 yeast beads into each plastic vial using a spoon and a pair of forceps.
4. At time = 0 minute, remove a small drop of sample from each vial with a plastic dropper and place it on the laminated spotting plate sheet.
5. Close the plastic vial and swirl it gently from time to time.
6. Repeat *Step 4* at time points 5, 10, 15 and 20 minutes.
7. When you have collected all the samples, dip the glucose paper test strip into each sample.
8. Observe and record the colour change, if any, after 1 minute.
9. Determine the glucose concentration from the colour chart.

#### Notes for teachers



- Remind the technician to adjust the relative concentration of sucrose solution as different brands of glucose test strips have different sensitivity.
- Laminated data collection sheets are commonly used in microscale activities.
- Scan the QR code for copy of the *Data Collection Sheet*.



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

### 任務 3:

- 閱讀以下實驗步驟以進行探究:

#### 實驗步驟

##### 酵母凝膠珠的製備

1. 將 10 mL 10%的酵母(在小瓶中)加入到盛有 10 mL 升 2%的海藻酸鈉溶液的 50 mL 試管中。
2. 倒置 50 mL 試管將溶液充分混合，使之成為酵母-海藻酸鈉溶液。
3. 用支架和夾子夾住塑料滴管(無蓋)。
4. 將 50 mL 2%的  $\text{CaCl}_2$  (氯化鈣)倒入一個塑料杯中/100 mL 燒杯。
5. 組裝圖 1 中所示的裝置。
6. 在塑料滴管中加入海藻酸鈉酵母溶液(當滴管接觸到  $\text{CaCl}_2$  溶液並落到燒杯底部時應形成一個珠子)。
7. 等待 5 分鐘，直到珠子變硬。
8. 用塑料勺子丟棄任何漂浮的酵母凝膠珠。
9. 用篩子收集珠子。
10. 用洗瓶中的蒸餾水沖洗幾次塑料杯上的酵母凝膠珠。

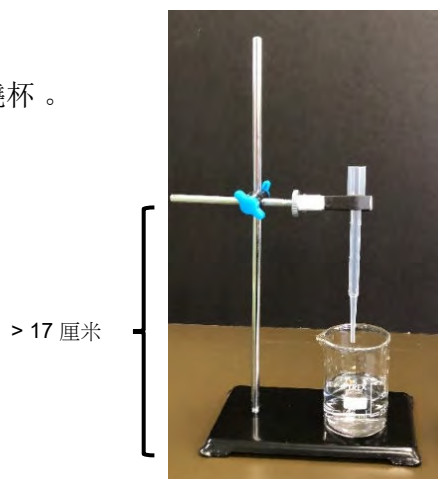


圖 1

##### 酵母凝膠珠在緩衝溶液中的培育

1. 在 4 個不同的培養皿中加入 5 mL 的緩衝溶液(pH=3.0, 5.0, 7.0, 9.0)。
2. 用勺子將至少 15 個酵母凝膠珠輕輕移入每個緩衝溶液(pH=3.0, 5.0, 7.0, 9.0)。
3. 等待至少 5 分鐘。

##### 測試轉化酶的活性

1. 將 5 mL pH 值為 3.0 的蔗糖溶液轉移到一個塑料小瓶中。
2. 用不同 pH 值的蔗糖溶液(5.0、7.0 和 9.0)重複步驟 1。
3. 用一個勺子和一把鑷子將 15 個酵母凝膠珠轉移到每個塑料瓶中。
4. 在時間為 0 分鐘時，用塑料滴管從每個小瓶中取出一小滴樣品，並將其放置在薄板狀的點滴板上。
5. 關上塑料小瓶，並不時地輕輕旋轉。
6. 在 5、10、15 和 20 分鐘的時間點重複步驟 4。
7. 當你收集完所有的樣品後，將葡萄糖紙測試條浸入每個樣品。
8. 觀察並記錄 1 分鐘後的顏色變化(如果有的話)。
9. 根據顏色圖確定葡萄糖的濃度。

掃描二維碼以獲取數據  
收集表的副本



## Teacher Notes 2

**Notes for teachers**

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

**Task 4**

1. Based on the data collected, describe and explain the effect of pH on yeast bead invertase activity.
2. Tom performed the same experiment. He found that all the glucose test strips of the samples at pH 7 and 9 at 0, 5, 10, and 20 minutes gave a negative result.

You found that your group's results are similar to the general trend observed in the class data. You suspected that Tom's results are anomalous data and had some errors.

- (a) By comparing your results with those of Tom, identify the possible errors in Tom's results.
  - (b) How would you further confirm that the results by Tom are anomalous?
  - (c) Explain the possible causes for the errors.
3. Tom would like to determine the optimum pH of yeast bead invertase accurately.
    - (a) Based on the data, explain the limitations of the experimental design in finding the optimum pH of yeast bead invertase.
    - (b) Describe how you would modify this experiment to obtain a more accurate estimate of the optimum pH of yeast bead invertase.

**Notes for teachers**

- Q.1 assesses students' ability to describe and explain data in simple data sets.
- Q.2 assesses students' ability to identify data inconsistent with the general trend or patterns observed within the class data and suggest ways to confirm if the data are anomalous as well as explanations for the occurrence.
- Q.3 assesses students' ability to assess the adequacy of the selection of the range and interval of the independent variable in determining the optimum pH of the yeast bead invertase, and suggest possible modifications to improve the accuracy of the designs to achieve this aim.

The following are some examples of student responses to Q.1:

### Sample 1

[Did you notice there are "+" sign on the chart of the test strip for each colour? I assume you did.]  
According to the experimental results, the rate of the colour intensity change from green to brown of the glucose test strips dipped into the target solution increases as the pH value increases. <sup>(looking at horizontal rows)</sup> ~~The~~ colour intensity of the glucose test strip starts to change from green to greenish, at 10 minutes for pH 3 solution, while it started to change in 5 mins for pH 9 solution. However, there is a abnormal data in the pH 5 solution, the colour intensity changes from green to brown colour at 10 mins which doesn't follow the trend of colour intensity change. <sup>so does pH 3's at 20 mins...</sup>

What's the point of this comparison? What's the aim of the expt? How does pH affect enzyme's activities based on yr knowledge?  
The result shows that the invertase activity increases as the pH value of the environment increases. <sup>Does this even make sense to you?</sup> The colour intensity of the glucose test strip changes fastest from green to greenish brown (at 5 mins) at pH 9 solution and turns out to show the deepest brown colour on the glucose test strip at the end (20 mins), showing that more glucose is produced by breaking sucrose by invertase at a higher rate at pH 9. pH 9 is the pH value that is the closest to the optimum pH value of invertase. <sup>how about pH 3 at 20 mins?</sup> At an environment of optimum pH level or very close to optimum pH, the invertase activity will be high and hence. <sup>the highest</sup> it can form more enzyme - substrate complex with sucrose at a higher rate to catalyse the break down of sucrose into glucose hence the content of glucose increase and that of sucrose decreases by time.

### Sample 2

As the pH of sucrose solution increased from 3 to 5, the color intensity of glucose test strip remained the same for the first 15 minutes. However, at the 20th minute, the color intensity of glucose test strip of pH 5 was higher than that of pH 3.

As the pH of sucrose solution increased from 5 to 9, most of the color intensity of glucose test strip at their respective time frame decreased, while only some remained unchanged. ??

As time went on, the color intensity of glucose test strips increased. ~~not for pH 9.~~

As the optimum pH of invertase is at pH 4.5, when pH of sucrose solution increased from 3 to 5, the pH was closer to the optimum pH. Hence as pH of sucrose solution increased from pH 3 to 5, a smaller proportion of invertase would be denatured, so the chance of formation of enzyme-substrate complex increases, more sucrose can be broken down to fructose and glucose, hence the invertase activity increases.

better writing is needed to avoid that alternative meaning that denaturation is reversible

However, as pH of sucrose solution increased from 5 to 9, the pH became further away from the optimum pH. Hence as pH of sucrose solution increased from pH 5 to 9, a larger proportion of invertase would be denatured, so the chance of formation of enzyme-substrate complex decreases, less sucrose can be broken down to fructose and glucose, hence the invertase activity decreases.

### Sample 3

Among all pH, <sup>who?</sup> pH7 took the shortest time to produce the largest amount of glucose. Also, after calculating the invertase activity by glucose amount/time, it is shown that the invertase activity increased from pH3 to pH7, but decreased from pH7 to pH9, the invertase activity was the highest in pH7. This indicates that pH7 is the optimum pH.

At optimum pH, which is pH7, invertase activity is at its maximum. While at unsuitable pH, invertase activity decreases because unsuitable pH causes denaturation of enzymes. Substrates can no longer fit into the active site of enzymes to form enzyme-substrate complex: as the shape of active sites are changed, this causes invertase to slowly lose its catalytic ability permanently and results in above results.



#### About the samples

- Sample 1 did not describe the trends and patterns observed in the data obtained and did not provide explanation for the trends and patterns observed.
- Sample 2 described the trends and patterns observed in the data obtained, but the description lacked clarity. In the explanation, it was wrongly stated that the optimum pH was 4.5, and the explanation did not explain why extreme pH would denature the enzyme.
- Sample 3 described the pH profile of the yeast bead invertase by relating it to the data obtained. It also explained the effect of extreme pH on the enzyme activity.

## 任務 4

### 參考問題

1. 根據獲得的數據,描述並解釋 pH 值對酵母凝膠珠中的轉化酶活性的影響。
2. 小明進行了相同的實驗。他發現在 pH 7 和 9 的樣本在 0、5、10 和 20 分鐘時,所有的葡萄糖試紙檢測結果均為陰性。

你發現你所屬小組的實驗結果與全班整體數據趨勢相似。你懷疑小明的結果為異常數據並可能存在錯誤。

- (a) 通過將你的結果與小明的結果進行比較,識別小明結果可能存在的錯誤。
  - (b) 你會如何進一步確認小明的結果是異常的?
  - (c) 解釋可能導致這些錯誤的原因。
3. 小明希望準確地確定酵母凝膠珠中的轉化酶活性的最佳 pH 值。
    - (a) 根據數據,解釋在確定酵母凝膠珠中的轉化酶最佳 pH 值時,現有實驗設計存在的局限性。
    - (b) 描述你將如何修改這個實驗,以更準確估計酵母凝膠珠中的轉化酶活性的最佳 pH 值。

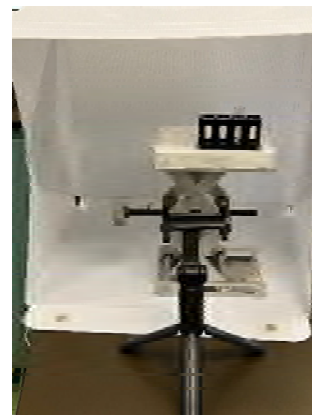


## Supplementary Resources

### Possible Modifications

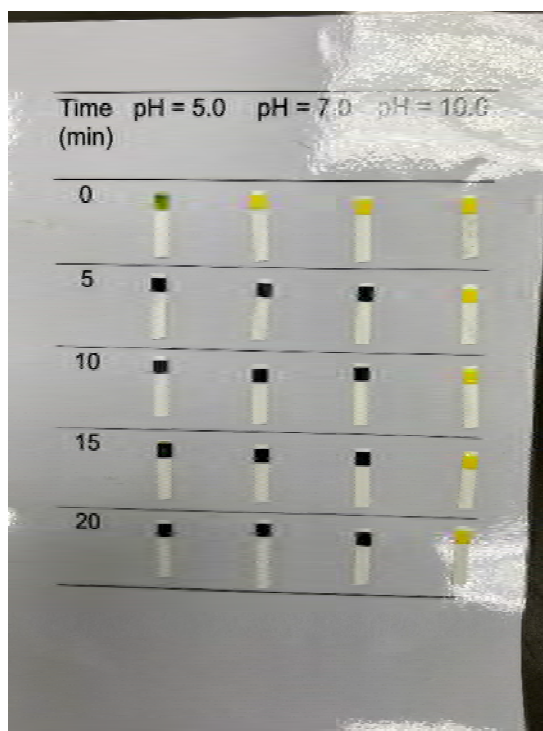
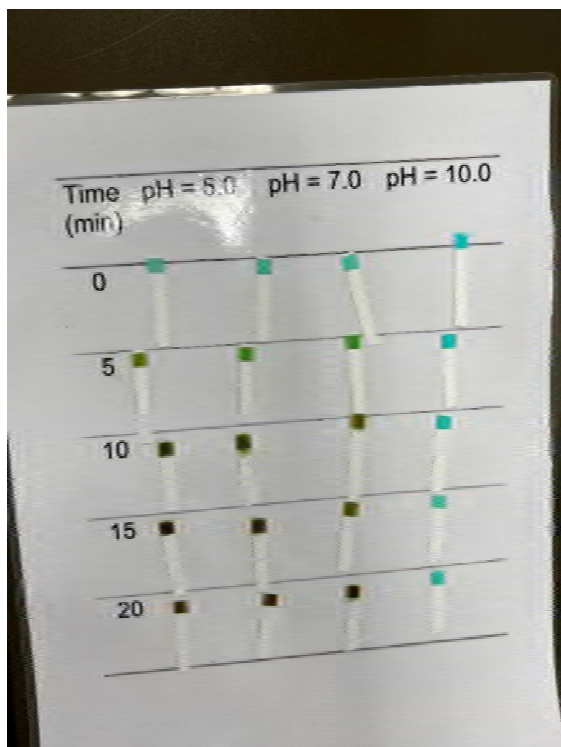
#### 1. Investigating the rate of yeast bead invertase quantitatively

- The reducing sugars produced by the invertase can be quantified using quantitative Benedict's test.
- A colorimeter can be used to determine the amount of reducing sugar produced.
- Details can be found in Hale (2023).



#### 2. Comparing the sensitivity of two brands of glucose test strips

- The sensitivity of different brands of glucose test strips can be compared.



### Materials for Task 3

#### Chemicals to be prepared

- 10% yeast extract (1 mL in 1.5 mL tube) (to be prepared on that day) (Add 15 g yeast in 150 mL distilled water. Add a spoonful of sugar. Stir on a magnetic stirrer. Wait for 30 minutes to activate the yeast. Keep stirring. Aliquot just before the experiment.)
- 2% sodium alginate (food grade). Add 200 mL of distilled or deionised water and a stir bar. Heat the solution. Slowly add some sodium alginate to dissolve it. Add more powder slowly (5 g in total). Make up volume to 250 mL. Store the solution at 4°C.



Scan the QR code to watch a video on how to prepare 2% sodium alginate.

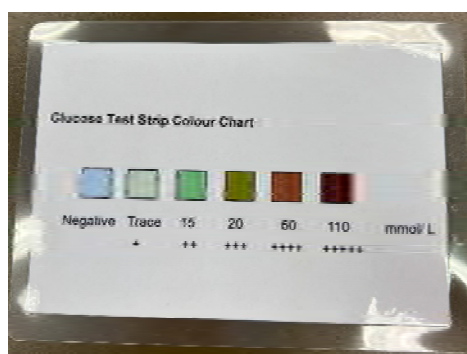


#### Materials for each group

• 10 mL 2% Sodium alginate in 50 mL tube	• Plastic forceps X 4	• Glucose test strips X 20
• 10 mL 10% Yeast in a plastic vial	• 3 mL Plastic disposable pipette (with part of the head cut)	• Rubbish bin
• 100 mL Beaker X2	• Mini petri dish X 12	• 50 mL 2% calcium chloride
• Spoon (for removing floating yeast bead)	• Stand and clamp	• Timer
• Sieve	• Wash bottle (with distilled water)	

#### Notes:

- Use 5% to 20% sucrose solution depending on the brand of glucose test strips.
- Do *not* use sodium phosphate buffer as alginate beads can react with the buffer.
- The time for *Step 8* (i.e., testing the invertase activity) depends on the brand of glucose test strips.



## References

- Bryer, P. J. (2016). Exploring catalase and invertase activity using sodium alginate–encapsulated yeast (yeast spheres). *Journal of Microbiology & Biology Education*, 17(3), 490–491.
- Hale, J. (2023) Using immobilised yeast synoptically at A-level. *School Science Review*, 104(387), 13–17.



# **Yeast Bead Catalase Investigation**

---



# Yeast Bead Catalase Investigation

## Overview

- The *Yeast Bead Catalase Investigation* is about the industrial application of immobilised yeasts to remove hydrogen peroxide from factory effluents.
- Immobilised yeasts, known as yeast beads, contain catalase (Bryer, 2016a, b).
- Students collect quantitative data (i.e., the time for the yeast beads to rise to the surface of the hydrogen peroxide solution) to determine the catalase activity for compare the inhibitory effects of different types of heavy metal ions under different concentrations.
- Students have the opportunity to design and carry out experiments in which they set up replicates, consider the importance of a larger sample size, identify significant assumptions in their experimental designs.
- Students also analyse data, construct graphical representations to compare data sets, and use the information to inform decisions in the application of effluent treatment.

## Teaching Plan

### Prerequisite knowledge (scientific ideas)

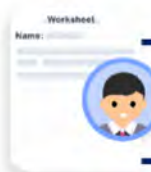
- The properties, actions and roles of enzymes
- Factors affecting the actions of enzymes
- The action of catalase on hydrogen peroxide

Lesson	Lesson sequence	Duration (mins)	Resources
<b>Stage 1 Preparing for the investigation</b>			
<ul style="list-style-type: none"> <li>• It is situated in an authentic context related to the industrial application of yeast beads in removing hydrogen peroxide from factory effluents (<b>Contextualisation</b>).</li> <li>• Students read information to familiarise themselves with the background of the investigation (<i>Reading Materials</i>).</li> </ul>			
Before Lesson 1	<ul style="list-style-type: none"> <li>• The teacher distributes <i>Worksheet 1</i> for students to complete at home so that they can be familiar with the background of the investigation.</li> </ul>		<i>Worksheet 1</i>
1	<ul style="list-style-type: none"> <li>• The teacher discusses the investigation context with students.</li> <li>• The teacher provides feedback on students' responses in <i>Worksheet 1</i>.</li> <li>• Students complete <i>Worksheet 2</i> to design an investigation.</li> <li>• Students perform mini-trial run.</li> </ul>	40	<i>Worksheet 2</i>
<b>Stage 2 Designing the investigation</b>			
<ul style="list-style-type: none"> <li>• Students perform mini-trial run (<i>Trial Run</i>).</li> <li>• Students interact with a virtual laboratory to familiarise themselves with the materials and apparatuses they use in the investigation (<i>Virtual Laboratory</i>).</li> <li>• Students use a template to design their own experimental set-ups (<i>Investigation Planning Template</i>).</li> <li>• Students have the chance to evaluate their own and their peers' experimental set-ups (<i>Self &amp; Peer Evaluation</i>).</li> <li>• Students' experimental designs of a similar investigation are collected and discussed in class (<i>Diagnostic Assessment</i>).</li> </ul>			
2	<ul style="list-style-type: none"> <li>• The teacher provides feedback on students' experimental designs in <i>Worksheet 2</i>.</li> </ul>	40	Student Samples 1
3	<ul style="list-style-type: none"> <li>• The teacher presents the main investigation context and discusses with students questions related to their experimental designs.</li> <li>• The teacher provides students with the laboratory manual for preparation at home.</li> </ul>	40	Teacher Notes 1

<b>Stage 3 Carrying out the investigation</b>			
<ul style="list-style-type: none"> <li>Students use microscale instrumentation that reduces the time of the experiments (<b>Microscale Instrumentation</b>).</li> <li>Students collect more complex data sets by setting up replicates (<b>Complex Data Set</b>).</li> </ul>			
3	<ul style="list-style-type: none"> <li>Teacher asks questions to help students connect their lab experience and related ideas/scientific inquiry skills.</li> <li>Students carry out the investigation.</li> </ul>	40	Laboratory Manual
<b>Stage 4 Explaining and evaluating data</b>			
<ul style="list-style-type: none"> <li>Students record and share data using <i>Google Spreadsheet</i>. (<i>Digital Tool</i>)</li> <li>Students use data to make claims about the inhibitory effects of different types of heavy metal ions under different concentrations and make decisions (<b>Decision-making Task</b>).</li> </ul>			
Before Lesson 4	<ul style="list-style-type: none"> <li>Students complete data reporting and analysis at home.</li> <li>The teacher collects and marks student responses.</li> </ul>		Teacher Notes 2
4	<ul style="list-style-type: none"> <li>The teacher provides feedback on students' performance related to data reporting and analysis.</li> </ul>	40	Teacher Notes 2

### Important Notes

- Students should wear safety goggles and lab coats during the experiment.
- Students should avoid contact with hydrogen peroxide, as it can discolour clothing.



## Instructional Materials

### Stage 1 Preparing for the investigation

#### Student Worksheet 1



##### Notes for teachers

- Teachers can distribute *Worksheet 1* and ask students to read the background information related to the investigation at home.
- Students' responses can be collected using a *Google Form*.
- Scan the QR code to get a copy of the *Google Form*.



#### Task 1

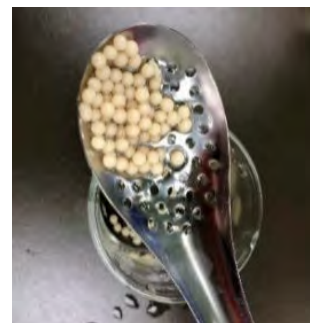
- Read the following information and the source materials in the data file.
- Answer the questions that follow.

#### Scenario

Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) is widely used as a bleaching agent in textile industries. Hydrogen peroxide residues should be broken down to harmless substances before discharge to the environment.

Yeast (*Saccharomyces cerevisiae*) is a rich source of catalase, which is an enzyme that catalyses the breakdown of hydrogen peroxide into oxygen and water. Scientists make use of yeasts to remove hydrogen peroxide residues in wastewater. Yet, industrial liquid waste often contains heavy metal ions that can inhibit catalase activity of yeast beads.

Read the information in the *Data File* to familiarise yourself with the background of this investigation.



Yeast beads

### Data file

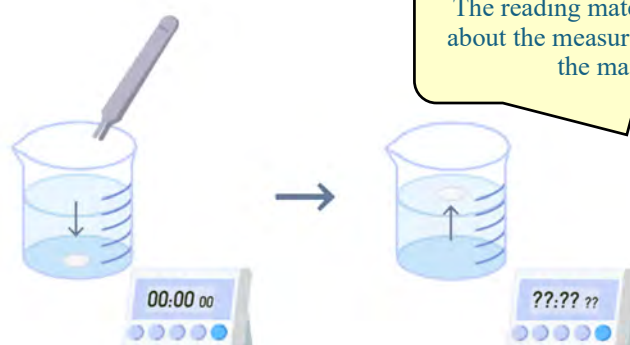
Your biology teacher asks you to read the following source materials to prepare yourself for designing an investigation related to studying catalase.

#### **Source 1:** Measuring the activity of catalase

Almost all organisms contain catalase. Catalase speeds up the breakdown of hydrogen peroxide, a toxic by-product of some metabolic reactions, into oxygen and water.

The activity of catalase in different tissues can be studied using a simple method involving the following procedures:

- Put filter paper discs into extract of different tissues
- The filter paper discs are then put into plastic vials containing equal volumes of hydrogen peroxide
- Start timing when the filter paper disc reaches the solution
- Note the time ( $t$ ) required for the filter paper disc to rise to the surface of the solution



The reading materials provide information about the measurement methods relevant to the main investigation.

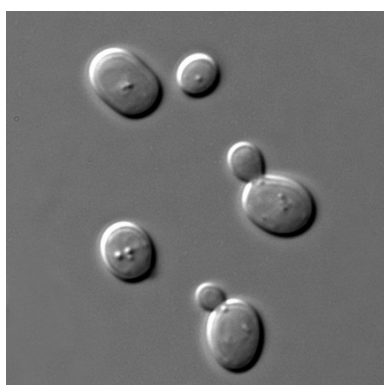


Scan the QR code to see what happens to a filter paper disc with fresh potato juice.

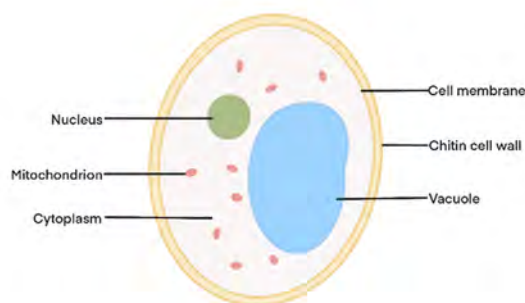


#### **Source 2:** What are yeast and yeast beads?

Yeast (*Saccharomyces cerevisiae*) is a eukaryotic organism. A eukaryotic cell has a true nucleus and membrane-bound organelle. Although yeast cells have cell wall, the chemical composition of cell wall of yeast cells is different from that of plant cells. The following diagrams show yeast cells under a light microscope and a drawing of yeast cell respectively.



Yeast cells under microscope



Drawing of a yeast cell

Yeast cells contain catalases. The catalase can break down hydrogen peroxide into oxygen and water.

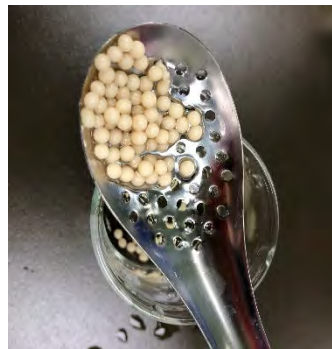
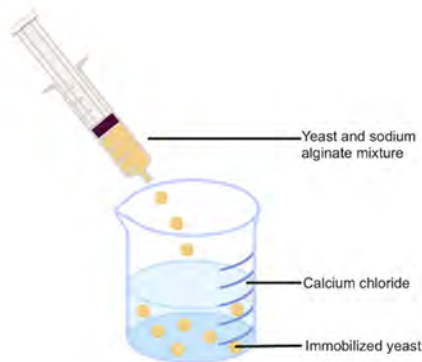


Scan the QR code to see what happens when a dialysis bag containing yeast solution is put into a beaker of hydrogen peroxide solution.



Scientists make use of yeast for many industrial applications including brewing of beer, making of bread and etc. In some applications, scientists immobilise whole yeast cells to form yeast beads using sodium alginate and calcium chloride.

The reading materials provide information about yeast beads, which may be unfamiliar to the students.



Yeast in alginate solution + Calcium chloride  $\longrightarrow$  Yeast beads

*(Yeast immobilised in insoluble calcium alginate)*

Immobilised yeasts are also active. The yeast beads can be collected and reused after reaction.

Answer the questions below *after* reading the source materials.

- Write a word equation for the action of catalase on hydrogen peroxide.
- Suggest an animal organ in which catalase is present in great abundance and from which the enzyme can be obtained. Explain why this organ has so much catalase.
- With reference to *Source 1*, explain why the paper discs rise to the surface of the hydrogen peroxide solution.
- How is the time taken for the paper disc to rise to the surface of the solution related to the activity of catalase?
- With reference to *Source 2*, state *two* observations when a dialysis bag containing yeast solution is put into a beaker of hydrogen peroxide solution.

### 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

過氧化氫( $H_2O_2$ )是一種廣泛應用於紡織工業的漂白劑。過氧化氫殘留物在排放到環境之前應該被分解成無害的物質。

酵母(*Saccharomyces cerevisiae*)是過氧化氫酶的豐富來源，過氧化氫酶是一種催化過氧化氫分解為氧氣和水的酶。科學家利用酵母去除廢水中的過氧化氫殘留物。

然而，工業廢液往往含有可抑制酵母珠中過氧化氫酶活性的重金屬離子。在這項探究實驗中，你的目標是研究不同類型的重金屬對過氧化氫酶活性的影響。

請閱讀資料檔案內的資料，以便熟悉這次探究實驗的背景。

### 資料檔案

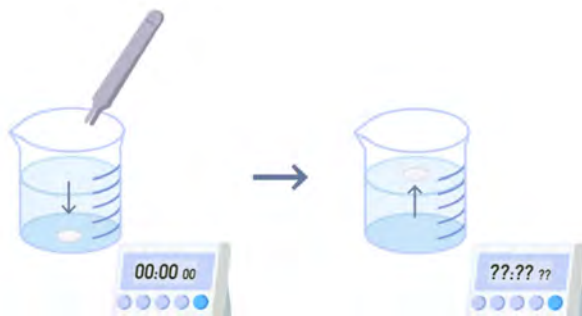
你的生物老師要求你閱讀下面的資料，為設計一個與酵母過氧化氫酶有關的科學探究做準備。

#### 資料 1: 過氧化氫酶活性測定

幾乎所有生物都含有過氧化氫酶。過氧化氫是一些代謝反應產生的有毒副產物。過氧化氫酶加速過氧化氫的分解為氧氣和水。

過氧化氫酶在不同組織中的活性可以用一種簡單的方法研究，這涉及以下步驟：

- 將濾紙片放入不同組織的提取物中
- 然後將濾紙片放入裝有等量過氧化氫溶液的塑料小瓶中
- 在濾紙片到達溶液的時候開始計時
- 注意濾紙片上升到溶液表面所需的時間 ( $t$ )

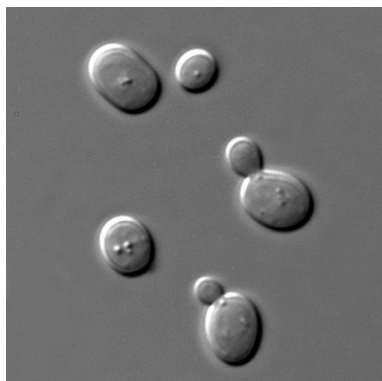


掃描二維碼以查看吸附了新鮮土豆汁的濾紙片放入過氧化氫溶液後會發生什麼事情。

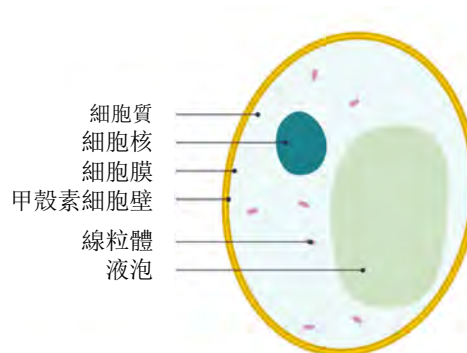


## 資料 2: 什麼是酵母和酵母珠?

酵母(*Saccharomyces cerevisiae*)是一種真核生物。真核細胞有一個真正的細胞核和多種具有膜結構的細胞器。雖然酵母細胞有細胞壁，但其細胞壁的化學組成成分與植物細胞的不同。下圖分別為顯微鏡下的酵母細胞圖和酵母細胞繪圖<sup>1</sup>。



顯微鏡下的酵母細胞圖



酵母細胞繪圖

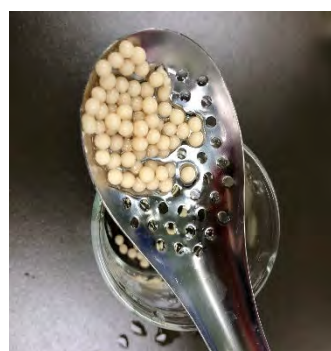
酵母細胞含有過氧化氫酶。過氧化氫酶能催化過氧化氫分解成氧氣和水。



掃描二維碼，看看當一個含有酵母溶液的透析袋被放入一個裝有過氧化氫溶液的燒杯中會發生什麼。



科學家利用酵母進行許多工業應用，包括釀造啤酒、製作麵包等。在某些應用中，科學家使用海藻酸鈉和氯化鈣使整個酵母細胞固定，以形成酵母珠。



海藻酸溶液中的酵母 + 氯化鈣 → 酵母珠

(酵母在非溶性的海藻酸鈣中固定)

固定化的酵母仍然有活性。酵母珠在反應後可被回收及重用。

閱讀資料後回答以下問題。

- (a) 寫出過氧化氫酶對過氧化氫的文字方程。
- (b) 建議一種含有大量過氧化氫酶、且此酶可被提取的動物器官。解釋為什麼這個器官有這麼多的過氧化氫酶。
- (c) 參考資料 1，解釋為什麼濾紙片會上升到過氧化氫溶液的表面。
- (d) 濾紙片上升到溶液表面的時間與過氧化氫酶的活性有什麼關係？
- (e) 參考資料 2，指出當一個含有酵母溶液透析袋被放入一個裝有過氧化氫溶液的燒杯時的兩個可觀察結果。



掃描二維碼以獲取 *Google Form* 的副本。



## Student Worksheet 2

### Notes for teachers



- Teachers distribute *Worksheet 2* and ask students to design the investigation at home.
- Teachers may ask students to perform a trial run before their design. See *Supplementary Resource* section for the material list.
- Students can see the materials and apparatuses in the *Virtual Laboratory*.
- Some student work samples are shown below to illustrate possible student thinking.

### Task 2

- Answer the questions that follow.
1. Briefly describe how you would use the following materials to design an experiment to achieve the above aim. You can draw your experimental design.

1M Zinc sulphate solution	Timer	Distilled water
1M Nickel chloride solution	Forceps	0.1% Hydrogen peroxide
1M Copper sulphate solution	Yeast beads	Plastic vials
Autopipette	Autopipette tips	10 ml measuring cylinder
Petri dish		



Scan the QR code to see these materials.



The virtual laboratory provides students with opportunities to get familiar with materials used in the investigation.

*Brief explanation of my design:*

The mini trial run provides opportunities for students to see how the dependent variable can be measured (i.e., the time for the yeast beads to rise).

### Mini trial run procedures

You may want to perform the following trial run before you design the investigations.

- Pour some hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) into a plastic vial.
- Use the forceps to transfer several yeast beads into the  $\text{H}_2\text{O}_2$  solution.
- Observe what happens.

**任務 2**

- 回答以下問題。

(a) 簡要描述你將如何使用以下材料來設計探究，以達到上述目標。你可以畫出你的實驗設計。

1M 硫酸鋅溶液	計時器	蒸餾水
1M 氯化鎳溶液	鑷子	0.1% 過氧化氫溶液
1M 硫酸銅溶液	酵母凝膠珠	塑料小瓶
自動移液管	自動移液管針尖	10 毫升量筒
培養皿		



掃描二維碼，以查看這些材料。



簡要說明我的設計:

**小型試行步驟**

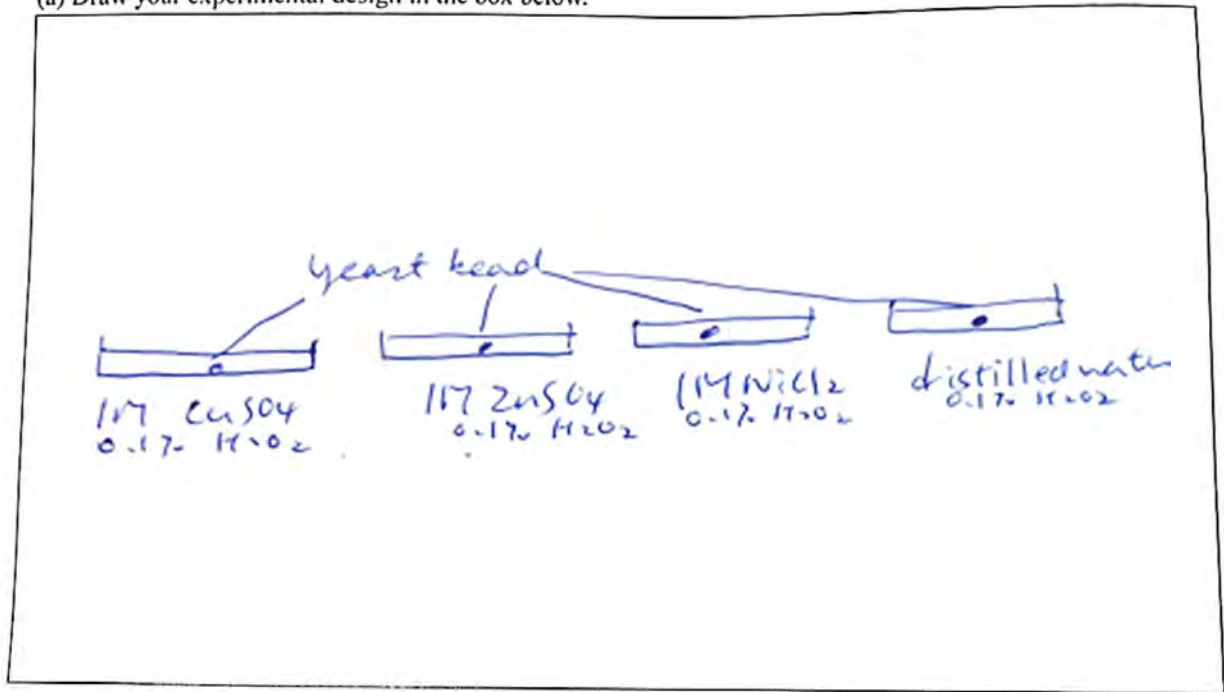
在設計探究之前,你可以進行以下試驗:

- 將一些過氧化氫倒入塑料小瓶中。
- 使用鑷子將幾顆酵母珠轉移到過氧化氫溶液中。
- 觀察發生的情況。

Examples of students' initial experimental designs

Sample 1

(a) Draw your experimental design in the box below.



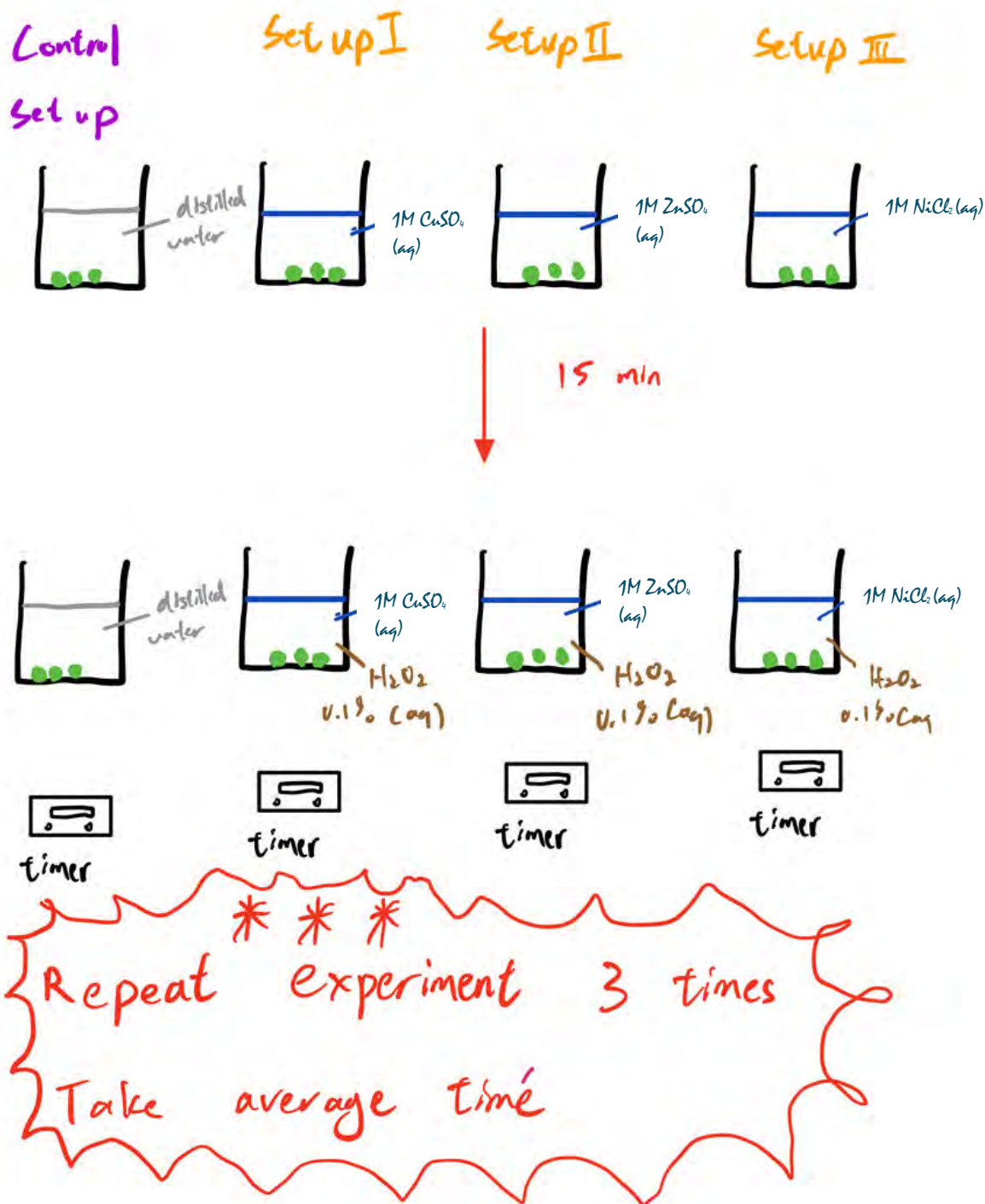
(b) Briefly explain your design:

- 1) set 4 set ups = 1M  $\text{CuSO}_4$ , 1M  $\text{ZnSO}_4$   
1M  $\text{NiCl}_2$ , distilled water  
All set ups with 0.1% hydrogen peroxide
- 2) Put the yeast heads into the mixture solution
- 3) use timer to time when the yeast heads reach the top of the petri dish

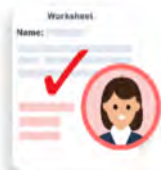
Students evaluate their own and their peers' designs on a similar experiment. Teachers provide feedback on important scientific thinking skills.

Sample 2

Many students tend to overlook the preincubation step in their experimental designs.



**Notes for teachers**



- Teachers can choose some students' diagrams (anonymised) of experimental setups for students to evaluate.
- Teachers can discuss the following scientific inquiry skills: (1) the importance of precautionary step; (2) the number of yeast beads in each vial; (3) the number of replicates they would set up.

**Notes to teachers**



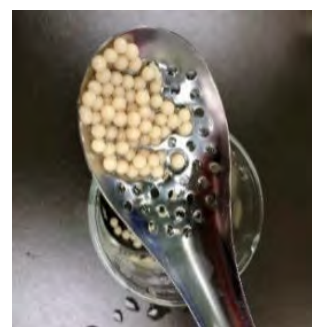
- After receiving feedback on their experimental designs, the following shows the main investigation context for students to design another investigation that involves continuous independent variable.
- There are some questions that teachers may use to guide students in thinking about or assessing the scientific inquiry skills related to experimental designs.
- Some student work samples are shown below to illustrate possible student thinking to some questions.

**Task 3**

**Scenario**

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is widely used as a bleaching agent in textile industries. Hydrogen peroxide residues should be broken down to harmless substances before discharge to the environment.

Baker’s yeast (*Saccharomyces cerevisiae*) is a rich source of catalase. Catalase is an enzyme that catalyses the breakdown of hydrogen peroxide into oxygen and water. Scientists make use of yeasts to remove hydrogen peroxide residues in wastewater. However, industrial liquid waste often contains heavy metal ions that can inhibit catalase activity of yeast beads. More yeast beads need to be used to achieve the same efficiency.



Yeast beads

Your biology teacher has asked you to design an investigation *to investigate the effect of different types of heavy metal ions on the activity of yeast bead catalase under different concentrations*. This information is important for determining the catalase activity of yeast beads in removing hydrogen peroxide in water with heavy metal.

**Design of investigation**

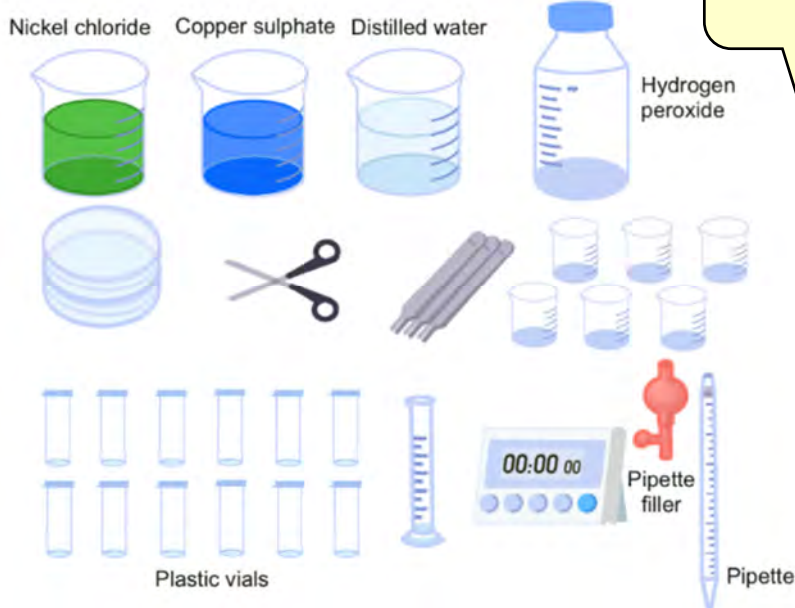
*Aim of the investigation*

- To investigate the effect of different types of heavy metal ions on the activity of yeast bead catalase under different concentrations

*Materials and apparatus:*

- You have been given the following materials and apparatus:

1M Nickel chloride solution	Forceps	Plastic vial
1M Copper sulphate solution	Petri dish	Timer
0.1% Hydrogen peroxide solution	1 mL Pipette	10 mL Measuring cylinder
Distilled water	Pipette filler	25 mL Beaker
Yeast beads	Scissors	



The diagrams provide visual scaffolds to help students understand the materials and apparatuses for the investigation.

Scan this QR code to see the materials.



### Possible questions

1. State how the dependent variable can be measured using the above materials and apparatus.

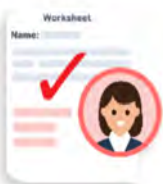
*Hints:* Make sure that you include the following parts in your answers:

- the measurement tools and the methods of measurement.
- the relationship between the measurement and the dependent variable

The checklist serves to scaffold students' responses.

2. Will you choose to use one single yeast bead or more than one yeast bead in each trial? Why?
3. Your teacher advised you not to use hydrogen peroxide solution that is too concentrated ( $>5\%$  hydrogen peroxide). Discuss the importance of using a suitable concentration of hydrogen peroxide solution in relation to the overall validity of the investigation.

### Notes for teachers



- Q.1 assesses students' ability to connect the measurement to the dependent variable.
- Q.2 assesses students' ability to explain how increasing the sample size can help reduce the impact of individual differences inherent in biological samples on the quality of the data.
- Q.3 assesses students' ability to explain design that relates to the validity of the design.

The following are some examples of students' responses to Q.1:

### Sample 1

Explain how the dependent variable could be measured using the above materials and apparatus.

Soak the yeast beads in the petri dishes and use forceps to put the beads inside the hydrogen peroxide. Use the pipette and pipette filler connect it to a water trough and an inverted measuring cylinder. Use the timer to measure the time taken for colourless bubbles to appear.

### Sample 2

Explain how the dependent variable could be measured using the above materials and apparatus. *respectively*

After adding same amount of yeast bead into four beakers, time the timer immediately and stop the timer after the yeast bead rise to the surface of the mixture.

### Sample 3

Explain how the dependent variable could be measured using the above materials and apparatus.

Use the timer to measure the time taken for the paper disk to rise to the surface of the solution. The shorter the time taken for the paper disk to rise to the surface, the higher the activity of yeast bead catalase.



#### **About the samples**

- Sample 1 proposed a set-up that required additional materials and apparatus not provided in the list. It is also not feasible to measure the time it takes for the colourless gas bubbles to appear.
- Sample 2 described the measurement (i.e., time for the yeast beads to rise to the surface of the solution) but did not state the relationship between the measurement and the relative rate of catalase activity.
- Sample 3 confused yeast beads with paper discs (information in the *Data File*.)

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

### Sample 1

Will you choose to use one single yeast bead or more than one yeast bead in each trial? Why?

More than one yeast bead because the higher amount of yeast beads have a larger surface area for the catalase in the yeast beads to react and it can decrease the time needed

### Sample 2

Will you choose to use one single yeast bead or more than one yeast bead in each trial? Why?

I will choose more than one yeast bead in each trial because more yeast beads have a larger sample size. Therefore, the reliability of the results could be increased by minimizing the individual differences.

### Sample 3

Will you choose to use one single yeast bead or more than one yeast bead in each trial? Why?

More than one yeast bead. Different yeast bead may contain different amount of catalase. By using more than one yeast bead, the reliability of the results could be increased by minimizing the individual differences.



#### **About the samples**

- Sample 1 wrongly believed using more than one yeast can lead to a shortening of the duration of the experiment. The surface area to volume ratio of each yeast does not change when more than one yeast bead is used.
- Sample 2 and Sample 3 related to the reliability of the data. Sample 2 described the differences between the yeast beads in terms of the differences in the amount of catalase. Increasing the number of yeast beads in each trial can reduce the impact of the inherent variations in catalase in the yeast beads.

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

### Sample 1

Your teacher advised you not to use hydrogen peroxide that is too concentrated (>5% hydrogen peroxide). Discuss the importance of using a suitable concentration of hydrogen peroxide in relation to the overall validity of the investigation.

As hydrogen peroxide is acidic, a too concentrated hydrogen peroxide will affect the action of catalase by denaturing it. A suitable concentration of hydrogen peroxide ensures the catalase in the yeast beads are able to work in a suitable pH.

### Sample 2

(h) Your teacher advised you not to use hydrogen peroxide that is too concentrated (>5% hydrogen peroxide). Discuss the importance of using a suitable concentration of hydrogen peroxide in relation to the overall validity of the investigation.

Catalase works best in a certain concentration of hydrogen peroxide. Too concentrated hydrogen peroxide will affect the rate of catalase activity and affect the overall validity of the investigation.

### Sample 3

Your teacher advised you not to use hydrogen peroxide that is too concentrated (>5% hydrogen peroxide). Discuss the importance of using a suitable concentration of hydrogen peroxide in relation to the overall validity of the investigation.

Because too concentrated hydrogen peroxide will lead to higher rate of catalase reaction, so, the rate of yeast bead to reach the surface will be too fast and ~~the~~ it will be too hard to measure the difference of rate of reaction between different solutions. Suitable concentration can ensure the result is obvious to compare.



#### **About the samples**

- Sample 1 wrongly stated that more concentrated hydrogen peroxide solution is more acidic.
- Sample 2 did not pinpoint what the changes in the rate of catalase activity would be when too concentrated hydrogen peroxide is used and its effect on the measurement.
- Sample 3 was able to relate the difficulty of discerning the differences if the rate is too high for both treatments.

### 任務 3

#### 情境

過氧化氫( $H_2O_2$ )在紡織工業中被廣泛用作漂白劑。過氧化氫殘留物在排放到環境中之前應被分解成無害物質。

麵包酵母(*Saccharomyces cerevisiae*)是過氧化氫酶的豐富來源。過氧化氫酶是一種催化過氧化氫分解為氧氣和水的酶。科學家們利用酵母菌來清除廢水中的過氧化氫殘留物。然而，工業液體廢物通常含有重金屬離子，會抑制酵母凝膠珠中的過氧化氫酶活性。因此，為了達到同樣的催化效率，需要使用更多的酵母凝膠珠。



酵母凝膠珠

你的生物老師要求你設計一項實驗，探究不同類型的重金屬離子在不同濃度下對酵母凝膠珠中過氧化氫酶活性的影響。這項實驗提供的資訊對於確定酵母凝膠珠中去除含重金屬的水中的過氧化氫的過氧化氫酶活性非常重要。

#### 實驗設計

##### 實驗目的

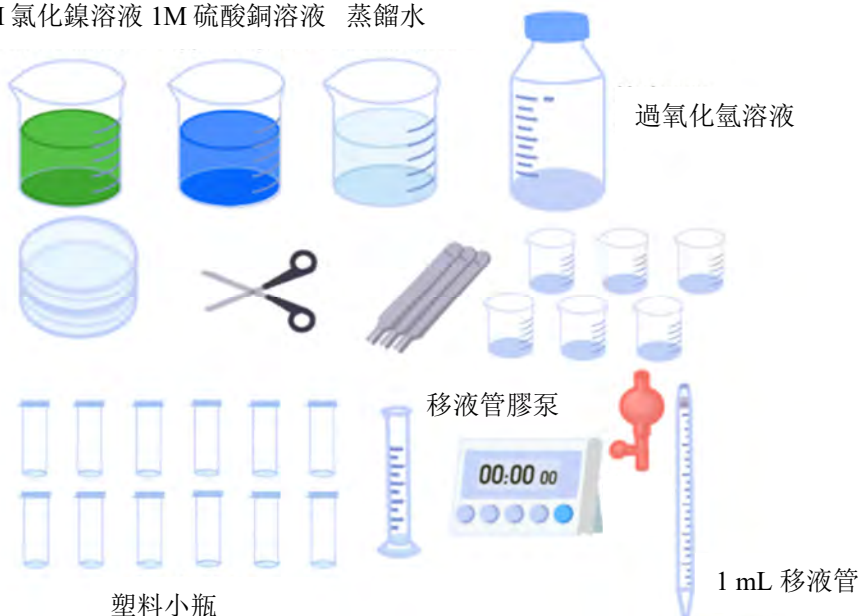
- 探究不同類型的重金屬離子在不同濃度下對酵母凝膠珠中過氧化氫酶活性的影響

##### 物料和儀器

- 你有以下實驗物料和儀器。

1M 氯化鎳溶液	鑷子	塑料小瓶
1M 硫酸銅溶液	培養皿	計時器
0.1%過氧化氫溶液	1 mL 移液管	10 mL 量筒
蒸餾水	移液管膠泵	25 mL 燒杯
酵母凝膠珠	剪刀	

1M 氯化鎳溶液 1M 硫酸銅溶液 蒸餾水



掃描此二維碼以  
查看實驗材料



### 參考問題

1. 說明如何使用上述材料和儀器量度因變量。  
*提示：請確保你的答案包括以下部分。*
  - 量度工具和測量方法。
  - 量度方法和因變量之間的關係
2. 在每次試驗中，你會選擇使用一個的酵母凝膠珠還是一個以上的酵母凝膠珠？為什麼？
3. 你的老師建議你不要使用濃度過高的過氧化氫溶液 (例如 >5%的過氧化氫溶液)。試討論使用適當濃度的過氧化氫溶液對實驗的整體信度的重要性。

**Notes to teachers**

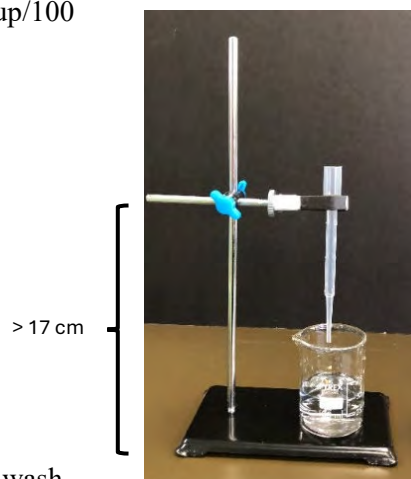
- Teachers can distribute the manual for students to read and prepare before the investigation.
- It is suggested that half of the class investigate the effect of copper ions while the other half study the effect of nickel ions.
- Teachers can ask questions to check if students fully understand the procedures (e.g., how many yeast beads will you need in this experiment? Why?).
- The *Supplementary Resource* section contains the material list.
- Scan the QR code to view the process of the experiment.

**Task 4**

- Read the following procedures to carry out the investigation.

**Procedure****Preparation of yeast beads**

1. Add 10 mL of 10% yeast (in a vial) to 10 mL of 2% sodium alginate solution in a 50 mL tube.
2. Mix the solution well by inverting the 50 mL tube to make a yeast–sodium alginate solution.
3. Hold the plastic dropper (without cap) with a stand and clamp.
4. Pour 50 mL 2% CaCl<sub>2</sub> (calcium chloride) into a plastic cup/100 mL beaker.
5. Assemble the set up shown in *Figure 1*.
6. Add the yeast-sodium alginate solution to the plastic dropper (a bead should form when the drop comes into contact with the CaCl<sub>2</sub> solution and falls to the bottom of the beaker).
7. Wait 5 minutes until the beads have hardened.
8. Discard any floating yeast beads with a plastic spoon.
9. Collect the beads with a sieve.
10. Wash the beads several times with distilled water from a wash bottle over a plastic cup.

*Figure 1*

### ***Inhibition of catalase activity***

1. Add the heavy metal solution (e.g., 5 mL depending on the size of the petri dish) with different concentrations (1M, 0.5 M, 0.25M) and distilled water to four different petri dishes.
2. Use forceps to gently move at least 15 yeast beads into each labelled petri dish for at least 5 minutes.

### ***Testing the catalase activity***

1. Measure 10 mL of 0.1% hydrogen peroxide solution with a measuring cylinder and pour the solution into a plastic vial. (You can use the dropper to transfer the solution).
2. Repeat *Step 1* three times.
3. Use the forceps to carefully transfer five yeast beads from each petri dish into each plastic vial.
4. Start the timer as soon as the yeast beads touch the surface of the hydrogen peroxide solution or as soon as they touch the bottom of the vial.
5. Record the time when all the beads have reached the surface of the hydrogen peroxide solution.
6. Repeat your measurements at least two more times.
7. Report your group data in this *Google Sheet* by scanning the QR code.



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



#### 任務 4

- 閱讀以下實驗步驟以進行探究:

#### 實驗步驟

##### 酵母凝膠珠的製備

- 將 10 mL 10%的酵母(在小瓶中)加入到盛有 10 mL 2%的海藻酸鈉溶液的 50 mL 的試管中。
- 倒置 50 mL 試管將溶液充分混合，使之成為酵母-海藻酸鈉溶液。
- 用支架和夾子夾住塑料滴管(無蓋)。
- 將 50 mL 2%的  $\text{CaCl}_2$ (氯化鈣)倒入一個塑料杯中/100 mL 燒杯。
- 組裝圖 1 中所示的裝置。
- 在塑料滴管中加入海藻酸鈉酵母溶液(當滴管接觸到  $\text{CaCl}_2$  溶液並落到燒杯底部時應形成一個珠子)。
- 等待 5 分鐘，直到珠子變硬。
- 用塑料勺子丟棄任何漂浮的酵母珠。
- 用篩子收集珠子。
- 用洗瓶中的蒸餾水沖洗幾次塑料杯上的酵母凝膠珠。

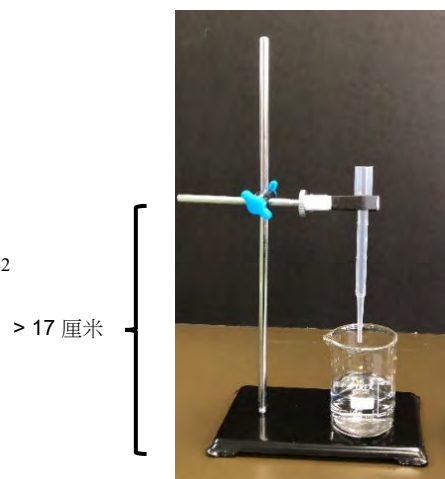


圖 1

##### 過氧化氫酶活性的抑制

- 將不同濃度的重金屬溶液(1M、0.5M、0.25M)和蒸餾水倒入四個不同的培養皿中(5 mL 視乎培養皿的大小)。
- 用塑料小匙及鑷子在每一個已標記的培養皿中緩緩加入至少 15 粒酵母凝膠珠，並至少靜候 5 分鐘。

##### 測量過氧化氫酶活性

- 用量筒測量 10 mL 0.1%過氧化氫溶液，並將溶液倒入塑料小瓶中。(你可以使用滴管轉移溶液。)
- 重複步驟 1 三次。
- 使用鑷子小心地將每個培養皿中的五個酵母珠轉移到每個塑料小瓶中。
- 酵母凝膠珠接觸過氧化氫溶液表面或接觸小瓶底部時，立即啟動計時器。
- 記錄所有酵母凝膠珠到達過氧化氫溶液表面的時間。
- 至少再重複兩次測量。
- 掃描二維碼,在此 *Google Sheet* 報告你小組的數據。

掃描二維碼以獲取  
*Google Sheet* 的副本



## Teacher Notes 2

**Notes for teachers**

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

**Task 5****Possible questions**

1. Based on the class data, which data set concerning the effect of 0.5 M of the two heavy metal ions on the yeast bead catalase activity is more variable? Explain your answer.
2. Plot a graph to show the class data.
  - Which type of graph would you choose to show the effect of different types of heavy metals on the activity of catalase under different concentrations? Why?
  - Which axis, the x-axis or the y-axis, should be the independent and dependent variables respectively?
  - What should be a suitable title for your graph?
3. Describe and compare the effect of different concentrations of the two heavy metal ions on the activity of catalase in the yeast beads.
4. If 1,000 yeast beads are typically used to remove hydrogen peroxide from industrial effluents without heavy metals, how many yeast beads would be needed to achieve the same removal efficiency in effluents containing 0.1 M nickel chloride and 0.1 M copper sulphate, respectively?

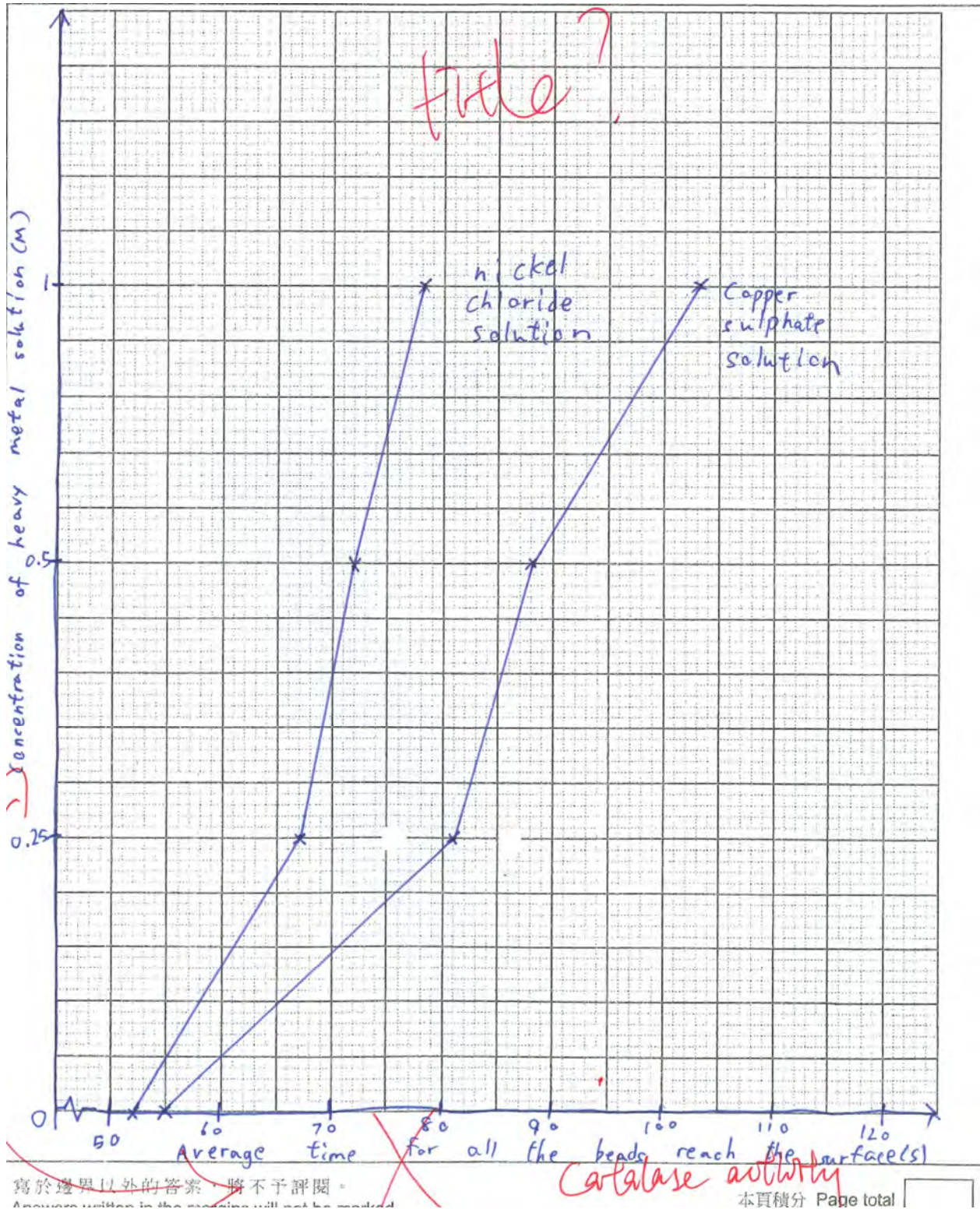
Some reminders are added to guide students in constructing graphical representations appropriately.

**Notes for teachers**

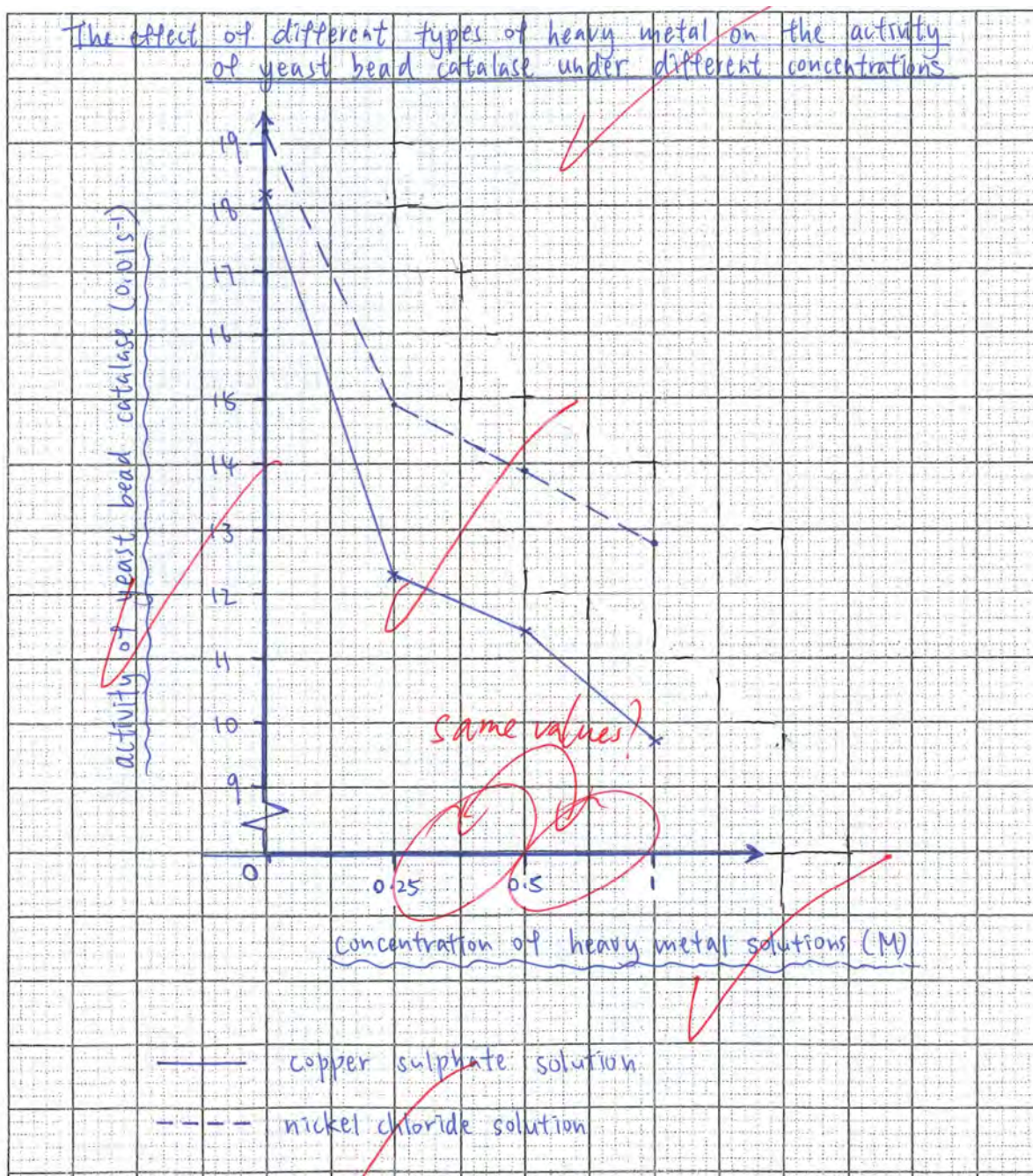
- Q.1 assesses students' ability to apply basic statistics to compare the variability of datasets.
- Q.2 assesses students' ability to construct appropriate graphical representations.
- Q.3 assesses students' ability to describe and compare more complex data sets involving more than one independent variable.
- Q.4 assesses students' ability to read data from the graph and perform simple calculation based on their data.

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

Sample 1



## Sample 2



### About the samples

- Sample 1 has various mistakes in the graph plotting. These include: (1) missing a title for the graph; (2) reversing the x-axis and y-axis; (3) using a measurement parameter rather than the dependent variable on the axis.
- Sample 2 shows a scaling issue in the graph. The interval between 0.25 M and 0.5 M is the same as the interval between 0.5 M and 1.0 M, which is not an appropriate scaling.

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

### Sample 1

For nickel chloride solution, the activity of catalase is decreased from 0.0192 to 0.0128. And for copper sulphate solution, the activity of catalase is also decreased from 0.0181 to 0.00971.  
*when ...*

### Sample 2

With copper sulphate solution, the time for all the yeast beads reach the solution's surface becomes longer, which means that copper sulphate affects catalase to carry out enzymatic reaction. With nickel chloride solution, the time for all the yeast beads reach the solution's surface also becomes longer but shorter than copper sulphate solution does. It means that nickel chloride affects the activity of catalase. Yet, it affects less comparing with copper sulphate.

### Sample 3

The activity of catalase in the yeast beads decrease when the concentration of copper sulphate solution increase from 0M to 1M. The activity of catalase in yeast beads decrease when the concentration of nickel chloride solution increase from 0M to 1M.

The effect of increasing concentrations of copper sulphate solution that lead to the decrease in activity of catalase is longer than that of nickel chloride solution.



#### About the samples

- Sample 1 did not clearly identify how the different concentrations of copper/nickel ions influenced the yeast bead catalase activity.
- Sample 2 did not specify the range of heavy metal ion concentrations over which there was a decrease in yeast bead catalase activities. There was also no explicit comparison of the effects of the two different heavy metal ions.
- Sample 3 showed that as the concentration of heavy metal ions increased, there was a decrease in yeast bead catalase activity. It also identified that the copper ions caused a greater decrease in activity compared to the nickel ions. However, the description of the differential effects between the two heavy metal ions could be more nuanced.

## 任務 5

### 參考問題

1. 根據全班數據，那一組關於摩爾濃度為 0.5 M 的兩種重金屬離子對酵母凝膠珠中的過氧化氫酶活性影響的數據可變性較大？解釋你的答案。
2. 以圖表的形式呈現全班數據。
  - 你會選擇那種類型的圖表來展示不同類型的重金屬離子在不同濃度下對酵母凝膠珠中過氧化氫酶活性的影響？為什麼？
  - X 軸和 Y 軸應分別代表哪些因變量及自變量？
  - 您的圖表應該有一個合適的標題是什麼？
3. 描述並比較兩種重金屬離子在不同濃度下對酵母凝膠珠中過氧化氫酶活性的影響。
4. 如果在沒有重金屬離子的工業廢水雖要使用 1,000 粒酵母凝膠珠以去除過廢水中的過氧化氫,那麼在含有 0.1 M 氯化鎳和 0.1 M 硫酸銅的廢水中,需要分別使用多少粒酵母凝膠珠才能達到同樣的去除效率？



## Supplementary Resources

### Possible Modifications

#### 1. Studying plant material beads

- Catalase can be found in many different tissues of plants and animals. Crude extract from plant materials can also be used to make alginate beads (Andrews et al., 2019). Potato, banana and cucumber beads can be prepared using the following protocol:

#### Procedure

##### *Preparation of alginate beads using crude extract from plant materials*

1. Weigh 20 g of potato/cucumber.
2. Cut the potato/cucumber into small pieces.
3. Add 20 mL of distilled water to the potato/cucumber.
4. Blend the potato/cucumber and the distilled water with a blender.
5. Filter the potato/cucumber juice with a muslin bag.
6. Add 10 mL of 3% or 4% sodium alginate solution to 10 mL of filtered potato/cucumber juice.
7. Mix the mixture well.
8. Store at 4°C to remove air bubbles.
9. Add the potato/cucumber juice-sodium alginate solution to the plastic dropper (a bead should form when the drop comes into contact with the CaCl<sub>2</sub> solution and falls to the bottom of the beaker) above a beaker of 2% calcium chloride.



Scan the QR code to watch a video on how to prepare plant material alginate beads.



#### *Important notes*

- It is important to make sure that the plant extract is dense enough for the beads to sink to the bottom.
- Storing the plant extract–alginate mixture overnight at 4°C can effectively remove the air bubbles (the beads will float if they contain many air bubbles).

### Technician Notes

#### 1. Materials for Task 2

##### *Materials to for each group*

- 1% H<sub>2</sub>O<sub>2</sub> (50 ml) in 100 mL beakers
- 3% H<sub>2</sub>O<sub>2</sub> (50 ml) in 100 mL beakers (Handle with care!)
- 5 plastic vials
- Forceps X2
- Irregular yeast beads in a petri dish



## 2. Materials for Task 4

### Chemicals to be prepared

- 10% yeast extract (1 mL in 1.5 mL tube) (to be prepared on that day) (Add 15 g yeast in 150 mL distilled water. Add a spoonful of sugar. Stir on a magnetic stirrer. Wait for 30 minutes to activate the yeast. Keep stirring. Aliquot just before the experiment.)
- 2% sodium alginate (food grade). Add 200 mL of distilled or deionised water and a stir bar. Heat the solution. Slowly add some sodium alginate to dissolve it. Add more powder slowly (5 g in total). Make up volume to 250 mL. Store the solution at 4°C.
- 0.1% hydrogen peroxide (150 mL per group) You may perform a trial run with 0.2% H<sub>2</sub>O<sub>2</sub> as the catalase activity depends on the quality of the yeast. It is desirable if the yeast beads rise within 1 minute in the control (i.e., without metal ion treatment).



Scan the QR code to watch a video on how to prepare 2% sodium alginate.



### Materials for each group

• 10 mL 2% Sodium alginate in 50 mL tube	• Plastic forceps X 4	• Plastic vials X 12
• 10 mL 10% Yeast in a plastic vial X 1	• 3 mL Plastic disposable pipette (with part of the head cut)	• 10 mL Measuring cylinder
• 100 mL Beaker X 2	• Mini-petri dish X 4	• 5 mL 1 M CuSO <sub>4</sub> /NiCl <sub>2</sub> solution in 15 mL tube
• Spoon (for removing floating yeast bead)	• 3 mL Disposable dropper (for measuring H <sub>2</sub> O <sub>2</sub> )	• 5 mL 0.75 M CuSO <sub>4</sub> /NiCl <sub>2</sub> solution in 15 mL tube
• Sieve	• Stand and clamp	• 5 mL 0.25 M CuSO <sub>4</sub> /NiCl <sub>2</sub> solution in 15 mL tube
• 50 mL 2% Calcium chloride	• Wash bottle (with distilled water) X 1	• 5 mL Distilled water in 15 mL tube
• Timer	• 0.1% Hydrogen peroxide (>120 mL)	• Rubbish bin

### Notes

- Do *not* use tap water to prepare heavy metal solutions.



Scan the QR codes to watch the videos.



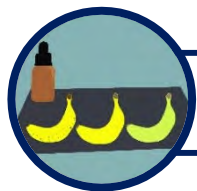
## References

- Andrews, K., Beaumont, P., & Louis, M. (2019). Catalase activity in immobilised yeast. *School Science Review*, 100(373), 13–16.
- Bryer, P. J. (2016a). A twist on measuring catalase. *Science Teacher*, 83(6), 69–73.
- Bryer, P. J. (2016b). Exploring catalase and invertase activity using sodium alginate–encapsulated yeast (yeast spheres). *Journal of Microbiology & Biology Education*, 17(3), 490–491.



# Banana Ripening Investigation

---



# Banana Ripening Investigation

## Overview

- The *Banana Ripening Investigation* is about why bananas become sweet during ripening.
- The investigation involves hypothesis testing, in which students propose an explanatory hypothesis.
- Students design food tests to investigate the biochemical changes that occur during the ripening process.
- Students are given the opportunity to design and carry out experiments in which they make predictions from the hypothesis, determine appropriate ranges and intervals for data collection, and consider the generalisability of their data.

## Teaching Plan & Key Features

Lesson	Lesson sequence	Duration (mins)	Resources
<b>Stage 1 Preparing for the investigation</b>			
<ul style="list-style-type: none"> <li>• Students observe what happened to the overripe banana to raise their curiosity of the biochemical changes during the ripening process.</li> </ul>			
1	<ul style="list-style-type: none"> <li>• The teacher performs a demonstration to show the fluorescent blue ring around the black spot on an overripe banana.</li> <li>• The teacher invites students to propose possible biochemical changes that may occur during the fruit ripening process in <i>Worksheet 1</i>.</li> </ul>	40	<i>Worksheet 1</i>
<b>Stage 2 Designing the investigation</b>			
2	<ul style="list-style-type: none"> <li>• The teacher distributes <i>Worksheet 2</i> and introduces the investigation context.</li> <li>• The teacher discusses with students questions related to the experimental design.</li> <li>• The teacher provides students with the laboratory manual for preparation at home.</li> </ul>	40	<i>Worksheet 2</i> , Teacher Notes 1
<b>Stage 3 Carrying out the investigation</b>			
<ul style="list-style-type: none"> <li>• Students use microscale instrumentation that reduces the time of the experiments (<b>Microscale Instrumentation</b>).</li> </ul>			
3	<ul style="list-style-type: none"> <li>• Teacher asks questions to help students connect their lab experience and related ideas/scientific inquiry skills.</li> <li>• Students carry out the investigation.</li> </ul>	40	Laboratory Manual
<b>Stage 4 Explaining and evaluating data</b>			
<ul style="list-style-type: none"> <li>• Students share their data on <i>Padlet (Data-sharing Web Platform)</i>.</li> <li>• Students evaluate their data to determine if the hypothesis is supported or refuted and consider how to gather additional evidence.</li> </ul>			
Before Lesson 4	<ul style="list-style-type: none"> <li>• Students complete data reporting and analysis at home.</li> <li>• Teacher collects and marks student responses.</li> </ul>		Teacher Notes 2
4	<ul style="list-style-type: none"> <li>• Teacher provides feedback on students' performance related to data reporting and analysis.</li> </ul>	40	Teacher Notes 2

## Important Notes

- This investigation is considered relatively simple. It is more suitable for use in Secondary 3 or Secondary 4.



## Instructional Materials

### Stage 1 Preparing for the investigation

#### Student Worksheet 1



##### Notes for teachers

- Teachers perform a demonstration to show the fluorescent blue rings around the black spots on overripe banana.
- Teachers can ask students to propose changes that might have occurred during banana ripening that led to their observations and other possible changes.
- Scan the QR code to see a video clip.



#### Task 1

Watch the demonstration and answer the following questions:

1. What do you observe in the overripe banana?
2. What do you think might have happened during the banana ripening process?
3. Based on your daily-life experience, what other changes might have occurred during the ripening of banana?

The demonstration arouses students' curiosity about the process of fruit ripening.



##### Notes for teachers

- Ripening is a catabolic process that involves a lot of biochemical process and physiological changes.
- Black spots are visible on the skin of a banana as it becomes overripe. Under ultraviolet light in darkness, a fluorescent blue ring can be observed around each black spot. This is formed from the breakdown of chlorophyll during the ripening process.
- This can be used as the basis for investigative practical work activities, where students are challenged to hypothesize about what they observe and the reasons for their observations.
- The website <https://www.saps.org.uk/teaching-resources/resources/1306/why-do-bananas-fluoresce-an-unexpected-view-of-chlorophyll/> provides an excellent resource related to this topic.

#### 學生工作紙 (一)

#### 任務 1

- 觀看示範並回答以下問題:
1. 你可從過熟的香蕉觀察到甚麼?
  2. 你認為香蕉在成熟過程中可能發生了什麼變化?
  3. 根據你的日常生活經驗,香蕉在成熟過程中可能發生的其他變化有哪些?

## Student Worksheet 2

**Notes to teachers**

- Teachers can distribute *Worksheet 2* and instruct students to design their experimental set-ups.
- Teachers can show students the materials and apparatuses to facilitate their design.

**Task 2**

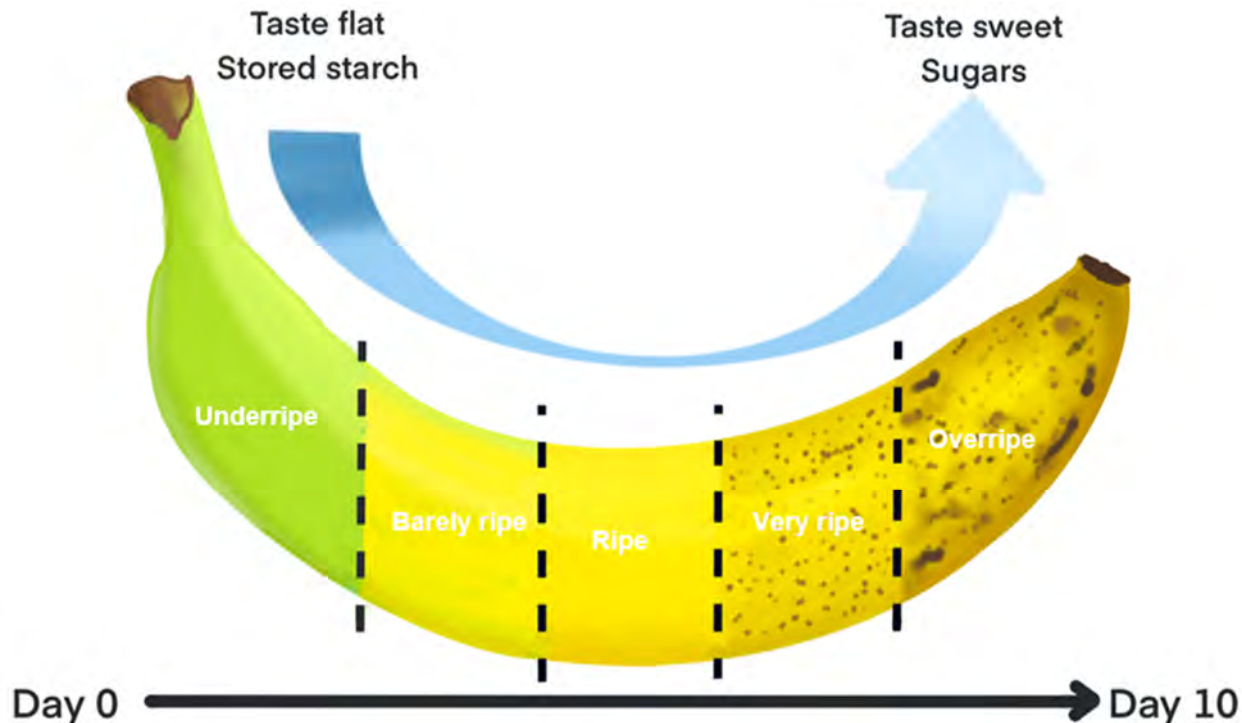
- Read the following information and answer the questions that follow.

**Scenario**

Godfrey bought some bananas. He ate a green one and complained that it tasted flat. His biology teacher told him that green bananas are not yet ripe and suggested to Godfrey that he should store the bananas and eat them until the bananas turn yellow. Godfrey ate the bananas that were stored for different days and noticed that the bananas that were stored longer tasted sweeter.






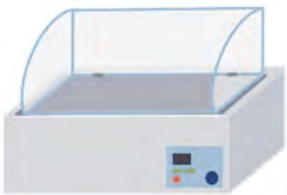

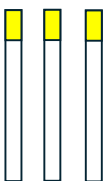









Godfrey wondered *why* bananas become sweeter when they ripen further. He hypothesized that biochemical changes occur during the ripening process (*Figure 1*).

To test this hypothesis, he investigated how the starch and sugar contents of bananas changed with the degree of their ripeness.



*Figure 1.* Godfrey's hypothesis about the biochemical process that make ripe bananas sweeter than unripe bananas

Design an investigation to test Godfrey's hypothesis using the following materials:

<p>Unripe bananas</p> 	<p>Muslin cloth</p> 	<p>Mortar and pestle</p> 
<p>Benedict's solution</p> 	<p>Filter funnel</p> 	<p>Water bath</p> 
<p>DCPIP solution</p> 	<p>Protein test paper</p> 	<p>Iodine solution</p> 
<p>Measuring cylinder</p> 	<p>Beaker</p> 	<p>Test tubes</p> 
<p>Refrigerator</p> 	<p>Wash bottle with distilled water</p> 	<p>Electronic balance</p> 
<p>Knife</p> 	<p>White tile</p> 	<p>The diagrams provide visual scaffolds to help students understand the materials and apparatuses for the investigation. Students need to choose the relevant materials for the investigation.</p>

**任務 2**

- 閱讀以下資訊並回答隨後的問題。

**情境**

高飛買了一些香蕉。他埋怨所吃的一條青色香蕉淡而無味。他的生物科老師指出青色的香蕉尚未熟透，並建議他把香蕉存放至變成黃色後才進食。高飛隨後吃了存放了不同日子的香蕉，並發現香蕉存放時間愈長，味道愈見香甜。

高飛好奇為什麼香蕉愈成熟會變得愈香甜。他提出了一個假說，認為成熟過程中進行了一些生化改變。

為測試這項假說，他想探究香蕉的糖和澱粉含量怎樣隨著成熟時間而改變。

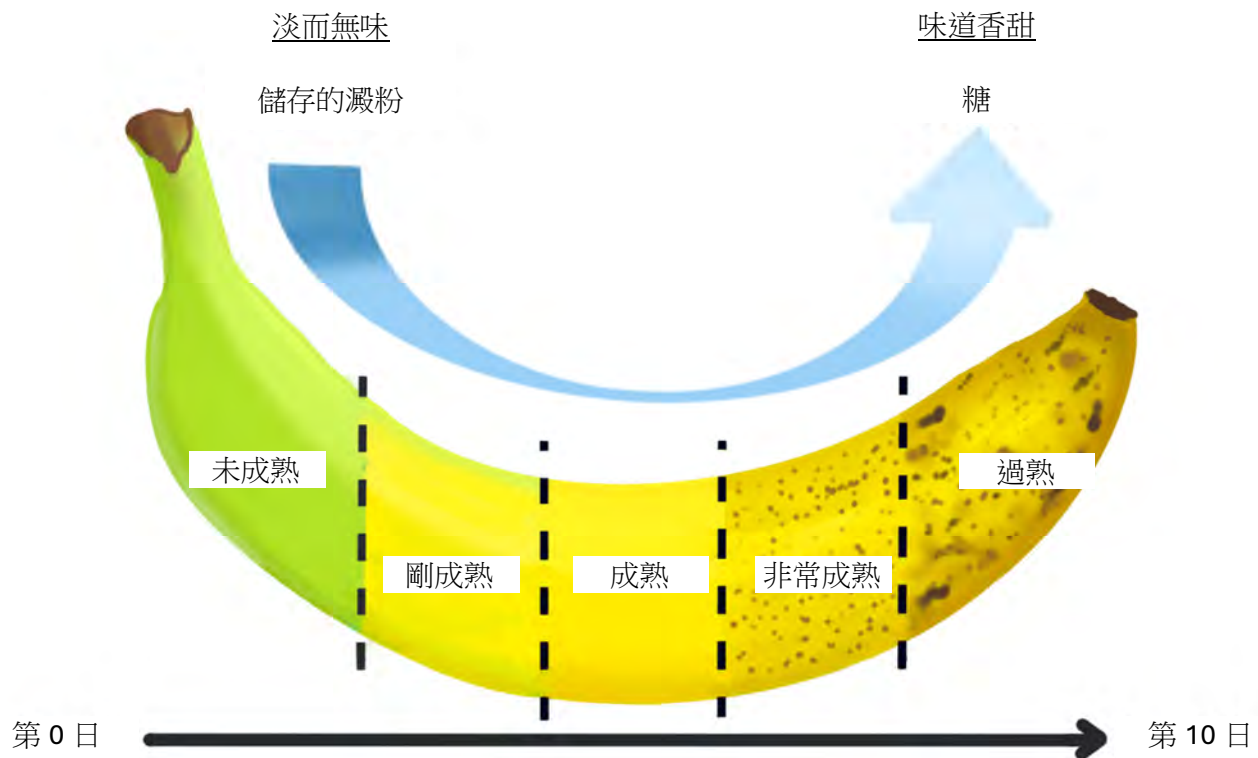



















圖 1： 高飛對成熟香蕉較未成熟香蕉更香甜背後的生化過程的假說

使用下列實驗材料設計一項探究以檢測高飛的假說。

<p>未成熟的香蕉</p> 	<p>紗布</p> 	<p>研鉢及研杵</p> 
<p>本立德溶液</p> 	<p>漏斗</p> 	<p>水浴</p> 
<p>DCPIP 溶液</p> 	<p>蛋白質試紙</p> 	<p>碘液</p> 
<p>量筒</p> 	<p>燒杯</p> 	<p>試管</p> 
<p>雪櫃</p> 	<p>洗滌瓶及蒸餾水</p> 	<p>電子秤</p> 
<p>刀</p> 	<p>白色瓷磚</p> 	



**Notes for teachers**

- The following are some questions that teachers may use to guide students in thinking about or assessing scientific inquiry skills related to their experimental designs.
- Student work samples are shown below to illustrate possible student thinking to some questions.

**Possible questions**

- (a) Propose a hypothesis to explain *why* bananas become sweeter when they ripen.  
 (b) If Godfrey’s hypothesis is correct, what are the predicted results of the experiment?

2. Below are two suggestions from Godfrey’s classmates:

Mary: Use the same banana, cutting a slice of banana on different storage days for testing.  
 Tom: Use different bananas stored for different days for testing.

Discuss the strengths and limitations of each design.

	Strength	Limitation
Mary’s design		
Tom’s design		



**Notes to teachers**

- Q.1(a) and (b) assess students’ ability to propose a hypothesis and make predictions based on their hypothesis.
- Q.2 assesses students’ ability to identify the strengths and limitations of alternative designs (i.e. within and between subject designs).

The following are some examples of students' responses to Q.1(b):

Sample 1

The higher the degree of ripeness of the banana, the higher sugar content and lower starch content of the bananas.

Sample 2


本立德溶液在實驗最終會變色  
碘液在實驗最終會變色

Sample 3

香蕉會隨時間愈香甜，時間越久，本立德測試中的磚紅色沉澱物越多，時間越短，反之越少

Sample 4

在實驗~~的~~起初，使用本立德~~測試~~<sup>測試</sup>和碘液測試，  
碘液變色的量隨時間變得愈來愈少，  
本立德溶液變色的量隨時間變得愈來愈多



**About the samples**

- Sample 1 did not describe the results of the chemical tests. Instead, it describes the inference from the results of the tests.
- Sample 2 described the predicted results without relating them to which samples.
- Sample 3 correctly described the predictions of the results of the Benedict's test but did not mention the predictions about the iodine test.
- Sample 4 mentioned both tests, but the predicted results were unclear.

**任務 3**

**參考問題**

1. (a) 提出一項假說，以解釋為什麼香蕉愈成熟會變得愈香甜。
- (b) 若高飛的假說正確，這個實驗的預期結果是什麼？
3. 高飛的同學就探究的設計提出了兩項不同的建議：

瑪莉： 使用同一條香蕉，但在不同存放日數切下一片香蕉片作試驗

湯姆： 使用存放了不同日數的不同香蕉作試驗

分別討論這些設計的優勢和限制。

	優勢	限制
瑪莉的設計		
湯姆的設計		

**Notes for teachers**

- Teachers can distribute the manual for students to read and prepare before the investigation.
- Teachers can provide students with videos demonstrating how to perform the food tests before they carry out the experiment. These instructional videos can be easily sourced from textbook publishers' audio-visual resources.
- Teachers can ask questions to check if students fully understand the procedures.
- The *Supplementary Resource* section contains the list of materials.
- Scan the QR code to view the process of the experiment.

**Task 4**

- Read the following procedures to carry out the investigation.

**Procedure****Preparation of the banana samples**

1. Label the bananas with a storage duration of 0, 2, and 4 days as samples *A*, *B*, and *C*, respectively.
2. Weigh 10 g of banana sample *A* using an electronic balance.
3. Put the sample into a plastic bag.
4. Add 20 mL of distilled water, and seal the plastic bag.
5. Mash the banana in a plastic bag to a pulp.
6. Filter the mashed materials through a double layer of moist muslin cloth over a filter funnel and collect the filtrate (i.e., the extract) in a 100-mL beaker.
7. Repeat *Step 2* and *Step 3* with the other two banana samples.

**Test for reducing sugar: Benedict's test**

8. Add 1 cm<sup>3</sup> of filtrates of each banana sample into three test tubes.
9. Add 2 cm<sup>3</sup> of Benedict's solution to each tube. Shake the contents gently to mix well.
10. Place the test tubes in the mini water bath.
11. Wait for 5 minutes, and shake the test tubes at intervals.
12. Observe and compare any colour changes in the solution and the amount of precipitate formed.

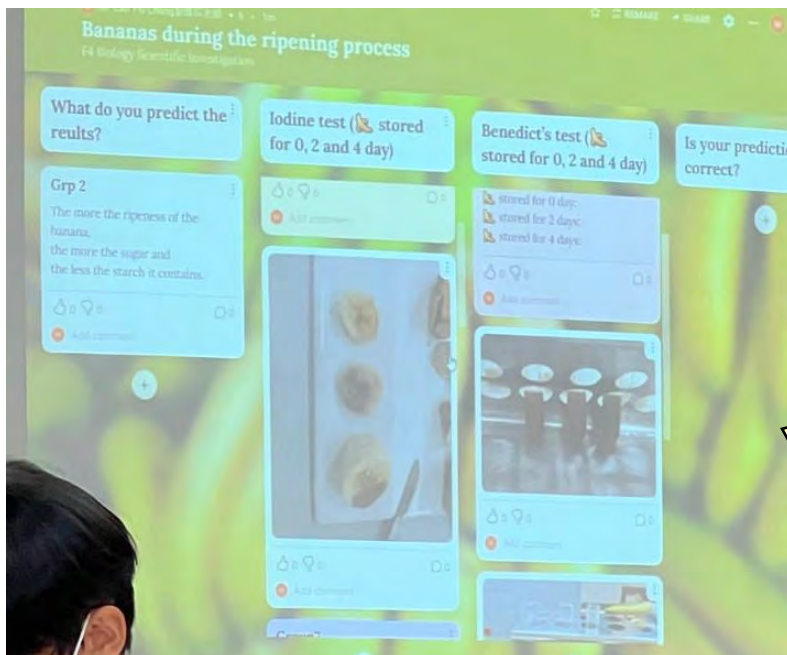
**Test for starch: Iodine test**

13. Cut a slice of banana from each banana sample on a white tile.
14. Add 10 drops of iodine solution to the samples with the dropper bottle.
15. Observe and compare the intensity of the blue-black colour.



### Notes for teachers

- A plastic bag can be used for mashing the banana, which saves time compared to grinding the banana using a mortar and pestle.
- Test tubes can be replaced with glass vials or microcentrifuge tubes.
- Teachers may ask students to take photographs of their experimental results. These photographs can then be used by students to check if their results match their initial predictions, and can also be shared with their classmates.



*Padlet* is a real-time collaborative web platform that allows students to share photographs, text, and other content with their peers.

**任務 4**

- 閱讀以下實驗步驟以進行探究:

**實驗步驟****準備香蕉樣本**

1. 把存放第 0 日、第 2 日和第 4 日的香蕉分別標示為樣本 A、B 和 C。
2. 使用電子秤，稱重 10 g 的香蕉樣本 A。
3. 將樣本置於膠袋中。
4. 將 20 mL 蒸餾水加進膠袋中，然後把膠袋封好。
5. 用手將膠袋內的香蕉壓成果蓉。
6. 將濕潤的雙層紗布置於漏斗上，然後將果蓉倒在紗布上過濾。以燒杯收集濾液。
7. 使用其他兩個香蕉樣本，重複步驟 2 和 3。

**本立德試驗：測試還原糖的存在**

8. 將 1 cm<sup>3</sup> 來自不同香蕉樣本的濾液分別加進三枝試管中。
9. 將 2 cm<sup>3</sup> 本立德溶液加到各小玻璃瓶中，輕輕搖動試管以混和溶液。
10. 將各玻璃小瓶置於迷你水浴。
11. 等待 5 分鐘，其間可間中輕輕搖動試管。
12. 觀察各溶液的顏色變化及所生成的沉澱物量。

**碘液試驗：測試澱粉的存在**

13. 從各香蕉樣本切下一片香蕉片放在白色瓷片上。
14. 將 10 滴碘液加到各香蕉片上。
15. 觀察和比較各樣本的藍黑色深度。

## Teacher Notes 2



### Notes for teachers

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

### Task 5

#### Possible questions

1. Based on the data obtained, evaluate whether the proposed hypothesis is supported.
2. Ada found that reducing sugars could not be detected in all the banana samples. She believed that this was because the ripening process was too slow and that 4 days were not enough for the ripening process. How would you change the experimental design to verify if her thought was right?

Tick '✓' the correct box below and explain your choice.

Modification:

- Repeating the experiment with bananas stored for 0, 1, 2, 3, and 4 days.
- Repeating the experiment with bananas stored for 0, 4, and 8 days

My explanation:

3. Your classmate found that the banana samples that ripened after 2 days and the samples that ripened after 4 days had similar colour intensity and gave a similar amount of precipitate in the Benedict's test, based on visual inspection.

What would you suggest him to do to more accurately determine if there is a difference in the amount of reducing sugars in the two samples? Explain your answer.



### Notes for teachers

- Q.1 assesses students' ability to evaluate whether the hypothesis is supported, refuted or remains undetermined according to the data.
- Q.2 assesses students' ability to suggest further data collection to address the limitations of the experimental design.
- Q.3 assesses students' ability to suggest valid improvements to reduce the measurement errors.

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

### Sample 1

- 5.(a) 艾達進行實驗後發現，所有的香蕉樣本都沒有檢測到還原糖。她認為香蕉的成熟過程太慢，四天的時間對香蕉的成熟過程並不足夠。你會如何改動實驗設計以驗證她的看法是否正確？在適當的方格內加“✓”以顯示你的選擇。解釋你的選擇。

改動：

- 以存放了 0 天、1 天、2 天、3 天和 4 天的香蕉重覆進行實驗  
 以存放了 0 天、4 天和 8 天的香蕉重覆進行實驗

我的解釋：

將不同存放時間的香蕉進行本尼迪克特試，能有效檢測到不同齊色色的改變，如果第四天的香蕉是兩重紅色沉澱物，證明出香蕉成熟過程足夠並和前三天的本尼迪克特試驗結果對比，並且這個改動可以更正香蕉觀測到變化，準確得知成熟時間

### Sample 2

改動：

- 以存放了 0 天、1 天、2 天、3 天和 4 天的香蕉重覆進行實驗  
 以存放了 0 天、4 天和 8 天的香蕉重覆進行實驗

我的解釋：

因為艾達應為存放 4 天對存放香蕉的成熟過程不足，所以可以用存放 8 天的香蕉和 4 天的再次重覆實驗作比較。

### Sample 3

改動：

- 以存放了 0 天、1 天、2 天、3 天和 4 天的香蕉重覆進行實驗  
 以存放了 0 天、4 天和 8 天的香蕉重覆進行實驗

我的解釋：

用存放 8 天的香蕉更能和存放 4 天的香蕉有鮮明的對比，結果量度減少誤差，提升精確性。  
而只相若一天的存放時間，<sup>及 0 天</sup>，大大增加量度結果時的誤差。  
<sub>全結果仍相似</sub>  
同時艾達想證明存放 4 天的香蕉成熟度不足，第一次實驗中已做過快，因此增加香蕉存放天數後如生成比第一天的香蕉更多的紅色沉澱物則能證明其存放時間香蕉成熟度不足。  
<sub>存放</sub>



#### About the samples

- Sample 1 incorrectly believed that using a narrower range and interval of the independent variable could produce positive Benedict's results even though the bananas had not yet ripened.
- Sample 2 correctly suggested lengthening the duration of the storage of the banana which could provide more time for the ripening process.
- Sample 3 further suggested comparing the amount of precipitate in the sample from Day 8 and that in the sample from Day 4.

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

### Sample 1

- (b) 你的另一位同學發現香蕉成熟第 2 日和第 4 日在本立德試驗下，所觀察到顏色深度非常相似並且沉澱量非常接近。為更準確地判斷兩個樣本的還原糖含量是否有差異，你會建議他怎樣做？

我會建議他用多點的本立德溶液  
和在放試管久一點去觀察溶液的沉澱  
及顏色便可以詳細分別兩試管顏色的不同

### Sample 2

- (b) 你的另一位同學發現香蕉成熟第 2 日和第 4 日在本立德試驗下，所觀察到顏色深度非常相似並且沉澱量非常接近。為更準確地判斷兩個樣本的還原糖含量是否有差異，你會建議他怎樣做？

利用光度計測試吸光度，吸光度更高的含更多沉澱物。

### Sample 3

- (b) 你的另一位同學發現香蕉成熟第 2 日和第 4 日在本立德試驗下，所觀察到顏色深度非常相似並且沉澱量非常接近。為更準確地判斷兩個樣本的還原糖含量是否有差異，你會建議他怎樣做？

我建議他用吸光度計檢測兩個沉澱物，越干，沉澱物越深色，便可得知還原糖那個的

或者用電子秤量度兩者沉澱物的份量，越干，沉澱物越多，便能得知兩者還原糖量是



#### About the samples

- Sample 1 incorrectly believed that increasing the volume of the Benedict's test and lengthening the time for the Benedict's test could more accurately detect the minute differences in the amount of reducing sugars. Note that the protocol used excess Benedict's solution and heated the samples sufficiently.
- Sample 2 suggested an alternative strategy, the use of a colorimeter to detect the differences in the samples.
- Sample 3 provided two alternative methods. The sample could further improve by providing an explanation for why these methods are more sensitive in detecting the differences.

## 任務 5

### 參考問題

1. 根據你所獲得的數據，評估數據是否能支持假說。
2. 艾達進行實驗後發現，所有的香蕉樣本都沒有檢測到還原糖。她認為香蕉的成熟過程太慢，四天的時間對香蕉的成熟過程並不足夠。你會如何改動實驗設計以驗證她的看法是否正確？在適當的方格內加“✓”以顯示你的選擇。解釋你的選擇。

改動：

- 以存放了 0 天、1 天、2 天、3 天和 4 天的香蕉重覆進行實驗
- 以存放了 0 天、4 天和 8 天的香蕉重覆進行實驗

我的解釋：

3. 你的另一位同學發現香蕉成熟第 2 日和第 4 日在本立德試驗下，所觀察到顏色深度非常相似並且沉澱量非常接近。

為更準確地判斷兩個樣本的還原糖含量是否有差異，你會建議他怎樣做？解釋你的答案。

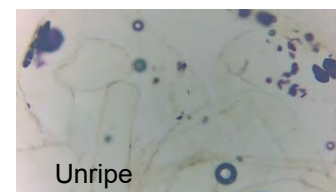
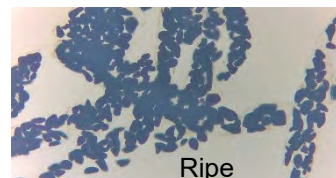


## Supplementary Resources

### Possible Modifications

#### 1. Preparation of temporary microscope slides of unripe and ripe banana samples

- Gently smear samples of the unripe and ripe bananas onto separate microscope slides.  
(Do *not* use a knife in order to avoid spilling the cellular contents.)
- Instruct students to examine the slides under the microscope.
- See Tamarkin (2015) for a detailed description.



#### 2. Investigating the ripening process of bell peppers

- Green, yellow, and red bell peppers are the same vegetable at different stages of ripeness. Green peppers are unripe while red peppers are fully ripened. Yellow peppers fall somewhere in the middle of the ripening process.
- Bell peppers can be used to study the biochemical changes that occur as a result of the ripening process (e.g., changes in vitamin C, reducing sugar and enzyme content [e.g., catalase]).
- See Olędzki & Harasym (2023) for an example.

### Technician Notes

#### 1. Materials for Task 1

Handheld UV light torch	Overripe banana	Black box
-------------------------	-----------------	-----------

#### 2. Materials for Task 4

##### *Materials for each group*

Banana from day 0	Electronic balance	Filter funnel
Banana stored for 2 days	Plastic bag	100 mL beaker
Banana stored for 4 days	Muslin cloth	Test tubes
Benedict's solution	Mini water bath	Iodine solution (dropper bottle)
White tile	Knife	Autopipette (P-1000)
Autopipette tip (P-1000)	Rubbish bin	

### References

- Olędzki, R., & Harasym, J. (2023). Boiling vs. microwave heating—The impact on physicochemical characteristics of bell pepper (*Capsicum annuum L.*) at different ripening stages. *Applied Sciences*, 13(14), 1–14.
- Tamarkin, D. (2015). Exploring carbohydrates with bananas. *The American Biology Teacher*, 77(8), 620–623.



# Lipase Inhibitor Investigation

---



# Lipase Inhibitor Investigation

## Overview

- The *Lipase Inhibitor Investigation* is about the search for an anti-obesity agent. Students investigate the inhibitory effects of different types of bitter melon seed extracts on lipase activity using the milk–pH indicator system (Royal Society of Biology Nuffield Foundation, 2019).
- Students are given the opportunity to design and carry out experiments in which they set up controls, consider the need for replicates, and identify limitations of using visual inspection to determine the end point of a reaction and an *in vitro* system to study the effects of the seed extracts on enzyme activity *in vivo*.

## Teaching Plan & Key Features

### Prerequisite knowledge (scientific ideas)

- Food substances and energy requirement in humans
- Digestion and absorption of fats in humans

### Prerequisite manipulative skills

- Using an autopipette to transfer a small volume of solution

Lesson	Lesson sequence	Duration (mins)	Resources
<b>Stage 1 Preparing for the investigation</b>			
<ul style="list-style-type: none"> <li>• It is situated in an authentic context related to the search for anti-obesity drugs (<b>Contextualisation</b>).</li> <li>• Students read information about the background of the investigation (<i>Reading Materials</i>).</li> </ul>			
Before Lesson 1	<ul style="list-style-type: none"> <li>• The teacher distributes <i>Worksheet 1</i> for students to complete at home so that they can be familiar with the background of the investigation.</li> </ul>		<i>Worksheet 1</i>
1	<ul style="list-style-type: none"> <li>• The teacher discusses the investigation context with students.</li> <li>• The teacher provides feedback on students' responses in <i>Worksheet 1</i>.</li> <li>• The teacher distributes <i>Worksheet 2</i> for students to complete at home.</li> </ul>	40	<i>Worksheet 2</i>
Before Lesson 2	<ul style="list-style-type: none"> <li>• The teacher distributes <i>Worksheet 2</i> for students to complete at home.</li> </ul>		<i>Worksheet 2</i>
<b>Stage 2 Designing the investigation</b>			
<ul style="list-style-type: none"> <li>• Students interact with a virtual laboratory to familiarise themselves with the materials and apparatuses they would use in the investigation (<i>Virtual Laboratory</i>).</li> <li>• Students use a template to design their own experimental set-ups (<i>Investigation Planning Template</i>).</li> <li>• Students have the chance to evaluate their own and their peers' experimental set-ups (<i>Self &amp; Peer Evaluation</i>).</li> </ul>			
2	<ul style="list-style-type: none"> <li>• Teacher provides feedback on students' experimental designs in <i>Worksheet 2</i>.</li> </ul>	40	Student Samples 1
3	<ul style="list-style-type: none"> <li>• The teacher discusses with the students some questions related to the experimental design.</li> <li>• The teacher provides students with laboratory manual for preparation at home.</li> </ul>	40	Teacher Notes 1

<b>Stage ③ Carrying out the investigation</b>			
<ul style="list-style-type: none"> <li>Students use microscale instrumentation that reduces the time of the experiments (<i>Microscale Instrumentation</i>).</li> <li>Students collect more complex data sets by setting up replicates (<b>Complex Data Set</b>).</li> <li>Students use camera to collect data (<i>Digital Tool</i>).</li> </ul>			
4	<ul style="list-style-type: none"> <li>Teacher asks questions to help students connect their lab experience and related ideas/scientific inquiry skills.</li> <li>Students carry out the investigation.</li> </ul>	40	Laboratory Manual
<b>Stage ④ Explaining and evaluating data</b>			
<ul style="list-style-type: none"> <li>Students use <i>Google Sheet</i> for data recording and manipulation (<i>Digital Tool</i>).</li> <li>Students use data to identify seed extracts with the highest inhibitory effect on lipase activity.</li> <li>Students considering the limitations of using an <i>in vitro</i> system to study the effects of the seed extracts on enzyme activity <i>in vivo</i>.</li> </ul>			
Before Lesson 5	<ul style="list-style-type: none"> <li>Students complete data reporting and analysis at home.</li> <li>Teacher collects and marks student responses.</li> </ul>		Teacher Notes 2
5	<ul style="list-style-type: none"> <li>Teacher provides feedback on students' performance related to data reporting and analysis.</li> </ul>	40	Teacher Notes 2

### Important Notes

- Students are *not* required to learn the detailed mechanism of enzyme inhibition. Rather, they are expected to use data to support their claims about the inhibitory effect.
- Students should avoid skin contact with the solutions and quickly rinse any splashes of lipase solution or sodium carbonate from their skin.



## Instructional Materials

### Stage 1 Preparing for the investigation

### Student Worksheet 1



#### Notes for teachers

- The teachers can distribute *Worksheet 1* and ask students to read the background information related to the investigation at home.
- Students' responses can be collected using a *Google Form*.

#### Task 1

- Read the following information and source materials in the *Data File*.
- Answer the questions that follow.

Obesity is a major risk factor of cardiovascular diseases, musculoskeletal disorders, and some cancers. According to the World Health Organization, approximately 650 million adults were obese, and more than 1.9 billion were overweight in 2016.

Orlistat is a drug approved by the United States Food and Drug Administration for the long-term treatment of obesity; it inhibits lipase activity in the alimentary canal. Orlistat reduces the absorption of dietary fat in the human body. However, it may cause side effects such as gastrointestinal discomfort.

Scientists are now searching for natural alternatives as anti-obesity drugs. Read the information in the *Data File* to familiarise yourself with the investigation background.



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



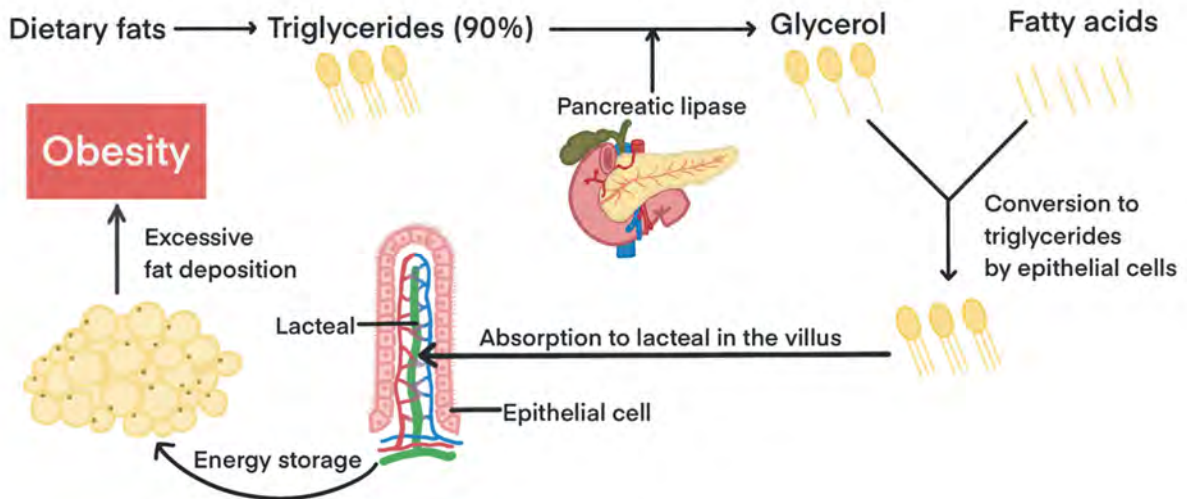
Teachers can diagnose students' difficulties in understanding the relevant content and methods for measuring lipase activity, and then provide feedback before students design the experiments.

## Data File

Your biology teacher asks you to read the following source materials to prepare you to design a scientific investigation related to lipase activity:

### Source 1:

Most dietary fats are made up of triglycerides. Dietary fat cannot be directly consumed by the human body and must be digested for absorption. Fats consumed by the human body are digested by lipases in the alimentary canal and broken up into smaller molecules, fatty acids, and glycerol for absorption into the body. *Figure 1* shows the action of lipases in fat absorption in the human body.



*Figure 1.* Metabolic pathways related to fats in the human body

Lipase inhibitors are substances that can inhibit lipase activity by reducing the breakdown of fats and their absorption into the human body.

**Source 2:**

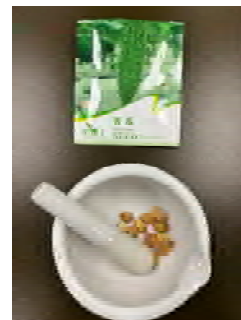
**Student scientists in Hong Kong discover anti-obesity agents present in bitter melon seed extract**

Some plant tissues contain lipase inhibitors such as polyphenols and saponins.

A team of secondary school student scientists screened more than 60 plant samples to identify natural inhibitors that effectively reduce pancreatic lipase activity *in vitro*. They performed a simple experiment using whole milk and an alkaline solution containing a pH indicator (which is blue at alkaline pH values and yellow at acidic pH values). They first prepared different seed extracts by grinding the seeds with a pestle in a mortar using water and a spoonful of sand.



Scan the QR code to access the material used by the student scientists.



The plant extract was then incubated with alkaline pancreatic lipase containing the pH indicator for 5 minutes. After adding whole milk to initiate fat digestion, the time taken for the reaction mixture to change colour from blue to yellow was recorded. The recorded data were then used to determine the activity of the pancreatic lipase preincubated with different plant extracts.



Scan the QR code to watch their investigation.



The student scientists' findings revealed that bitter melon (*Momordica charantia*) seed extracts contain pancreatic lipase inhibitors. Their findings show potential for addressing the global obesity problem.

Answer the questions below *after* reading the source materials:

- Explain why inhibiting the lipase activity in the alimentary canal can help reduce body weight.
- In which part of the alimentary canal can you find pancreatic lipase? Explain the conditions that favour the pancreatic lipase activity in this part.
- Whole milk contains triglycerides. Write a word equation to show the actions of pancreatic lipase on triglycerides in whole milk.
- Explain why whole milk containing an alkaline solution and the pH indicator described in *Source 2* would turn from blue to yellow after the addition of pancreatic lipase.
- How is the time taken for the alkaline solution to turn from blue to yellow related to the rate of lipase activity?
- After reading the source material, propose *one* investigation question related to the material you have read.

## 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

過度肥胖是心血管疾病、肌肉骨骼疾病和某些癌症的主要危險因素。根據世界衛生組織的數據，2016 年約有 6.5 億成年人過度肥胖，超過 19 億人超重。

奧利司他是美國食品藥品監督管理局批准用於長期治療肥胖症的藥物，它能抑制消化道中的脂肪酶活性。奧利司他可以減少人體對膳食脂肪的吸收。然而，它也可能會引起副作用，例如胃腸道不適。

科學家們現在正尋找天然替代品作為抗肥胖藥物。請閱讀資料檔中的信息以熟悉此探究的背景。

### 資料檔案

你的生物老師要求你閱讀以下的資料，以準備設計一個有關於脂肪酶活性的科學探究。

### 資料 1

大多數膳食脂肪都由甘油三酯組成。膳食脂肪不能被人體直接使用，必須經過消化才能吸收。人體消耗的脂肪在消化道中被脂肪酶消化並分解成更小的分子脂肪酸和甘油，然後才被人體吸收。圖 1 展示了脂肪酶在人體吸收脂肪中的作用。

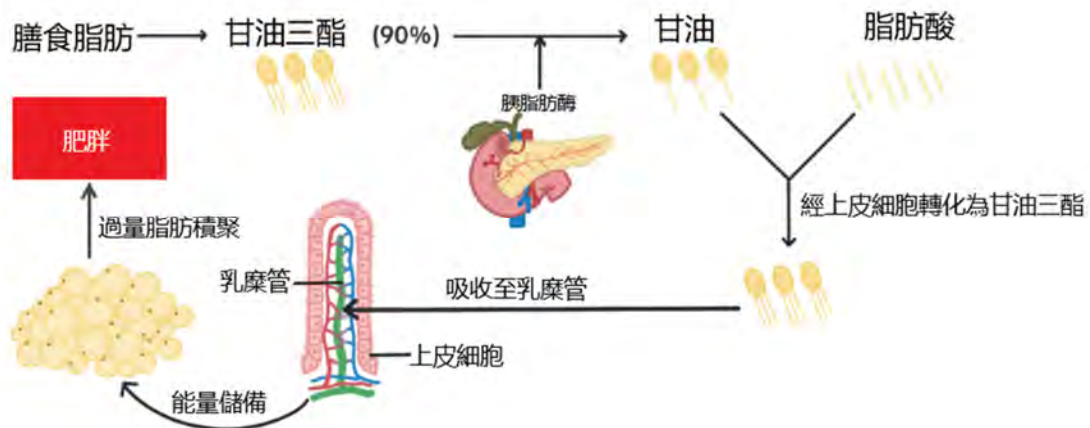


圖 1. 人體內有關脂肪的代謝途徑

脂肪酶抑制劑是通過減少脂肪的分解及人體對脂肪的吸收來抑制脂肪酶活性的物質。

## 資料 2

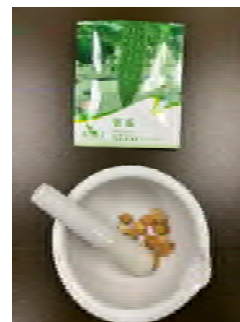
### 香港的學生科學家於苦瓜籽提取物中發現抗肥胖劑

有些植物組織中含有諸如多酚和皂苷的脂肪抑制劑。

一個中學生科學團隊篩選了 60 多個植物樣本以找出能在體外有效降低胰脂肪酶活性的天然抑制劑。他們使用全脂牛奶和含有 pH 指示劑（其在鹼性 pH 下呈藍色，而在酸性 pH 下呈黃色）的鹼性溶液進行了一個簡單的實驗。他們先把水和一勺沙子加入種子中，用研杵在研鉢中研磨種子，製備出不同的種子提取物。



掃描二維碼以取得學生科學家們使用的材料。



植物提取物隨後與含有 pH 指示劑的鹼性脂肪酶一起溫育 5 分鐘。添加全脂奶以開始脂肪消化後，記錄反應混合物從藍色變為黃色所需的時間。然後使用記錄的數據來確定與不同植物提取物預溫育的胰脂肪酶活性。



掃描二維碼以觀看他們的探究。



學生科學家們的研究結果表明，苦瓜(*Momordica charantia*)的種子提取物含有胰脂肪酶抑制劑。他們的發現展示了解決全球肥胖問題的潛在可能。

請閱讀材料後回答以下問題。

- 解釋為何抑制消化道中脂肪酶的活性有助於減輕體重。
- 在消化道的哪個部分可以找到胰脂肪酶？解釋該部分有利於胰脂肪酶活性的條件。
- 全脂牛奶含有甘油三酯。請以文字化學方程式來表示全脂牛奶中胰脂肪酶對甘油三酯的作用。
- 請解釋為甚麼添加胰脂肪酶後，資料 2 中描述的含有鹼性溶液和 pH 指示劑的全脂牛奶的會由藍色變為黃色。
- 鹼性溶液由藍變黃所需的時間與脂肪酶的活性如何相關？
- 請在閱讀材料後提出一個與你所讀材料相關的探究問題。



掃描二維碼以獲取 Google Form 的副本



## Student Worksheet 2

### Notes for teachers



- After discussing with students their responses in *Worksheet 1*, teachers can distribute *Worksheet 2* and ask students to design the investigation at home.
- An *Investigation Planning Template* can be provided to students.
- Student work samples are shown below to illustrate possible student thinking.
- Scan the QR code to get a copy of the *Google Form*.



### Task 2

- Answer the questions that follow.

### Scenario

Bitter melon (*Momordica charantia*) belongs to the Cucurbitaceae family. Different varieties of bitter melons have different shapes and bitterness. The Cucurbitaceae family is composed of different types of melons.

Your biology teacher asks you to design an investigation to compare the inhibitory effect of the seed extracts of three different types of melons within the Cucurbitaceae family on pancreatic lipase. The goal is to identify the seed extract sample with the highest inhibitory effect on pancreatic lipase activity.

You received the following materials:

Alkaline solution containing a pH indicator (blue under alkaline pHs and yellow under acidic pHs)	Glass vials	Test sample 1 [Bitter melon 1 ( <i>Momordica charantia</i> ) seed extract]
Pancreatic lipase	Timer	Test sample 2 [Bitter melon 2 ( <i>Momordica charantia</i> ) seed extract]
Orlistat (a drug that inhibits pancreatic lipase)	Distilled water	Test sample 3 [Angled Luffa ( <i>Luffa acutangula</i> ) seed extract]
Whole milk	Tablet (to be used as a camera)	Tablet stand
Autopipette	Autopipette tips	



Scan the QR code to view the materials.



The virtual laboratory provides students with opportunities to get familiar with materials used in the investigation.

- (a) Briefly describe how you would use the materials to design an investigation to achieve the aim. You can also draw your experimental design.  
(For this purpose, the *Investigation Planning Template* may be helpful.)

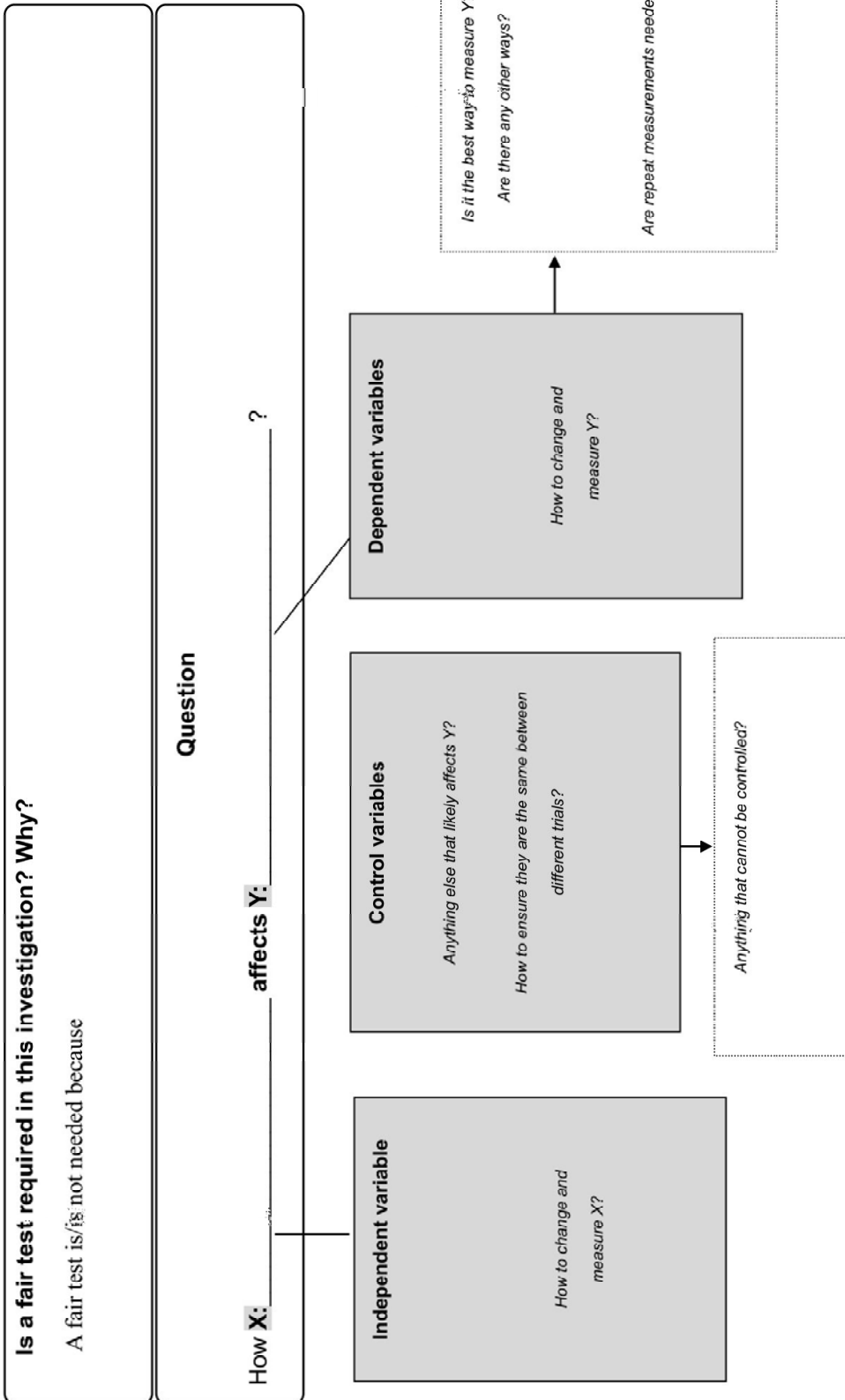
The *Investigation Planning Template* provides a structured framework and scaffolding to help students express their design decisions.

*Brief explanation of my design:*



Scan the QR code to get a copy of the *Investigation Planning Template*.





**任務 2**

- 回答以下問題。

**情景**

苦瓜 (*Momordica charantia*) 屬於葫蘆科。不同品種的苦瓜形狀和苦澀程度不同，苦味也不同。葫蘆科的瓜類也有不同的品種。

你的生物老師要求你設計一項探究，以比較葫蘆科三種不同瓜品種的種子提取物對胰脂肪酶的抑制作用。其目的是識別對胰脂肪酶活性抑制作用最高的種子提取物樣本。

你收到以下材料：

含 pH 指示劑的鹼性溶液（鹼性 pH 下呈藍色，酸性 pH 下呈黃色）	玻璃小瓶	試驗樣本 1 [苦瓜 1 ( <i>Momordica charantia</i> ) 種子提取物]
胰脂肪酶	計時器	試驗樣本 2 [苦瓜 2 ( <i>Momordica charantia</i> ) 種子提取物]
奧利司他 (一種抑制胰脂肪酶的藥物)	蒸餾水	試驗樣本 3 [棱角絲瓜 ( <i>Luffa acutangula</i> ) 種子提取物]
全脂牛奶	平板電腦 (用作攝影機)	平板電腦支架
自動移液器	自動移液器吸管尖	



你可以掃描二維碼查看這些材料



- (a) 簡要描述你將如何使用上述材料來設計一項探究實驗以實現上述目的。你也可以畫出你的實驗設計 (實驗策劃模板可能會對你有幫助)。

掃描二維碼以獲取 **實驗策劃模板** 的副本



掃描二維碼以獲取 **Google Form** 的副本



我的設計簡介：

## Student Samples 1 (Worksheet 2)



### Notes for teachers

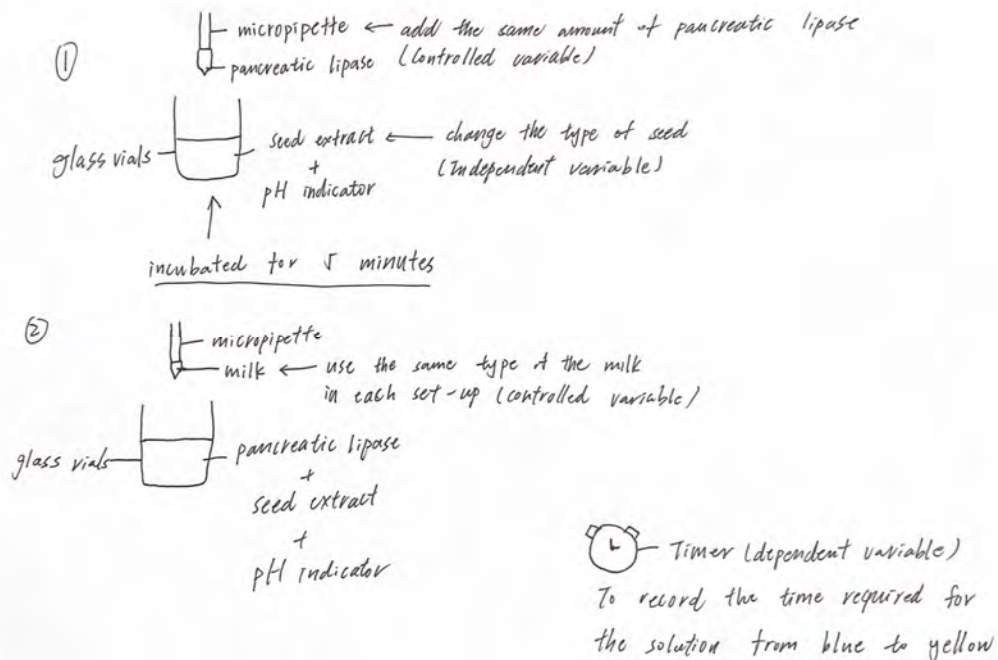
- After collecting students' designs, teachers can select student drawings (anonymised) for discussion.
- The following shows three samples with varying sophistication in responses. Some guiding questions can be included to facilitate students' evaluation of experimental designs.

### Examples of students' experimental designs

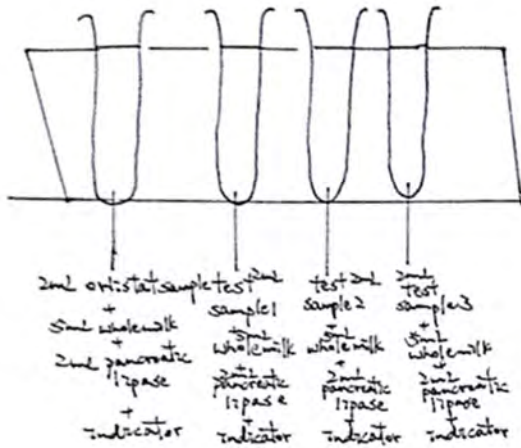
#### Possible guiding questions

- Which design(s) accurately represent the independent variable and provide methods for manipulating it? Why does your group think so?
- Which design(s) accurately represent the dependent variable and specify the parameters for measuring it? Why does your group think so?
- Which design(s) demonstrates the correct sequence for adding the chemicals? Why does your group think so?
- Which design(s) incorporate the appropriate control set-up(s), if necessary? Why does your group think so?
- What are the ways to enhance the designs to ensure that the data collected are accurate and reliable?

#### Design 1:



**Design 2:**



- ① Pipette 5ml whole milk to 4 vials respectively
- ② Pipette 2ml orlistat, test sample 1, test sample 2, test sample 3 to 4 vials respectively.
- ③ Put each sample into vials, record the time of indicator colour change afterwards  
Orlistat → sample 1 → sample 2 → sample 3
- ④ Repeat the tests twice
- ⑤ Repeat steps above for 3 different temperatures

Independent variable = Test sample 1, 2, 3 and temperature  
 ↳ temperature: put glass vials for required temperature of water bath for 5 mins before tests, incubate them throughout the test.

Dependent variable: Time for indicator colour change  
 's' as unit, the smaller the value, the larger the inhibit effect of the sample  
 Use timer to record required time.

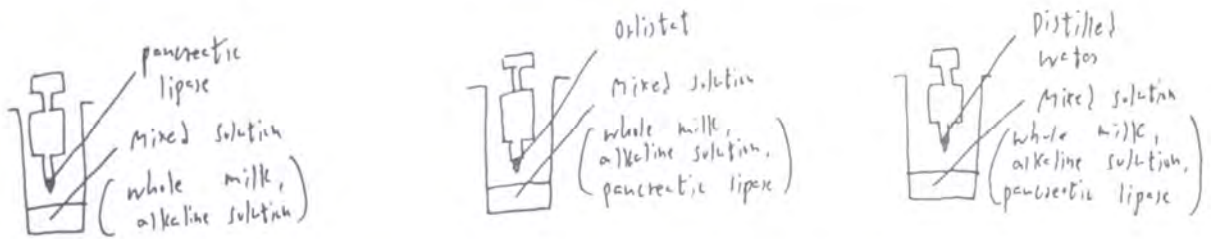
Control variable: ① amount of pancreatic lipase used  
 ↳ more enzymes will increase the catalyzing rate to the breakdown reaction which greatly lowers the required time by lowering the activation energy. The time recorded may be underestimated.  
 ② Total surface area of test sample used  
 ↳ the larger the surface area, the higher rate of inhibitory effect.  
 Time recorded may be underestimated.

Design Consideration → ↳ crush in same period / length of time (5mins) to ensure it's more or less the same.

Assumption: Other component in the seed extract does not contain inhibitory effect on pancreatic lipase.

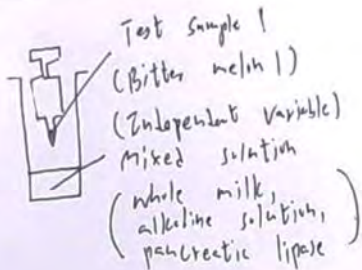
**Design ③ :**

To investigate the effect of three different types of melon within the Cucurbitaceae family on the pancreatic lipase activities.

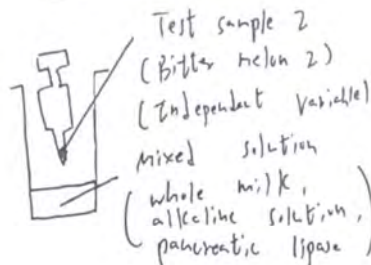


Note: Set a timer for each setup for one minute once the solution in the micropipette tip is added to the mixed solution inside the glass vial.

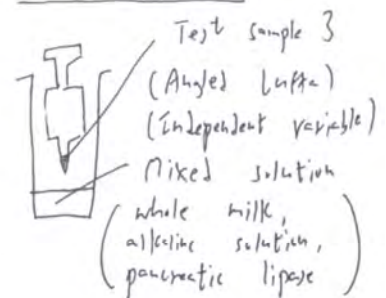
Test sample 1



Test sample 2



Test sample 3



Note: Set a timer for each setup for one minute once the test sample in the micropipette tip is added to the mixed solution inside the glass vial.

Control variables: Initial pancreatic lipase and concentration of test sample.  
This is to ensure the accuracy of the experiment.

Bacteria may enter the solution through air during experiment.

**Notes for teachers**

- Teachers can capture and represent student thinking using public displays (e.g. whiteboards) and then work with students to explore their divergent thinking.

Group	Laura	Zoe	Neda	Kelly	Candy	Cindy	Jess	Rafael	Lily	Sonia
Q 1.	1.3	3	2.3	1.3	1.3	1	1.3	1.3	1.3	1.3
Q 2.	X	1	1.2	2	2	2	1	2	1.2	1.2
Q 3.										
Q 4.										



**Notes for teachers**

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

**Task 3**

**Possible questions**

1. The following shows two methods to measure the dependent variable:

<i>Method A:</i>	Record the colour of the alkaline solution containing the pH indicator in glass vials, both <i>with</i> and <i>without</i> the test samples, after 10 min.
<i>Method B:</i>	Measure the time it takes for the colour of the alkaline solution containing the pH indicator to change (i.e. reach the end point) in the glass vials, both <i>with</i> and <i>without</i> the test samples.

Your teacher suggests that you should use *Method B*.

- (a) What is the limitation of using *Method A* to compare the inhibitory effects of different types of seed extract on pancreatic lipase activity?
- (b) Explain how the inhibitory effects of different types of seed extract on pancreatic lipase can be compared using *Method B*.
2. Jeffrey proposes two methods for setting up a control to compare the degree of inhibition on alkaline lipase activity:

*Set-up A:* Replacing the test samples with orlistat.

*Set-up B:* Replacing the alkaline lipase with boiled alkaline lipase in the glass vials containing the test samples.

Which set-up, *A* or *B*, enables a more accurate determination of the degree of inhibition? Explain your answer.

(Put a '✓' in the appropriate box.)

I will choose  *Set-up A*    *Set-up B*

The reasons:



**Notes for teachers**

- Q.1 assesses students' ability to connect the methods of measurement to the dependent variable and the limitations related to the measurement method.
- Q.2 assesses students' ability to set up the control and explain its function.

The following are some examples of students' responses to Q.1(a):

### Sample 1

- (1) Your teacher suggests that you should use *Method B*. U  B  G  E   
What is the limitation of using *Method A* to compare the inhibitory effect of different types of seed extract on pancreatic lipase activity?

The colour present is based on personal judgement. Therefore, the result may not accurate. It is hard to compare the inhibitory effect of different types of seed extract on pancreatic lipase activity.

both methods involve personal judgment of colour change

### Sample 2

- (1) Your teacher suggests that you should use *Method B*. U  B  G  E   
What is the limitation of using *Method A* to compare the inhibitory effect of different types of seed extract on pancreatic lipase activity?

We cannot compare the inhibitory effect of different types of seed extract on pancreatic lipase activity if there is no colour change in pH indicator within 10 minutes by using Method A.

why?

### Sample 3

- (1) Your teacher suggests that you should use *Method B*. U  B  G  E   
What is the limitation of using *Method A* to compare the inhibitory effect of different types of seed extract on pancreatic lipase activity?

For method A, we cannot compare the colour intensity of set-ups if 10 minutes are not enough for lipase to digest all lipids, which will give same or similar colour intensity of all set-ups. We also cannot compare the colour intensity of all sample change from blue to yellow if no set-ups have colour change after 10 minutes. Thus, we cannot compare the degree of inhibition of lipase activity.

to investigate lipase activity are enough yellow of all set-ups.



#### About the samples

- Sample 1 incorrectly cited the limitation arising from the subjectivity of colour judgment, a limitation inherent in both methods.
- Sample 2 correctly identified the limitation but lacked details whereas Sample 3 provided a detailed explanation, such as the absence of colour difference because all lipids were digested within the specified time frame.

### 任務 3

#### 參考問題

1. 下面顯示了兩種不同測量因變數的方法:

<b>方法 A:</b>	10 分鐘後, 記錄玻璃小瓶中含有 pH 指示劑的鹼性溶液的顏色 (含有和不含測試樣本都需記錄)
<b>方法 B:</b>	測量玻璃小瓶中的含有 pH 指示劑的鹼性溶液顏色產生變化(即達到終點)所需的時間 (含有和不含測試樣本都需測量)

- (a) 你的老師建議你使用方法 B。使用方法 A 去比較不同種子提取物對胰脂肪酶活性的抑制效果可能會有什麼限制?
- (b) 解釋方法 B 如何比較不同種子提取物對胰脂肪酶活性的抑制效果。

2. 傑夫提出兩種設置對照裝置的方法來比較鹼性脂肪酶活性的抑製程度

裝置 A: 將樣本替換為奧利司他

裝置 B: 將裝有測試樣本的玻璃小瓶中的鹼性脂肪酶替換為煮沸的鹼性脂肪酶。

哪種裝置(A 或 B)能夠更準確地確定抑製程度? 解釋你的答案。

(將✓填在合適的方格內)

我會選擇

方法 A

方法 B

原因:

**Notes for teachers**


- It is suggested that seed extracts are prepared for students. See the *Supplementary Resource* section for the protocol.
- Students may also be asked to prepare the seed samples. If so, one more lesson will be required.
- 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.
- The *Supplementary Resource* section contains the list of materials.
- Scan the QR code to view the process of the experiment.


**Task 4**

- Read the following procedures to carry out the investigation.

**Procedure**
*Determining the inhibitory effects of seed extracts*

1. Use your mobile phone/tablet to start recording a video.
2. Label 15 vials (A1–3 to E1–3).
3. Add the seed extracts/orlistat/distilled water, alkaline solution containing the pH indicator, and pancreatic lipase into the vials, according to the following table:

Vial	Sample (mL)	Alkaline solution containing the pH indicator (mL)	Pancreatic lipase (mL)
A	Seed extract 1	1	2
B	Seed extract 2	1	2
C	Seed extract 3	1	2
D	Orlistat	1	2
E	Water	1	2

4. Incubate the vials at room temperature for 5 minutes.
5. Add 3 mL of whole milk to the vials, and shake the vials well.
6. Start the timer.
7. Shake the vials occasionally.
8. Repeat *Steps 4–8* two more times.
9. Stop the video recording when the colour of the solution in all of the vials turns yellow from blue.
10. Fill in the data in the *Google Sheet*.



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



The *Google Sheet* helps students process and visualise the data they collected.

#### 任務 4

- 閱讀以下實驗步驟以進行探究:

#### 實驗步驟

##### 測定種子提取物的抑制作用

1. 使用平板電腦/手機錄製影片。
2. 標記 15 個小瓶(A1–A3 到 E1–E3)。
3. 按照下表，將種子提取物/奧利司他/蒸餾水、含有 pH 指示劑的鹼性溶液和胰脂肪酶加入小瓶中:

小瓶	樣本 (mL)	含 pH 指示劑的鹼性溶液 (mL)	胰脂肪酶 (mL)
A	種子提取物 1	1	2
B	種子提取物 2	1	2
C	種子提取物 3	1	2
D	奧利司他	1	2
E	水	1	2

4. 將小瓶在室溫下放置 5 分鐘。
5. 將 3 mL 全脂牛奶加入小瓶並搖勻。
6. 開始計時。
7. 偶爾搖晃小瓶。
8. 重複步驟 4–8 兩次。
9. 當所有小瓶中溶液的顏色由藍變黃時，停止錄像。
10. 在 *Google Sheet* 中填寫數據。



掃描二維碼以獲取 *Google Sheet* 的副本。



## Teacher Notes 2

**Notes for teachers**

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

**Task 5****Possible questions**

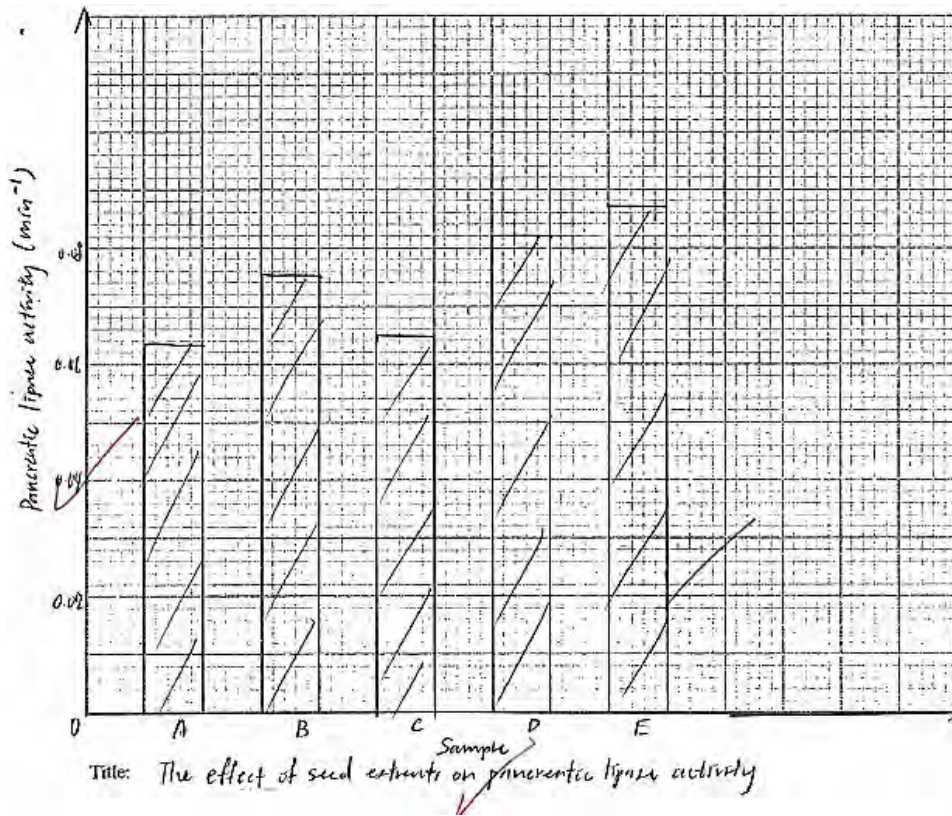
- Plot a graph to show the effect of the seed extracts on pancreatic lipase activity. Consider the following questions when plotting the graph:
    - Which type of graph (bar graph, line graph, pie chart, etc.) would you choose? Why?
    - Which axis (x-axis/y-axis) should contain the independent variable?
    - Which axis (x-axis/y-axis) should contain the dependent variable?
    - What would be a suitable title for your graph?
- Some reminders are added to guide students in constructing graphical representations appropriately.
- Boris found that an outlier was present in a replicate of one of the test samples. Suggest *one* possible reason for why this occurred.
  - Your classmate claims that the seed extract that showed the highest inhibitory effect on pancreatic lipase in this investigation should be used as an anti-obesity drug. Discuss whether you agree with this claim. (Put a '✓' in the appropriate box.)
    - Agree
    - Disagree
  - Suggest one *new* investigation that needs to be conducted before the seed extract(s) can be used as an anti-obesity drug.

**Notes for teachers**

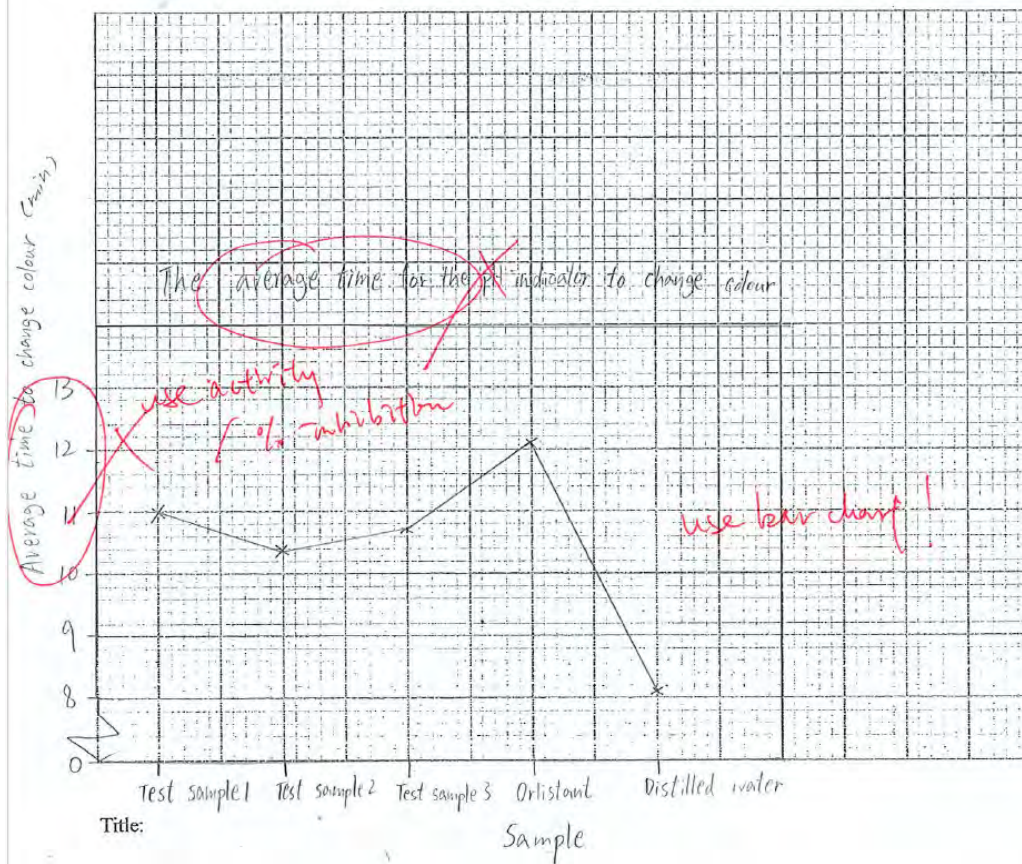
- Q.1 assesses students' ability to construct appropriate graphic representations.
- Q.2 assesses students' ability to propose reasons to explain the occurrence of an outlier.
- Q.3 assesses students' ability to identify the limitations of the generalisability of the results from an *in vitro* to an *in vivo* system.
- Q.4 assesses students' ability to generate a new investigation question that extends the present investigation.

The following are some examples of students' responses to Q.1:

**Sample 1**



**Sample 2**





### About the samples:

- Sample 1 mistakenly used a line graph for data presentation while Sample 2 correctly used a bar chart to present the data. A bar chart is appropriate as the independent variable is the type of seed extract, which is a categorical variable.
- Sample 1 also has a proper title and labelling of the x and y axes.

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

#### Sample 1

- (2) Boris found that an outlier was present in one of the replicates of one of the test samples. Suggest *one* possible reason for why this occurred. E2: U  B  G  E

The colour change is difficult to observe. *will it result in a great difference in one of the data?* We can't exactly tell when the colour changes the time.

#### Sample 2

- (2) Boris found that an outlier was present in one of the replicates of one of the test samples. Suggest *one* possible reason for why this occurred. E2: U  B  G  E

The sample used does not have equal amount of inhibitory effect within the same sample used. The part of sample *used the set up* taken has much more or much less inhibitor than other part of the same sample. *due to...*

#### Sample 3

- (2) Boris found that an outlier was present in one of the replicates of one of the test samples. Suggest *one* possible reason for why this occurred. E2: U  B  G  E

There might be human error occurs. The person might have made mistake in adding a different amount of milk into the sample, which *means a different amount of lipid is added, the* requires a longer or shorter period of time for the breakdown of lipid and the colour change.



### About the samples

- Sample 1 proposed a reason that was not sufficiently convincing in explaining the occurrence of the outlier.
- Sample 2 proposed a plausible reason for the occurrence of the outliers but lacked a detailed explanation.
- Sample 3 not only provided a plausible reason but also offered a more thorough explanation.

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

### Sample 1

Discuss whether you agree with this claim. (Put a "✓" into the appropriate box.) E11: U  B  G  E

- Agree  
 Disagree

The investigation is carried out *in vitro* that may not have the same effect *in vivo*. any examples? :-

### Sample 2

Discuss whether you agree with this claim. (Put a "✓" into the appropriate box.) E11: U  B  G  E

- Agree  
 Disagree

We still <sup>do not</sup> have enough data, as it is a experiment outside <sup>our body.</sup> we do not set this experiment *in body*, we do not know its effect will affected by the body temperature (37°C), the substance in body such as acid in stomach, under a totally different situation *in body*, the effect of the seed extract may ~~be~~ cannot reflect in our body.



#### About the samples

- Both samples correctly disagreed with the claim. Both samples identified the limitations of generalising results produced in an *in vitro* system to the *in vivo* conditions.
- Sample 2 further provided an explanation for the difference between the *in vivo* and *in vitro* conditions.

The following are some examples of students' responses to Q.4:

### Sample 1

- (i) Suggest *one* new investigation that needs to be conducted before the seed extract(s) in (g) can be used as an anti-obesity drug.

G12: U  B  G

Investigate whether have side effect on human's body.  
how...

### Sample 2

- (i) Suggest *one* new investigation that needs to be conducted before the seed extract(s) in (g) can be used as an anti-obesity drug.

G12: U  B  G

Carry out the whole investigation in vivo (e.g. white rat)  
or at a set temperature of 37°C.

### Sample 3

- (i) Suggest *one* new investigation that needs to be conducted before the seed extract(s) in (g) can be used as an anti-obesity drug.

G12: U  B  G

To investigate whether the extracts will inhibit other  
enzymes, like amylase, present in pancreatic juice.



#### About the samples

- All the samples were able to generate a new investigation question that expanded upon the current investigation. However, the question posed by Sample 1 was somewhat vague.

## 任務 5

### 參考問題

1. 繪製圖表以顯示種子提取物對胰脂肪酶活動的影響。

繪製圖表時請考慮以下問題：

- 你會選擇那種類型的圖表(條形圖、折線圖或餅狀圖等)? 為甚麼?
- 哪個軸( $x$  軸/ $y$  軸)應包含自變量?
- 哪個軸( $x$  軸/ $y$  軸)應包含因變量?
- 你的圖表適宜用甚麼標題?

2. 小明在其中一個重複實驗中找到了離群值。試提出一個可能的原因

3. 你的同學聲稱本次探究中對胰脂肪酶抑制作用最強的種子提取物應用作抗肥胖藥物。試解釋你是否認同他的說法。

4. 建議在種子提取物可以用作抗肥胖藥物之前所需進行的一項新的研究。

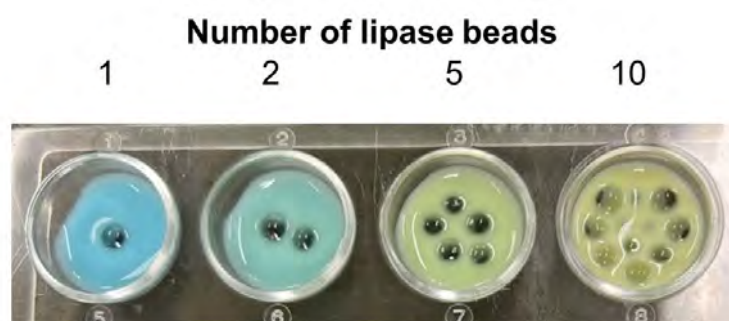


## Supplementary Resources

### Possible Modifications

#### 1. Using immobilised lipase beads to investigate lipase activity

- Lipase can be immobilised using 3% sodium alginate solution. Immobilised lipase beads can be used to study lipase activity using a milk–pH indicator system.
- The following shows the sample results of the investigation that examined the effect of increasing the number of lipase beads on the digestion of milk.
- See Chan et al. (2024) for procedures of how to make immobilised enzyme beads.



### Technician Notes

#### 1. Materials for Task 4

##### Preparation of seed extract

1. Weigh 2 g of seeds using an electronic balance.
2. Place the seeds in a mortar and pestle.
3. Add a spoonful of sand.
4. Add 10 mL of distilled water.
5. Grind the seeds into powder.
6. Filter the extract/Centrifuge the extract at top speed (13, 500 rpm) to obtain the supernatant. (A grinder can be used to grind the seeds.)

##### Chemicals to be prepared

- Alkaline solution with a pH indicator (a master mix comprising 100 mL of 2% sodium carbonate [2 g of sodium carbonate in 100 mL distilled water] and 200 mL of 0.04% bromothymol blue [0.1 g of bromothymol blue in 16 mL of 0.01 M sodium hydroxide, with the volume made up to 250 mL with distilled water])
- 5% porcine pancreatic lipase (0.5 g in 10 mL distilled water)
- 10 mg/mL orlistat (120 mg tablet dissolved in 1 mL of absolute ethanol, with the volume made up to 12 mL with distilled water)

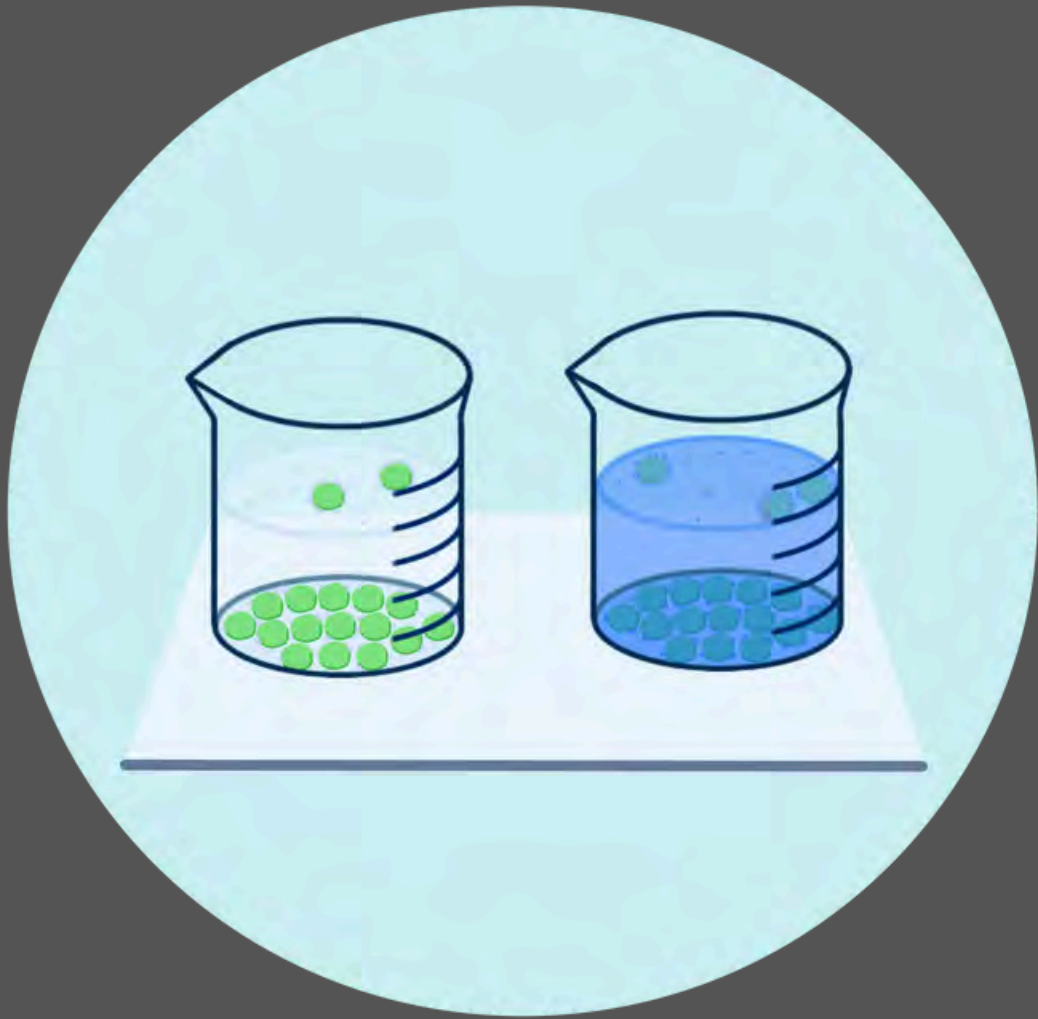
### Materials for each group

• Whole milk (>35 mL)	• Vials X 15	• Tablet stand
• Distilled water (>3 mL)	• Timer	• Tablet/mobile phone
• Seed extract 1 to 3 (>3 mL)	• Orlistat (>3 mL)	• Rubbish bin
• Autopipette (P-1000)	• Autopipette tips (P-1000)	• Labels
• Pen	• Alkaline solution with a pH indicator (>30 mL)	• Pancreatic lipase solution (>30 mL)



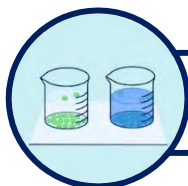
### References

- Chan, K. K. H., Ho, D. T. S., & Lau, D. S. P. (2024). Using amylase beads to investigate factors affecting enzyme activity. *The American Biology Teacher*, 86(3), 153–160.
- Chan, P. C., & Chan, K. K. H. (2023). Inquiry on a potential anti-obesity agent: Investigating pancreatic lipase inhibitors in seed extracts *The American Biology Teacher*, 85(5):265–269
- Royal Society of Biology Nuffield Foundation. (2019). Investigating effect of temperature on the activity of lipase. <https://practicalbiology.org/bio-molecules/factors-affecting-enzyme-activity/investigating-effect-of-temperature-on-the-activity-of-lipase>



# **Photosynthesis Inhibitor Investigation**

---



# Photosynthesis Inhibitor Investigation

## Overview

- This *Photosynthesis Inhibitor Investigation* is about determining the mode of action of different herbicides.
- Students perform the leaf flotation method (Cookson & Price, 1982) to study the effect of herbicides on the photosynthesis of leaf discs (Hill & Steucek, 1985; Zemedkun et al., 2019).
- Students are given the opportunity to design and carry out experiments in which they consider the accuracy and reliability of methods of measuring the dependent variable, pooling class data, and the limitations of the methods in determining the mode of action of the herbicides.

## Teaching Plan & Key Features

*Prerequisite knowledge (scientific ideas)*

- The process of photosynthesis
- The relationship between photosynthesis and respiration

Lesson	Lesson sequence	Duration (mins)	Resources
<b>Stage 1 Preparing for the investigation</b>			
<ul style="list-style-type: none"> <li>• Students read information to familiarise themselves with the background of the investigation (<i>Reading Materials</i>).</li> <li>• Student questions about the <i>Reading Materials</i> are addressed in class (<i>Diagnostic Assessment</i>).</li> </ul>			
Before Lesson 1	<ul style="list-style-type: none"> <li>• The teacher distributes <i>Worksheet 1</i> for students to complete at home for them to be familiar with the background of the investigation.</li> <li>• The teacher collects student questions about the investigation context using a <i>Google Form</i>.</li> </ul>		<i>Worksheet 1</i>
1	<ul style="list-style-type: none"> <li>• The teacher addresses student questions about the investigation context in a <i>Google Form</i>.</li> </ul>	40	Student Samples 1
<b>Stage 2 Designing the investigation</b>			
<ul style="list-style-type: none"> <li>• Students physically interact with the materials and apparatuses before designing the investigation.</li> </ul>			
2	<ul style="list-style-type: none"> <li>• The teacher discusses with students questions related to their experimental designs.</li> <li>• The teacher provides students with the laboratory manual for preparation at home.</li> </ul>	40	Teacher Notes 1
<b>Stage 3 Carrying out the investigation</b>			
<ul style="list-style-type: none"> <li>• Students use cameras to record data (<i>Digital Tool</i>).</li> </ul>			
3	<ul style="list-style-type: none"> <li>• Teacher asks questions to help students connect their lab experience and related ideas/scientific inquiry skills.</li> <li>• Students carry out the investigation.</li> </ul>	40	Laboratory Manual
<b>Stage 4 Explaining and evaluating data</b>			
<ul style="list-style-type: none"> <li>• Students use data to deduce the mode of action of the herbicides (<b>Problem Solving Task</b>).</li> <li>• Students identify the limitations of the data collected in answering the investigation question.</li> </ul>			
Before Lesson 4	<ul style="list-style-type: none"> <li>• Students complete data reporting and analysis at home.</li> <li>• The teacher collects and marks student responses.</li> </ul>		Teacher Notes 2
4	<ul style="list-style-type: none"> <li>• The teacher provides feedback on students' performance related to data reporting and analysis.</li> </ul>	40	Teacher Notes 2

## Important Notes

- Students should wear safety goggles and lab coats during the experiment.
- Students should avoid skin contact with the solutions with herbicides.



## Instructional Materials

### Stage 1 Preparing for the investigation

#### Student Worksheet 1



##### Notes for teachers

- Teachers can distribute *Worksheet 1* and instruct students to read the background information related to the investigation at home.
- Teachers collect student questions about the investigation using a *Google Form*.
- Scan the QR code to get a copy of the *Google Form*.



#### Task 1

- Read the following information and source materials in the *Data File*.

#### Scenario

Herbicides, also called weedkillers, are substances used to manipulate or control unwanted plants (or weeds). Different herbicides have different modes of action based on the mechanism by which the herbicide controls susceptible plants. Identifying this mode of action is important for selecting the right herbicide for each crop.

In this investigation, you will collect data to determine the modes of action of three herbicides. Read the information in the *Data File* to familiarise yourself with the context. You will use your biological knowledge of photosynthesis and the design of valid and reliable experiments, as well as the information in the *Data File* to complete this investigation.

Complete the *Google Form* after you have read the *Data File*.

#### Questions in the Google Form

- (a) Self-assess your understanding about the information in the *Data File*.
- 0 – I don't get it.
  - 1 – I kind of get it.
  - 2 – I get it, but I need help to explain it.
  - 3 – I get it, and I can explain it.
  - 4 – I get it, and I can teach it to my friends.

Students self-assess their understanding of the reading materials and raise questions. Teachers can then address students' difficulties based on their responses.

- (b) List *at least* one question you have about the investigation.

## Data File

Your biology teacher asks you to read the following source materials to prepare yourself for designing an investigation related to the study of herbicides.

### **Source 1:** Mode of action of herbicides

Herbicides are chemicals that prevent or stop the normal growth and development of weeds. Herbicides can increase crop yield. However, improper use can lead to crop inquiry, herbicide-resistant weeds, health risks, and environmental damage. Herbicides have different modes of action based on the mechanism by which the herbicide disrupts the normal growth and development of susceptible plants. Three common modes of action can be distinguished:

#### (1) Cell division inhibitors

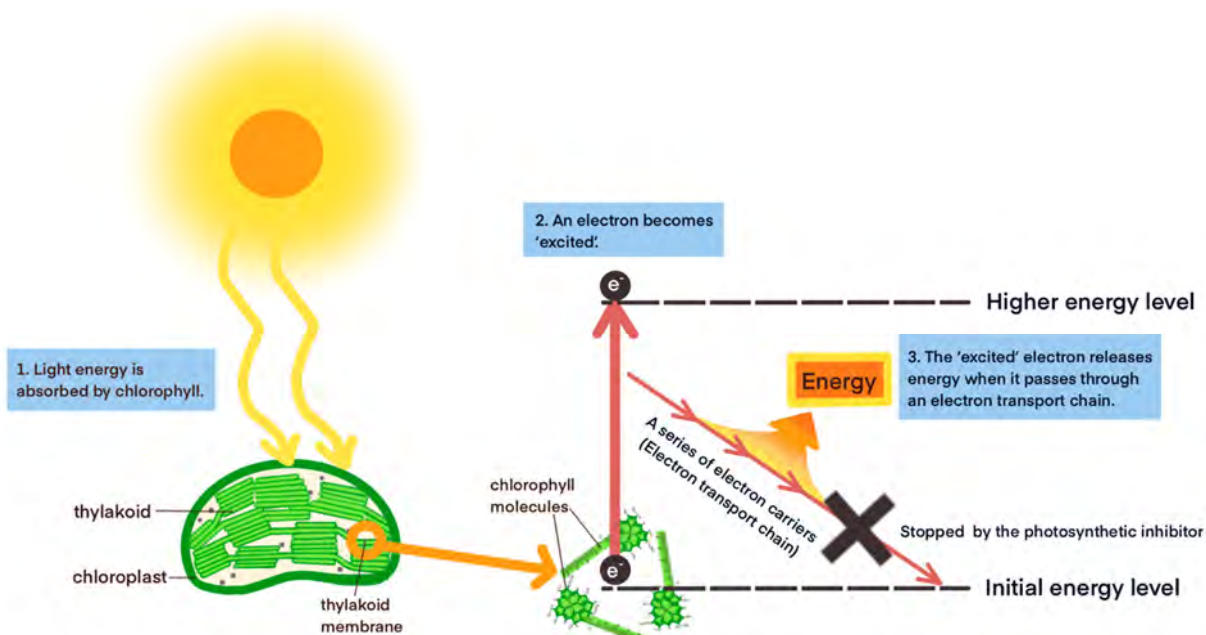
- This group of herbicides inhibits cell division and new growth. For example, some herbicides interfere with the process that leads to the correct arrangement of chromosomes during cell division. This group of herbicides can stop cell division at the root and root tips.

#### (2) Photosynthesis inhibitors

- These herbicides inhibit photosynthetic pathways. For example, some herbicides bind to an electron carrier involved in the light-dependent stage of photosynthesis. This interrupts the electron transport chain and thus the production of NADPH and ATP. These herbicides are often used when the weed has already germinated and developed photosynthetic activity.

#### (3) Synthetic plant growth regulators

- This group of herbicides mimics plant growth regulators. For example, some herbicides mimic the action of auxins. When these herbicides are applied to the leaves, they are transported to the meristems and cause uncontrolled growth. Other longer-term effects include leaf discolouration.



## Source 2: Testing for photosynthesis inhibitors

### *What is the leaf flotation method?*

- The leaf disc flotation method is a simple and quick way to identify photosynthesis inhibitors using leaf discs. If a photosynthesis inhibitor is present in the test solution, oxygen production is slowed down or stopped because the process of photosynthesis is inhibited, and the leaf discs do not float/float more slowly.

### *How is the leaf flotation method performed?*

- Leaf discs are punched from fully expanded leaves. The leaf discs floating on the test solutions in a beaker are infiltrated under vacuum for 5 minutes to remove the air in the air spaces of the leaf discs.



Scan the QR code to see how leaf discs are vacuumed.



The beaker is then illuminated with a light source.

### *How can we compare the inhibitory effect of different photosynthesis inhibitors?*

- The time it takes for a leaf disc to float to the surface of the test solution is an indicator of the photosynthesis rate of that leaf disc. As there are individual differences between leaf discs, some leaf discs float faster, while others float later.



Scan the QR code to see what happens to leaf discs under light illumination.



- The time that passes until 50% of the leaf discs float is called **ET50**. At this point, 50% of the leaf discs are floating. The time it takes for half of the leaf discs to float to the surface of the medium can be compared with that of the control. This ratio is called the **retardation index (RI)**.

### *How can the validity and reliability of the method be improved?*

- All treatments should be repeated. Each replicate should consist of 10 or more leaf discs. The experiment should include a control to which no photosynthesis inhibitor is added.

### 任務 1

- 閱讀以下資訊和資料檔案中的資料。

#### 情境

除草劑，又稱殺草劑，是用於操縱或控制不需要的植物(或雜草)的物質。根據除草劑如何控制易受影響的植物，不同的除草劑有不同的作用機制。識別除草劑的作用機制對於選擇適合每種作物的除草劑很重要。

在這項探究活動，你將收集數據以識別三種除草劑的作用機制。請閱讀資料檔中的資訊，熟悉相關背景。你將利用你對光合作用的生物學知識、設計有效可靠實驗的能力，以及資料檔案中的資訊來完成這項探究。

在閱讀資料檔後,完成 *Google Form*。

#### Google 表單的問題

- (a) 自我評估對資料檔案中資訊的理解程度。
- 0 - 我完全不明白
  - 1 - 我有點明白
  - 2 - 我明白,但需要一些幫助來幫助我解釋
  - 3 - 我明白,並且能解釋
  - 4 - 我明白,並能教導我的朋友
- (b) 列出至少一個你對這項探究有疑問的地方。



掃描二維碼以獲取 *Google Form* 的副本。



## 資料檔案

你的生物老師要求你閱讀以下資料，為設計研究除草劑的探究作準備。

### 資料 1: 除草劑的作用機制

除草劑是可以預防或阻止雜草正常生長和發育的化學物質。除草劑可以提高作物產量。然而，使用不當可能導致作物受損、引致雜草產生抗藥性、導致健康風險和環境損害。除草劑根據其干擾易受影響植物正常生長和發育的機制而有不同的作用機制。以下為三種常見的作用機制：

#### (1) 細胞分裂抑制劑

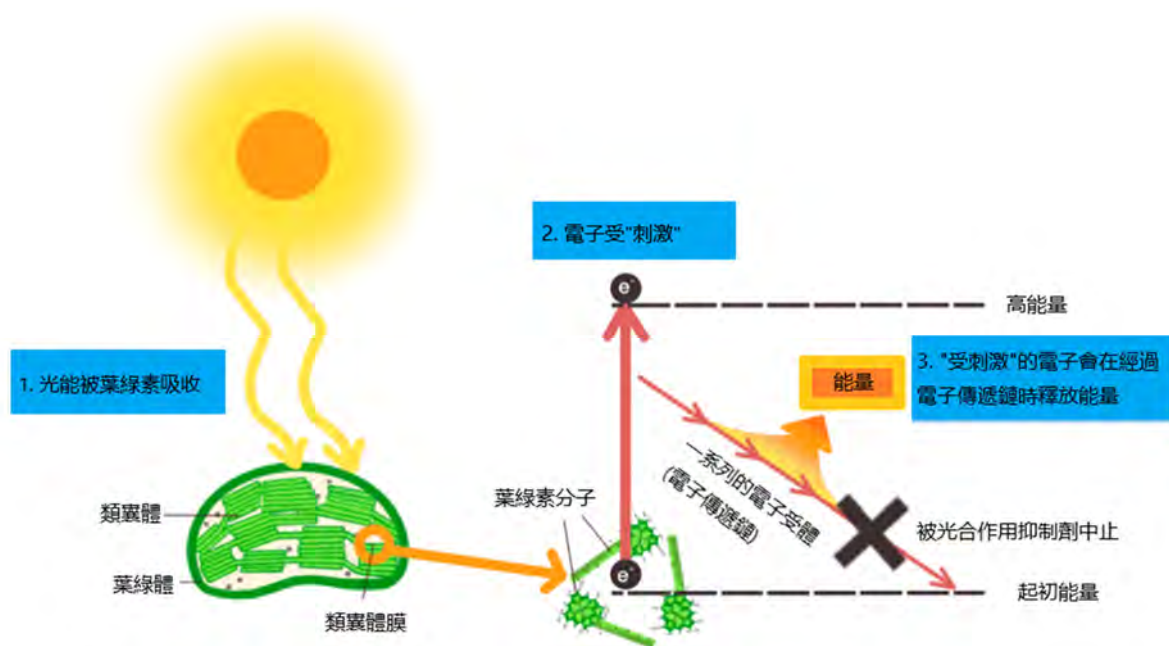
- 這類除草劑抑制細胞分裂和新生長。例如，一些除草劑干擾細胞分裂過程中染色體的正常排列。這類除草劑可以在根部和根尖停止細胞分裂。

#### (2) 光合作用抑制劑

- 這些除草劑抑制光合作用途徑。例如，一些除草劑會與參與光合作用光反應階段的電子載體結合。導致電子傳遞鏈中斷，從而影響 NADPH 和 ATP 的產生。這類除草劑通常在雜草已經發芽並具有光合作用能力時使用

#### (3) 合成植物生長調節劑

- 這類除草劑模仿植物生長調節劑的作用。例如，一些除草劑模仿生長素的作用。當這些除草劑施加到葉片上時，會被運輸到分生組織並引起失控生長。其他長期效果包括葉片變色。



## 資料 2: 測試光合作用抑制劑

### 什麼是浮葉法 (Leaf floatation method)?

- 浮葉法 (Leaf floatation method) 是一種簡單快速的方法，用於識別光合作用抑制劑。如果測試溶液中含有光合作用抑制劑，由於光合作用受到抑制，氧氣生產將降低甚至停止，導致小葉片不能浮起或浮起得較慢。

### 怎麼進行浮葉法?

- 首先從完全展開的葉子上打孔以取得小葉片。然後將這些小葉片浸泡在測試溶液中的燒杯裡，抽真空 5 分鐘，以去除小葉片內部的空氣。


 掃描二維碼，用於說明如何抽真空處理小葉片



- 然後將燒杯放在光源下進行照明。

### 如何比較不同光合作用抑制劑的抑制作用?

- 小葉片浮到測試溶液表面所需的時間，可以作為該小葉片光合速率的指標。由於小葉片之間存在個體差異，有些小葉片浮動較快，而有些則較慢浮動。
- 通過比較不同濃度或不同光合作用抑制劑的影響下，小葉片浮動到表面所需的時間，就可以評估各種抑制劑的相對抑制強度。浮動至表面所需的時間越長，代表光合作用受抑制得越強。這種相對比較的方法可以幫助確定哪種光合作用抑制劑的作用最為顯著。

 掃描二維碼，可以查看小葉片在光照下的情況。



- 直到 50% 的小葉片浮上來所經過的時間稱為 **ET50**。在這一時刻，50% 的小葉片已經浮起。葉片圓盤浮到表面所需的時間可以與對照組進行比較。這個比值稱為 **延遲指數 (RI)**。

### 如何提高浮葉法的有效性和可靠性?

- 所有實驗組都應重複進行。每個重複實驗應包括 10 個或更多小葉片。實驗中應包括一個未添加任何光合作用抑制劑的對照組。
- 通過增加重複次數和樣本數量，可以降低個體差異的影響，提高實驗結果的可靠性。同時設立對照組可以確保實驗條件的可比性，排除其他因素的干擾。這些措施可以有效提高葉片圓盤浮力法的有效性和可信度。

## QUESTIONS FROM STUDENTS

- What is RI?
- When we put the leaf discs into the small beaker, some are already floating on the water's surface. Do we include those floating ones in the total number of leaf discs when we calculate the RI?

## QUESTIONS FROM STUDENTS

- What's the difference between accuracy, reliability, and validity?



### Notes for teachers

- Teachers can address students' questions about the investigation context.
- The examples of student questions show that students struggle to understand certain terms related to scientific inquiry. Instead of telling students the definitions of the terms, teachers can use examples related to the present investigation to explain the ideas.

## Teacher Notes 1

### Notes for teachers



- After addressing the student questions, teachers can show students the materials and apparatuses to facilitate their design. See the *Supplementary Resource* section for a list of materials.
- There are some questions that teachers may use to guide students in thinking about or assessing the scientific inquiry skills related to experimental designs.
- Some student work samples are shown below to illustrate possible student thinking to some questions.

## Task 2

### Scenario

Herbicides, also called weedkillers, are substances used to manipulate or control unwanted plants (or weeds). Different herbicides have different modes of action based on the mechanism by which the herbicide controls susceptible plants. Identifying the mode of action of herbicides is important for selecting the right herbicide for each crop.

In this investigation, you will investigate the effect of three different herbicides on the photosynthesis of leaf discs. This information can help you determine the mode of action of the three herbicides.

You are given the following materials:

• Vacuumed spinach leaf discs	• 25-mL Measuring cylinder	• 25-mL Beaker
• Table lamp	• 1% Sodium hydrogencarbonate solution	• 1% Sodium hydrogencarbonate solution with herbicides X, Y, and Z
• Timer	• Forceps	

### Possible questions

1. Explain whether each of the following modifications can shorten the time of the experiment:

	Yes	No	Your reasons
(a) Use a table lamp with higher light intensity	<input type="checkbox"/>	<input type="checkbox"/>	
(b) Use a larger volume of sodium hydrogencarbonate solution	<input type="checkbox"/>	<input type="checkbox"/>	

2. Your biology teacher advised the class to put 10 leaf discs in each beaker and measure the time for the leaf discs to rise. The following shows the conversation between your classmates about how to measure the time:



**Paul**

Measuring the time for each of the 10 leaf discs to rise to the surface of the sodium hydrogencarbonate solution and taking the average.



**Jane**

Measuring the time for 5 of the 10 discs (50%) to float to the surface of the sodium hydrogencarbonate solution.



- (a) Explain why the method proposed by Paul can enhance the reliability of the results.  
 (b) Explain why the method proposed by Jane is more accurate than that proposed by Paul.
3. The retardation index (RI) refers to the ratio between the time it takes for half of the leaf discs to float to the surface in a solution containing a herbicide to the time taken by the control (a solution without herbicide).

$$\text{RI} = \frac{\text{Time for the leaf discs to float to the surface in a solution with a herbicide}}{\text{Time for the leaf discs to float to the surface in a solution without a herbicide}}$$

Explain how the RI can be used to determine the effect of three herbicides on the photosynthesis of leaf discs.



#### Notes for teachers

- Q.1 assesses students' understanding of the factors that can affect the rate of photosynthesis of the leaf discs.
- Q.2 assesses students' ability to explain the strategy for averaging the effect of variations within a sample and identify the strengths and limitations of the alternative measurement method of the dependent variable.
- Q.3 assesses students' ability to relate the RI to the dependent variable and apply their biological knowledge about photosynthesis.

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

### Sample 1

(a) Explain why the method proposed by Paul can enhance the reliability of the results

Paul uses 100% of the data but Jane only uses 50% of the data. The larger the number of sample, the higher the reliability.

### Sample 2

(a) Explain why the method proposed by Paul can enhance the reliability of the results

the more the leaf discs being tested, the more data we get. By taking average, we can eliminate the effect of the extreme cases and increase the reliability of the result.

### Sample 3

(a) Explain why the method proposed by Paul can enhance the reliability of the results

It is because Paul's method is repeating the experiment by having 10 leaf disc. However, Jane's method only need, measure the time of the fifth leaf disc float but without repeating.

### Sample 4

(a) Explain why the method proposed by Paul can enhance the reliability of the results

Since each leaf discs treated with weedkillers may have some differences in photosynthetic rate. By taking average time for leaf discs float with 10 leaf discs, this can minimize the individual differences between leaf discs as some discs float faster while others float later. Thus, reliability can be enhanced.

#### **About the samples**

- Sample 1 only addressed the concept of sample size but did not explain how a larger sample size can lead to more reliable data.
- Sample 2 incorrectly stated that the effect of the extreme cases would be eliminated. In fact, the influence of the extreme cases can be averaged out rather than eliminated.
- Sample 3 conflated including more samples within each trial with repeating the entire experiment.
- Sample 4 more clearly explained the variability in photosynthesis rate across different leaf discs, and how taking the average would allow the effect of these individual differences to be averaged out.



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

### Sample 1

(b) Explain why the method proposed by Jane is more accurate than that of Paul.

Jane's method is more accurate as it <sup>measure time for</sup> take the 5 discs-leaf discs float on the surface first. The results <sup>time</sup> is more accurate and with less difference between the time of first 5 leaf discs to float, getting a more accurate result as there are less individual differences.

### Sample 2

(b) Explain why the method proposed by Jane is more accurate than that of Paul.

As the method of Jane excluded the extreme cases, such as the leaf discs which floats in a very short or long time, and use the median of the result, therefore it is more accurate.

### Sample 3

(b) Explain why the method proposed by Jane is more accurate than that of Paul.

There might be leaf discs that take <sup>or short</sup> an extra long time to rise up, making extreme data. Hence, this method can eliminate the extreme data that makes the result inaccurate and the results can be closer to the true value, but Paul's method includes the extreme data <sup>calculations</sup> so the results obtained may deviate greatly from the true value, so Jane's method is more accurate.



#### **About the samples**

- Sample 1 did not clearly explain how using data with less variability can result in more accurate data.
- Sample 2 incorrectly stated that the method can eliminate the extreme cases. Indeed, this method does not disregard the extreme data points as the time for the first five leaf discs to rise to the surface of the solution is still measured.
- Sample 3 correctly noted that eliminating extreme data points can lead to results that are closer to the true underlying value, by reducing the distorting effect of outliers.

任務 2

情境

除草劑，又稱殺草劑，是用於操縱或控制不需要的植物(或雜草)的物質。根據除草劑如何控制易受影響的植物，不同的除草劑有不同的作用機制。識別除草劑的作用機制對於選擇適合每種作物的除草劑很重要。

在這項探究,你將研究三種不同除草劑對小葉片光合作用的影響。這些資訊可以幫助你確定這三種除草劑的作用機制。

你收到以下實驗材料:

真空處理過的菠菜小葉片	25 mL 量筒	25 mL 燒杯
檯燈	1%碳酸氫鈉溶液	含有除草劑 X、Y 和 Z 的 1% 碳酸氫鈉溶液
計時器	鑷子	

參考問題

1. 解釋以下改變是否能縮短實驗時間:

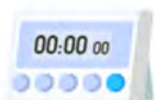
	能	不能	解釋
(a) 使用更強光照的檯燈	<input type="checkbox"/>	<input type="checkbox"/>	
(b) 使用更大體積的碳酸氫鈉溶液	<input type="checkbox"/>	<input type="checkbox"/>	

2. 你的生物老師建議每個燒杯裡放 10 片小葉片，並測量小葉片浮起所需的時間。以下是你的同學們討論如何測量時間



小明

測量每 10 片小葉片浮上碳酸氫鈉溶液表面所需的時間,並計算平均時間。



小美



測量 10 片小葉片中 5 片(50%)浮到碳酸氫鈉溶液表面所需的時間。

- (a) 解釋為什麼小明提出的方法可以增強結果的可靠性。
- (b) 解釋為什麼小美的方法比小明的方法更準確。
3. 延遲指數(Retardation Index, RI)是指含有除草劑溶液中一半小葉片浮到表面所需時間與對照組(不含除草劑)的時間之比。

$$RI = \frac{\text{含有除草劑溶液中小葉片浮到表面所需的時間}}{\text{不含除草劑的溶液中小葉片浮到表面所需的時間}}$$

解釋如何使用延遲指數(RI)來確定三種不同除草劑對小葉片光合作用的影響。

## 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 (e.g., how would you position the table lamp and the beakers? Why?).
- The *Supplementary Resource* section contains the list of materials.
- Scan the QR code to view the process of the experiment.

**Task 3**

- Read the following procedures to carry out the investigation.

**Procedure**

1. Cut at least 40 leaf discs from the green leaf of the spinach plant (*Spinacia oleracea*) with a hole punch. (Avoid the main leaf veins.)
2. Place the cut leaf discs on moist tissue paper to prevent them from drying out during preparation.
3. Pour 15 mL of 1% sodium hydrogencarbonate solution into a plastic cup.
4. Place at least 10 leaf discs in the plastic cup.
5. Repeat *Steps 3 and 4* using 1% sodium hydrogencarbonate with *Herbicides X, Y, and Z*, respectively.
6. Vacuum all the leaf discs for 1 minute.
7. Repeat *Step 6* 1 more time.
8. Use forceps to transfer 10 leaf discs into each 25-mL beaker containing 15 mL of the test solution and control.
9. Place all beakers under the table lamp provided. Make sure that all leaf discs receive even light intensity and heat from the table lamp.
10. Switch on the lamp, and immediately start the timer.
11. Record the time taken for 50% (i.e., 5 discs) of the leaf discs in each of the beakers to float to the surface of the solution (i.e., ET50).
12. Obtain data from two other groups.

**Notes for teachers**

- The vacuuming step can be done by using a syringe.



### 任務 3

- 閱讀以下實驗步驟以進行探究:

#### 實驗步驟

1. 使用打孔器從菠菜的綠色葉子上切下至少 40 片小葉片。(避開主葉脈)
2. 將切好的小葉片放在濕潤的棉花上，以防止在準備過程中乾燥。
3. 將 15 mL 1%碳酸氫鈉溶液倒入塑料杯中。
4. 將至少 10 片小葉片放入塑料杯中。
5. 重複步驟 3 及 4，分別使用含有除草劑 X、Y 和 Z 的 1%碳酸氫鈉溶液。
6. 對所有小葉片進行 1 分鐘的真空處理。
7. 重複步驟 6，再次進行真空處理。
8. 使用鑷子將 10 片小葉片轉移到每個裝有 15 mL 實驗溶液和對照溶液的 25 mL 燒杯中。
9. 將所有燒杯置於提供的檯燈下。確保所有小葉片都能受到均勻的光照強度和熱量。
10. 開啟檯燈，並立即啟動計時器。
11. 記錄每一個燒杯中有 50% (即 5 片) 小葉片浮到溶液表面所需的時間 (即 ET50)。
12. 從其他兩組獲取數據。

Teacher Notes 2



**Notes for teachers**

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

**Task 4**

A checklist (see p.19) can help students understand the requirements for creating a table.

**Possible questions**

1. Present your group and your classmates' results using a table.  
(Make sure that your table includes the retardation index and a title.)
2. Which of the following claims about the mode of action can be made?  
(Put a '✓' into the appropriate box(es). You can choose *one* or *more* answers.)

A.	Herbicide <i>X</i> is a photosynthesis inhibitor.	<input type="checkbox"/>
B.	Herbicide <i>Y</i> is not a photosynthesis inhibitor but a cell division inhibitor.	<input type="checkbox"/>
C.	Herbicide <i>X</i> can inhibit photosynthesis more than herbicides <i>Y</i> and <i>Z</i> .	<input type="checkbox"/>
D.	Both Herbicides <i>Y</i> and <i>Z</i> are plant growth regulators.	<input type="checkbox"/>

3. (a) Which of the following ways did your group use when pooling class data:
  - (1) Selecting groups with data similar to your group
  - (2) Selecting groups randomly

Explain your reasons for why this method is appropriate:

- (b) Explain why obtaining data from other groups can reduce the impact of measurement errors.



**Notes for teachers**

- Q.1 assesses students' ability to record data properly and to construct a result table that can clearly document the data.
- Q.2 assesses students' ability to make claims based on the data available. The method used in this investigation can only identify whether it is a photosynthesis inhibitor but not other modes of action.
- Q.3 and Q.4 assesses students' understanding of how to pool class data to reduce the impact of errors and the explanation.

The following are some examples of students' responses to Q.1:

**Sample 1**

1. Present your group and your classmates' results using a table:

(Make sure that your table include the retardation index and a title)

Time taken for leaf discs to float to the surface of sodium hydrogen carbonate with different herbicide				
	Herbicide X	Herbicide Y	Herbicide Z	without herbicide
Group 6	infinity	26min	13min 38s	infinity
Group 2	infinity	9min 2s	23min 18s	14min 7s
Group 1	infinity	9min	48min 7s	11min 49s
RI	infinity	1.4	1.92	1

**Sample 2**

1a) Present your group and your classmates' results using a table:

(Make sure that your table include the retardation index and a title)

	Time taken for solution X	RI of X	Time Taken for Solution Y	RI of Y	Time taken for solution Z	RI of Z	Time taken for control
Group 3	$\infty$	$\infty$	20min 49s = 1249s	$\approx 1.96$	4min 3s = 243s	$\approx 0.38$	10min 36s = 636s
Group 6	$\infty$	$\infty$	13min 23s = 803s	$\approx 0.60$	22min 40s = 1360s	$\approx 1.00$	22min 35s = 1355s
Group 2	$\infty$	$\infty$	19min 51s = 1191s	$\approx 0.76$	32min 33s = 1973s	$\approx 1.25$	26min 14s = 1574s
Effects of different herbicides acting with leaf disc discs on photosynthesis							

### Sample 3

14. Present your group and your classmates' results using a table:

(Make sure that your table include the retardation index and a title)

The time taken for the leaf discs to float in different herbicide

type of herbicide	Group		Colleen's gp		KVerdy's gp	
	time (s)	Retardation index	time (s)	Retardation index	time (s)	Retardation index
control	1670	1	1205	1	1810	1
X	>2009	>1.202	>2000	1.11	X	X
Y	1702	1.02	672	0.56	273	0.15
Z	312	0.19	1800	1.49	1908	1.03



#### About the samples

- These samples show varying sophistication in terms of their ability to construct a proper table.
- Common mistakes include the absence of titles, lack of units in the headings of the table columns/rows, and inclusion of calculation within the table.
- The following shows a checklist that may be provided to students to guide them to construct a table.

#### Checklist on how to construct a table

- 1 Use a ruler to construct a table that presents the raw data.
- 2 Place the independent variable in the first column and the dependent variable in the subsequent columns.
- 3 Do *not* include calculations in the table.
- 4 For each column, include a heading with the appropriate unit in brackets (e.g., enzyme activity [1/min]).
- 5 Do *not* include units within the body of the table, only in the column headings.
- 6 Record the raw data to a number of decimal places appropriate to the resolution of the equipment.
- 7 Record all the raw data of the same type to the same number of decimal places.
- 8 Record processed data up to one significant figure more than the raw data.
- 9 Include a title for the table.

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

### Sample 1

3. What of the following ways did your group use when pooling class data:
- A. Selecting groups with data similar to your group
  - B. Selecting groups randomly

Answer: B

*Explain your reasons*

Collecting data from some random groups can increase the reliability. It ensures all data, including some extreme data will be counted.

### Sample 2

3. What of the following ways did your group use when pooling class data:
- A. Selecting groups with data similar to your group
  - B. Selecting groups randomly

Answer: B

*Explain your reasons*

Selecting groups randomly can prevent selection bias and the result is not affected by human factor.

### Sample 3

3. What of the following ways did your group use when pooling class data:
- A. Selecting groups with data similar to your group
  - B. Selecting groups randomly

Answer: B

*Explain your reasons*

It can increase validity. If the data of your own group is inaccurate, selecting groups with data similar to your own group will also be inaccurate. The result will be inaccurate after taking average. As a result, the data about from other groups should be selected randomly for reducing the chance of obtaining inaccurate data.



#### **About the samples**

- All the samples chose the correct strategy to pool class data. However, they differed in the sophistication of their reasoning.
- Some samples included invalid reasons, such as including extreme data and removing the errors (i.e., not affected by human factors) (rather than reducing the impact of errors), as seen in Samples 1 and 2.

#### 任務 4

##### 參考問題

1. 使用表格以表達你的小組和同學的結果：

(你的表格應包括延遲指數及標題。)

2. 以下哪些關於作用模式的聲稱可以被認為是真實的？

(在下列方格加上‘✓’號以選出你的答案。你可以選擇一個或多個答案。)

A.	除草劑 X 是光合作用抑制劑	<input type="checkbox"/>
B.	除草劑 Y 不是光合作用抑制劑，而是細胞分裂抑制劑	<input type="checkbox"/>
C.	除草劑 X 比除草劑 Y 和除草劑 Z 更有效地抑制光合作用	<input type="checkbox"/>
D.	除草劑 Y 和除草劑 Z 都是植物生長調節劑	<input type="checkbox"/>

3. (a) 你的小組在彙編班級數據時使用了以下哪種方式：

- (1) 選擇數據與你的小組相似的組
- (2) 隨機選擇組

解釋你認為這方法是恰當的原因：

- (b) 解釋為什麼從其他小組獲取數據可以減少測量誤差的影響。



## Supplementary Resources

### Possible Modifications

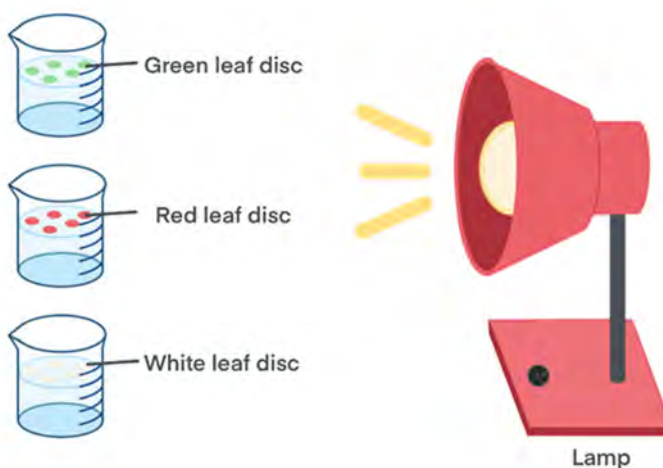
- 1. Sinking of floating leaf discs in darkness**
  - Teachers may ask students what would happen to the floating leaf discs if they are kept in darkness for 30 minutes and ask them to provide the reasons.
  - See Steucek & Hill (1985) for more information.
- 2. Investigating the effect of light colour on the photosynthesis of the leaf discs**
  - An LED (RGB light bulb) may be used to investigate the effect of light colour on the photosynthesis of the leaf discs. See also the *Cat Grass Investigation*.
  - Note that even though the effects of light colour on photosynthesis 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 evidence from their data rather than on the theory behind the effects.



Scan the QR code for more information.



- 3. Investigating the photosynthesis of different colours.**
  - The set-ups can also be used to investigate the photosynthesis of leaves with different colours.



### Materials for Task 4

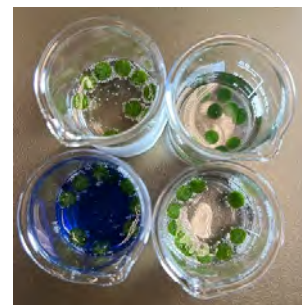
#### *Chemicals to be prepared*

- 1% sodium hydrogencarbonate solution (1 L) (Dissolve 10 g sodium hydrogencarbonate in 1 L distilled water.)
- Herbicide X (0.005% Atrazine)
  - Atrazine stock solution 1 mg/mL = 0.05 g in 50 mL DMSO
  - Add 50 mL of Atrazine (1 mg/mL) stock solution and 10 g of sodium hydrogencarbonate, then make up the volume to 1 L.
- Herbicide Y (0.005% 2,4-D)
  - 2,4-D stock solution 1 mg/mL = 0.05 g in 50 mL DMSO
  - Add 50 mL of 2,4-D (1 mg/mL) stock solution and 10 g of sodium hydrogencarbonate, then make up the volume to 1 L.
- Herbicide Z (0.001% DCPIP)
  - Add 10 mL of 0.1% DCPIP (0.1 g in 100 mL distilled water), 10 g sodium hydrogencarbonate solution, make up to 1 L.

#### *Materials for each group*

• Hole punch	• Forceps	• Pen
• Spinach leaves	• Petri dish	• 1% sodium hydrogencarbonate
• Plastic cup X 4	• Cotton wool (moist)	• 1% sodium hydrogencarbonate with Herbicide X
• Vacuum pump	• 25 mL beaker X 4	• 1% sodium hydrogencarbonate with Herbicide Y
• Vacuum chamber	• Timer	• 1% sodium hydrogencarbonate with Herbicide Z

*Note:* Select fresh spinach leaves.



## References

- Cookson, S. J. & Price, D. N. (1982). The leaf flotation method for measuring photosynthesis. *School Science Review*, 64(226), 84–87.
- Hill, R. J. & Steucek, G. (1985). Photosynthesis: II. An assay for herbicide resistance in weeds. *The American Biology Teacher*, 47(2), 99–102.
- Steucek, G. & Hill, R. (1985). Photosynthesis: I: An assay utilizing leaf disks. *The American Biology Teacher*, 47(2), 96–99.
- Zemedkun, D., Alaparmak, H. & Apte, S. (2019). The effect of herbicides on the rates of photosynthesis and respiration in spinach leaves. *Journal of High School Science*, 3(1):1–15.



# **Cat Grass Investigation**

---



# Cat Grass Investigation

## Overview

- The *Cat Grass Investigation* is situated in the context of growing cat grass indoors using artificial lighting.
- Students investigate the effect of different wavelengths of light from LED lamps on the photosynthetic rate by measuring the rate of Hill reaction (Spencer, 2018).
- Students have the opportunity to design and carry out an experiment in which they set up replicates, consider the importance of controls, and evaluate the generalisability of the data in making claims about plant growth.

## Teaching Plan & Key Features

*Prerequisite knowledge (scientific ideas)*

- The process of photosynthesis
- The relationship between photosynthesis, respiration, and plant growth

*Prerequisite manipulative skills*

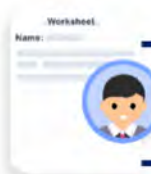
- Using an autopipette to transfer a small volume of solution

Lesson	Lesson sequence	Duration (mins)	Resources
<b>Stage 1 Preparing for the investigation</b>			
<ul style="list-style-type: none"> <li>• It is situated in an authentic, daily-life context related to the use of artificial lighting for growing cat grass (<b>Contextualisation</b>).</li> <li>• Students read information to familiarise themselves with the background of the investigation (<i>Reading Materials</i>).</li> <li>• Students' experimental designs of a similar investigation are collected and discussed in class (<i>Diagnostic Assessment</i>).</li> </ul>			
Before Lesson 1	<ul style="list-style-type: none"> <li>• The teacher distributes <i>Worksheet 1</i> for students to complete at home so that they can be familiar with the background of the investigation.</li> </ul>		<i>Worksheet 1</i>
1	<ul style="list-style-type: none"> <li>• The teacher discusses the investigation context with students.</li> <li>• The teacher provides feedback on students' responses in <i>Worksheet 1</i>.</li> <li>• Students complete <i>Worksheet 2</i> to design an investigation.</li> </ul>	40	<i>Worksheet 2</i>
<b>Stage 2 Designing the investigation</b>			
<ul style="list-style-type: none"> <li>• Students have the chance to evaluate their own and their peers' experimental set-ups (<i>Self &amp; Peer Evaluation</i>).</li> </ul>			
2	<ul style="list-style-type: none"> <li>• The teacher provides feedback on students' experimental designs in <i>Worksheet 2</i>.</li> </ul>	40	Student Samples 1
3	<ul style="list-style-type: none"> <li>• The teacher presents the main investigation context and discusses with students questions related to their experimental designs.</li> <li>• The teacher provides students with the laboratory manual for preparation at home.</li> </ul>	40	Teacher Notes 1

<b>Stage 3 Carrying out the investigation</b>			
<ul style="list-style-type: none"> <li>Students use microscale instrumentation that reduces the time of the experiments (<b>Microscale Instrumentation</b>).</li> <li>Students collect more complex data sets by setting up replicates (<b>Complex Data Set</b>).</li> </ul>			
4	<ul style="list-style-type: none"> <li>The teacher asks questions to help students connect their lab experience and related ideas/scientific inquiry skills.</li> <li>Students carry out the investigation.</li> </ul>	40	Laboratory Manual
<b>Stage 4 Explaining and evaluating data</b>			
<ul style="list-style-type: none"> <li>Students share data on the <i>Google Spreadsheet (Digital Tool)</i>.</li> <li>Students use data to support their claims about the effect of different wavelengths of light on the photosynthesis of cat grass and discuss the generalisability of the results.</li> </ul>			
Before Lesson 5	<ul style="list-style-type: none"> <li>Students complete data reporting and analysis at home.</li> <li>The teacher collects and marks student responses.</li> </ul>		Teacher Notes 2
5	<ul style="list-style-type: none"> <li>The teacher provides feedback on students' performance related to data reporting and analysis.</li> </ul>	40	Teacher Notes 2

### Important Notes

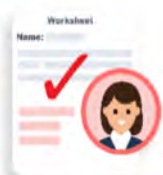
- Students are *not* required to learn the detailed reasons for the effects of different wavelengths of light on the photosynthesis of cat grass. Rather, they are expected to use their data to support their claims about the effects.
- Students are *not* expected to know the details of Hill reaction to successfully complete this investigation.



## Instructional Materials

### Stage 1 Preparing for the investigation

#### Student Worksheet 1



#### Notes for teachers

- Teachers can distribute *Worksheet 1* and instruct students to read the background information related to the investigation as a take-home assignment.
- Students' responses can be collected using a *Google Form*.
- Depending on the student performance, some questions can be discussed in class.

#### Task 1

- Read the following information and source materials in the *Data File*.
- Answer the questions that follow.

#### Scenario

The scenario is set in an everyday context (i.e., growing cat grass indoors).

Cat grass is a mixture of grasses grown from seeds, such as wheat, barley, oats, and rye. Cat grass is safer for cats to eat than outdoor grass, which may have been treated with pesticides. Cat grass is also a rich source of vitamins, minerals, and dietary fibres.

Wheatgrass is a type of cat grass that is commonly grown indoors. Wheatgrass seeds are sown in moist soil. After germination, artificial light, such as light-emitting diode (LED) lamps, are used to supply light for the seedlings to grow. Wheatgrass is ready for cats to eat around 2 weeks after germination.

In this investigation, you would like to investigate the photosynthesis and growth of wheatgrass.

Read the *Data File* to familiarise yourself with the background of the investigation. You will use your biological knowledge of photosynthesis and plant growth and how to design valid and reliable experiments to complete this investigation.



Source: <https://www.amazon.com/Cat-Planter-Hairball-Digestive-Manufactured/dp/B01JN19W9E>



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



## Data File

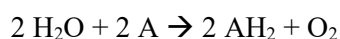
Your biology teacher asks you to read the following source materials to prepare yourself for designing an investigation related to studying photosynthesis and plant growth:

### **Source 1:** Hill reaction

The reading material contains relevant history of science.

In 1939, Robert Hill, a scientist working at the University of Cambridge, studied the process of photosynthesis. He discovered that when an artificial electron acceptor 'A' is introduced to isolated chloroplasts from broken plant cells under the illumination of light, the artificial electron acceptor, after accepting the electrons, is reduced (to AH<sub>2</sub>). Oxygen (O<sub>2</sub>) is evolved. This reaction is called the *Hill Reaction*:

Water + Electron acceptor → Reduced form of electron acceptor + oxygen



On the basis of his data, he proposed that electrons are produced in a certain biochemical process in isolated chloroplasts. Under illumination, the process evolves oxygen and reduces unknown substances (electron acceptors within the chloroplasts) that are not easily removed from the chloroplasts. This substance is not carbon dioxide.

### **Questions for thought**

1. We now know that the unknown substance receives the electrons produced in the biochemical process (i.e. the final electron acceptor in the photochemical process). What is this substance? *(You may want to scan this QR code to watch an animation if you are not sure about the process in the photochemical reaction.)*

Teachers can insert a QR code that shows an animation from readily available resources such as the textbook publisher.

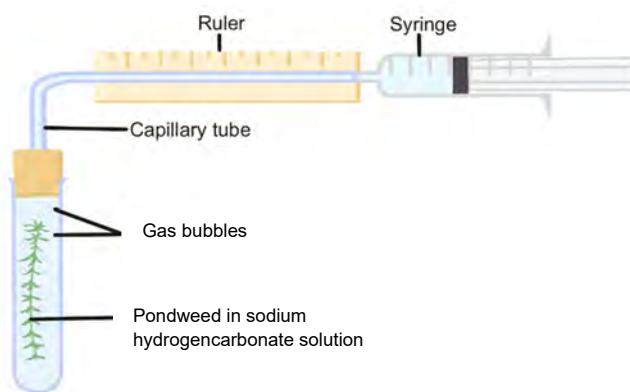
The reading material includes an animation to consolidate student learning of the relevant science concepts.

## Source 2: Measuring the rate of photosynthesis

Several methods can be used to measure the rate of photosynthesis.

### Method 1: Measuring the rate of oxygen release

Oxygen is produced during photosynthesis. The rate of photosynthesis of pondweed can be measured by placing the pondweed in sodium hydrogencarbonate solution and illuminating it with light. The oxygen released is collected with a capillary tube. The photosynthetic activity can be calculated from the amount of gas released over a certain period of time.



### Method 2: Measuring the rate of photochemical reaction

In photochemical reactions, electrons are generated from water under light illumination. DCPIP (2,6-dichlorophenol indophenol), a blue dye, can act as an artificial electron acceptor and becomes colourless when reduced (i.e., when it accepts electrons). When DCPIP is added to isolated chloroplasts, it is reduced by the electrons produced in the photochemical reaction of photosynthesis when the chloroplasts are illuminated. The higher the rate of photochemical reactions, the higher the rate at which DCPIP is reduced and turns colourless. The time it takes for the blue DCPIP to decolourise can be used to calculate photosynthetic activity.



Scan the QR code to see the action of DCPIP on isolated chloroplasts under light illumination.



The video provides conceptual assistance to understanding experimental design.

### Method 3: Measuring the rate of increase in dry mass

Photosynthesis produces carbohydrates, which lead to a gain in the mass of the plant. This method involves 'serial harvests', in which several plants are harvested and dried to constant mass and then weighed. This is repeated several times over a certain period. The increase in dry mass of the plants at different harvest times allow for the calculation of photosynthetic activity.

### Questions for thought

1. Respiration occurs all the time in plants. Which of the above method(s) measure(s) the balance between the rate of photosynthesis and the rate of respiration? Briefly explain your choice.

Method(s):

Your explanation:

### 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

貓草是由小麥、大麥、燕麥和黑麥等種子培育而成的混合草。與可能用殺蟲劑處理過的室外草相比，貓草對貓來說更安全。貓草也是維生素、礦物質和膳食纖維的豐富來源。

小麥草是一種通常在室內種植的貓草。小麥草種子播種在潮濕的土壤中。發芽後，使用發光二極管 (LED) 燈等人造光為幼苗的生長提供光照。小麥草在發芽後約 2 週即可供貓食用。

在這次探究中，你想研究小麥草的光合作用和生長。

閱讀資料檔案，熟悉探究背景。你將運用有關光合作用和植物生長的生物學知識，以及透過設計有效且可靠的實驗來完成這項研究。



Source: <https://www.amazon.com/Cat-Planter-Hairball-Digestive-Manufactured/dp/B01JN19W9E>



掃描二維碼以獲取 *Google Form* 的副本。



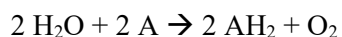
## 資料檔案

你的生物科老師要求你閱讀以下資料，為設計與研究光合作用和植物生長相關的探究做好準備。

### 資料 1: 希爾反應

1939 年，在劍橋大學工作的科學家羅伯特·希爾 (Robert Hill) 研究光合作用的過程。他發現在有光照的情況下，將人工電子受體「A」放入由破碎的植物細胞分離出的葉綠體之中，人工電子受體「A」會在接受電子後被還原(至  $\text{AH}_2$ )，並放出氧氣 ( $\text{O}_2$ )。該反應被命名為希爾反應：

水 + 電子受體  $\rightarrow$  還原的電子受體 + 氧氣



根據他的數據，他提出電子是在葉綠體中的某個生化過程中產生。在光照下，該過程釋放出氧氣，同時還原一些不易從葉綠體中去除的未知物質(葉綠體中的電子受體)。這物質不是二氧化碳。

### 思考問題

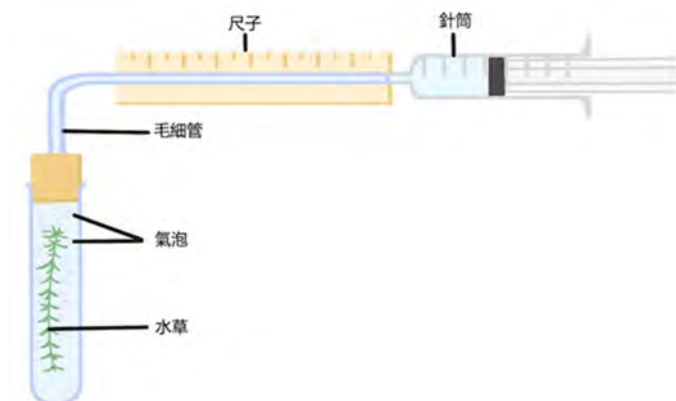
1. 我們現在已知道未知物質會接收生化過程中產生的電子(即光化學過程中的最終電子受體)。這是什麼物質？

(如果你對光化學反應的過程還不了解，可以掃描二維碼觀看動畫)

## 資料 2：測量光合作用的速率

有幾種方法可以用來測量光合作用的速率。


### 方法一：測量氧氣釋放率



光合作用的過程會產生氧氣。水草的光合作用速率可以通過將水草置於光照下的碳酸氫鈉溶液中來測量。釋放的氧氣由毛細管收集。一段時間內釋放的氣體量可以用於計算光合作用的速率。

### 方法二：測量光化學反應速率

在光化學反應中，電子是在光照下從水分子中產生。DCPIP 是一種藍色染料，可作為人工電子受體，在還原(即接受電子)時變為無色。當 DCPIP 添加到葉綠體中時，而葉綠體被光照時，它會被光化學反應中產生的電子還原。光化學反應的速率越高，DCPIP 被還原並變為無色的速率就越高。藍色 DCPIP 脫色的時間可用於計算光合作用的速率。

 掃描二維碼以查看葉綠體在光照下對 DCPIP 的作用。



### 方法三：測量乾質量的增加率

光合作用產生碳水化合物，導致植物質量增加。這種方法涉及「連續收穫」，收穫幾株植物並乾燥至恆定質量，然後量重。在一段時間內重複此步驟幾次。透過比較在不同時間所收穫的植物質量增加，繼而計算光合作用的速率。

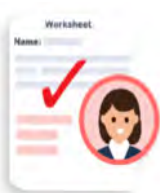
## 思考問題

1. 呼吸作用在植物中無時無刻都在發生。以上哪種方法能用於衡量光合作用速率和呼吸速率之間的平衡？簡要解釋你的選擇。

方法：

解釋：

## Student Worksheet 2



### Notes for teachers

- Teachers can provide feedback on student responses in *Worksheet 1* if necessary.
- Teachers can then distribute *Worksheet 2* and instruct students to design the investigation.
- Teachers can show students the materials and apparatuses to facilitate their design. See the *Supplementary Resource section* for a list of materials.
- Some student work samples are shown below to illustrate possible student thinking.

### Task 2

- Answer the questions that follow.

1. You are given the following information:

*Investigation question:*

“What is the effect of light intensity on the rate of photosynthesis of wheatgrass?”

*Materials and apparatus:*

DCPIP solution	Wheatgrass chloroplast extract	Capillary tubes
Table lamp	Aluminium foil	Timer
LED light bulb (White)	Ruler	Ice-bath
Autopipette tip	Micropipette	White tile
Camera		

- (a) Briefly describe how you would use the materials to design an investigate to achieve the aim. Draw your experimental design in the box below.

Students are allowed to draw *and* explain their design decisions.

Students designed a related experiment and explained their design decisions.

**任務 1**

- 回答以下問題。

1. 你獲得以下資訊:

*探究問題:*

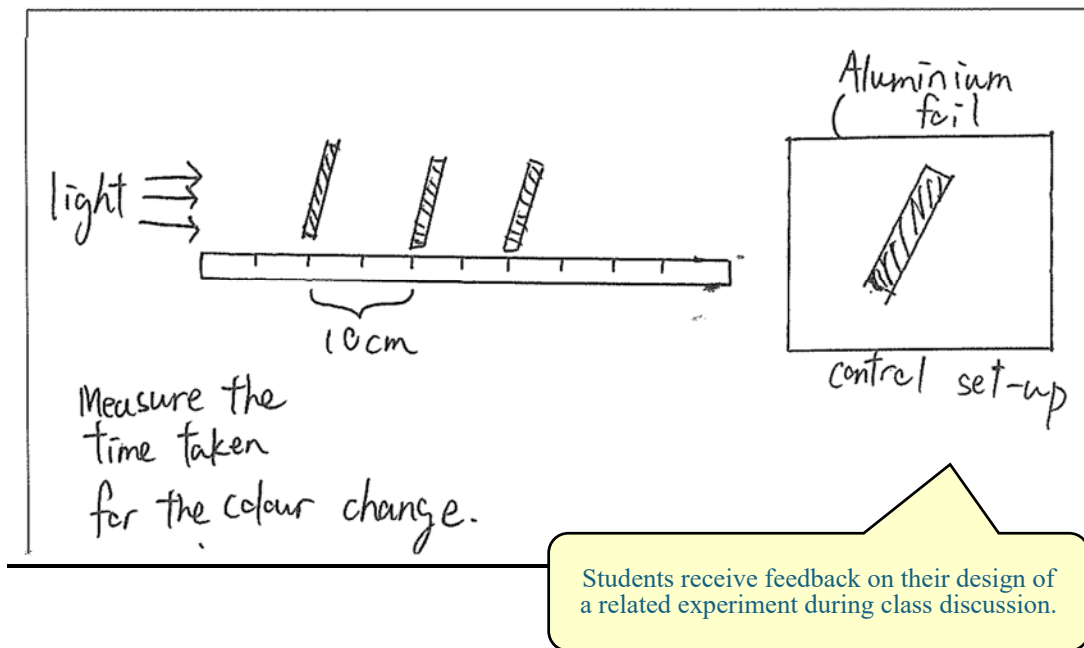
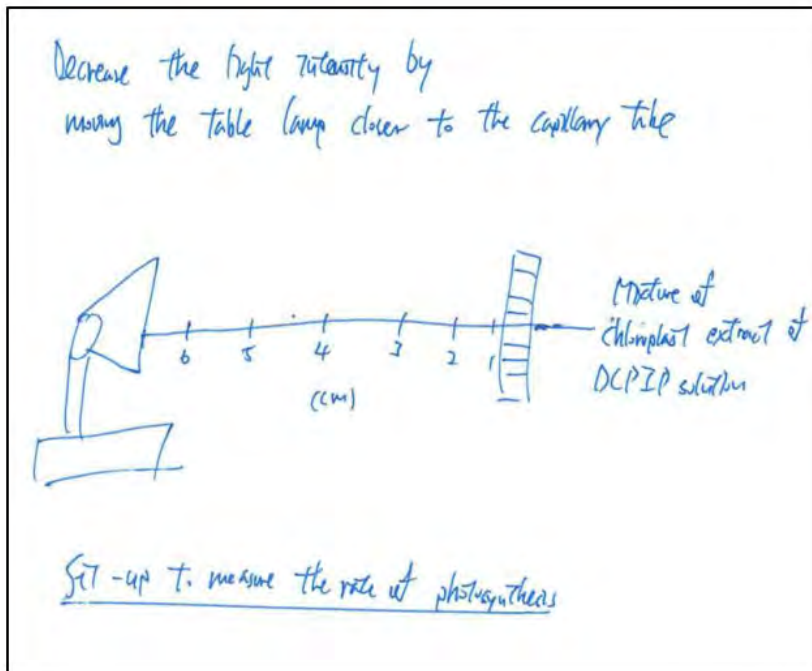
“LED 燈發出的光強度對小麥草的光合作用速率有什麼影響?”

*材料和儀器:*

DCPIP 溶液	小麥草葉綠體提取物	毛細管
檯燈	鋁箔	計時器
LED 燈泡 (白色)	尺子	冰浴
自動移液器吸頭	自動移液器	白色瓷磚
相機		

- (a) 簡要描述你會如何使用以上材料設計一項探究以實現目標。在下面空白的地方繪畫出你的實驗設計。

Examples of students' experimental designs



**Notes for teachers**

- Teachers can choose some students' diagrams (anonymised) of experimental set-ups for students to evaluate.
- Teachers can discuss students the following ideas such as how students manipulate the independent variable, whether replicates are set up, whether controls are needed, position of the table lamp.

**Notes for teachers**



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

**Task 3**

**Scenario**

Cat grass is a mixture of grasses grown from seeds, such as wheat, barley, oats, and rye. Cat grass is safer for cats to eat than outdoor grass, which may have been treated with pesticides. Cat grass is also a rich source of vitamins, minerals, and dietary fibres.

Wheatgrass is a type of cat grass that is commonly grown indoors. Wheatgrass seeds are sown in moist soil. After germination, artificial light, such as light-emitting diode (LED) lamps, are used to supply light for the seedlings to grow. Wheatgrass is ready for cats to eat around 2 weeks after germination.

In this investigation, you would like to study the effect of different wavelengths of light from LED lamps on the rate of wheatgrass photosynthesis. This information is important for determining the light conditions that maximise the growth of cat grass.



Source: <https://www.amazon.com/Cat-Planter-Hairball-Digestive-Manufactured/dp/B01JN19W9E>

**Design of investigation**

*Investigation question:*

“What is the effect of different wavelengths of light from LED lamps on the rate of photosynthesis of wheatgrass?”

*Materials and apparatus:*

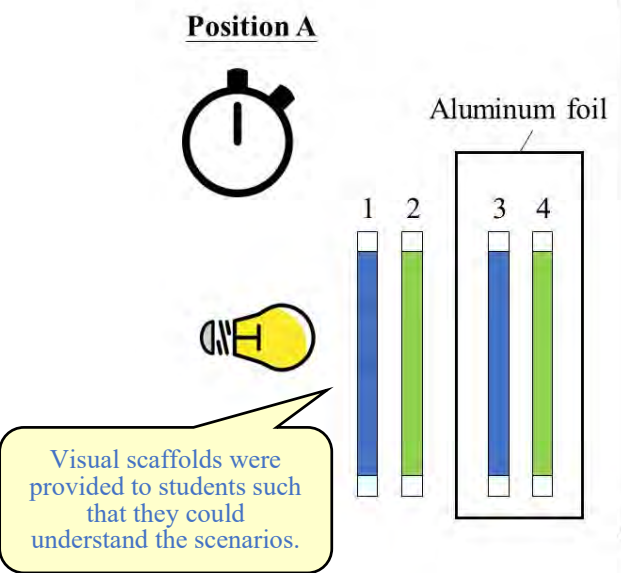
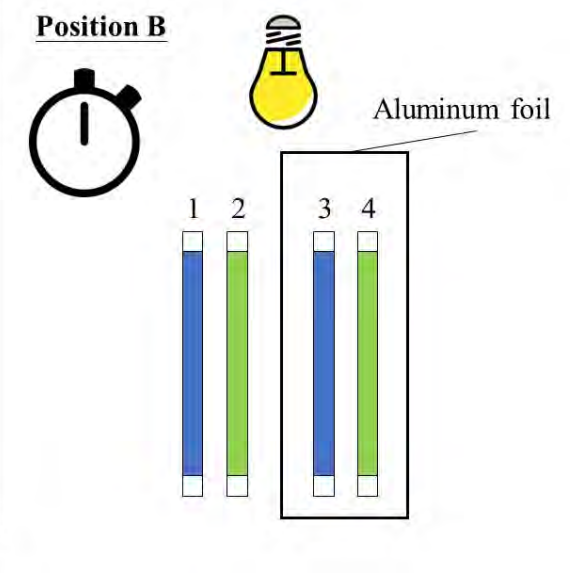
Students designed another experiment individually after the whole class discussion.

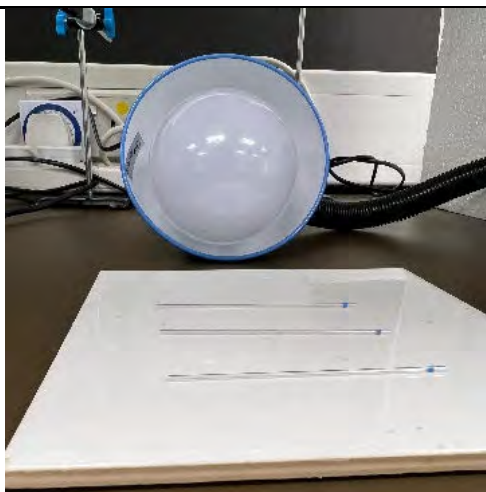
- You are given the following materials and apparatus:

DCPIP solution	Wheatgrass chloroplast extract	Capillary tubes
Table lamp	Aluminium foil	Timer
LED light bulb of different colours (Red, Green, Blue, White)	Ruler	Ice-bath
Autopipette tip	Autopipette	White tile
Camera		

**Possible questions**

- Your teacher suggests that you should use the following set-ups, but you are not sure in which position you should place the light source (see the diagrams and photos below).

Position A <i>(lamp placed horizontally)</i>	Position B <i>(lamp placed vertically above the capillary tubes)</i>
<p><b>Position A</b></p> 	<p><b>Position B</b></p> 



	<b>Tube 1</b>	<b>Tube 2</b>	<b>Tube 3</b>	<b>Tube 4</b>
Chloroplast extract	✓	✓	✓	✓
DCPIP	✓		✓	
Distilled water		✓		✓
Light	✓			

- (a) In which position (A or B) would you put the light source? Why?

Position (Put a '✓' into the correct box)	Reason
<input type="checkbox"/> A <input type="checkbox"/> B	

2. Johnny claims that the DCPIP solution would be reduced by other substances in the chloroplast extract.

Examining which tube (1, 2, 3, 4) would allow Johnny to verify his claims? Why?

3. Suggest *one* way you could modify the set-up to reduce measurement errors. Explain why the modification would reduce measurement errors.

How to reduce measurement error	Explanation of why this would reduce measurement error

#### Notes for teachers



- Q.1(a) assesses students' understanding of control variables. Placing the light bulb in position B ensures that all the tubes receive uniform light illumination. It is important to control the amount of light received as it can influence the rate of the Hill reaction.
- Q.1(b) assesses students' understanding of control set-up. If the DCPIP solution is reduced by other substances in the chloroplast extract, the DCPIP solution would lose colour in tube 3 even without light illumination.
- Q.1(c) assesses students' understanding of strategies to reduce measurement errors.

The following are some examples of students' responses to Q.1:

**Sample 1**

d1) Position B.  
To ensure that all the capillary tubes could get the same amount of light.

**Sample 2**

(d) (1) B so that all 4 capillary tubes receive similar amount of light energy. However, for position A, the capillary tube nearer to the lamp would receive more light energy than the rest. As the amount of light energy received by the chlorophyll is a control variable, it is also a factor affecting the rate of photosynthesis. Hence, it has to be the same in every set-up, so as to ensure the accuracy of the experiment. Hence the change in rate of photosynthesis can be attributed to the wavelength of light.



**About the samples**

- Both samples identified the correct lamp position and the importance of ensuring that all the capillary tubes receive the same amount of light illumination.
- Sample 2 additionally identifies light as a control variable which is a factor affecting photosynthesis (the variable to be measured).

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

**Sample 1**

q12) Tube 3. As tube 3 set up have chloroplast extract, DCPIP solution. There is no light.

**Sample 2**

2) Tube 3. If there are other substances that will reduce the DCPIP solution, the solution will decolorize even without light, which shows that without photosynthesis to give electron, the DCPIP solution will be reduced and decolorized due to the presence of other substances in chloroplast extract. However, if it won't decolorize, it means only the electron in chloroplast due to photosynthesis will reduce DCPIP. So, tube 3 allows Johnny to verify his claims.



### About the samples

- Both samples identified the correct tube.
- Sample 2 provides a full explanation of the function of this tube.

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

#### Sample 1

3.1) To repeat the experiment multiple times and calculate the average time taken for complete decolorization of DCPIP across the setups. *Explanation?*

#### Sample 2

(3) Repeat the experiment. *For how many times? Why repeating is important?*

#### Sample 3

d3.) Repeat the experiment 3 times and take the average, there are human error when stopping the timer hence repeating the experiment and taking the average can minimize the measurement error. G  
E



### About the samples

- All the samples identified a strategy (i.e., repeating the experiment). However, how repeating the experiment can reduce the impact of measurement errors is only explained in Sample 3.
- Students often have difficulties in explaining why repeating an experiment/measurement can reduce the impact of random errors.

### 任務 3

#### 情境

貓草是由小麥、大麥、燕麥和黑麥等種子培育而成的混合草。與可能用殺蟲劑處理過的室外草相比，貓草對貓來說更安全。貓草也是維生素、礦物質和膳食纖維的豐富來源。

小麥草是一種通常在室內種植的貓草。小麥草種子播種在潮濕的土壤中。發芽後，使用發光二極管 (LED) 燈等人造光為幼苗的生長提供光照。小麥草在發芽後約 2 週即可供貓食用。

在這項探究中，你想研究 LED 燈發出的不同波長的光對小麥草光合作用速率的影響。該資訊對於確定貓草生長的光照條件以使其生長最大化非常重要。



Source: <https://www.amazon.com/Cat-Planter-Hairball-Digestive-Manufactured/dp/B01JN19W9E>

#### 探究問題

LED 燈發出不同波長的光對小麥草的光合作用速率有什麼影響？

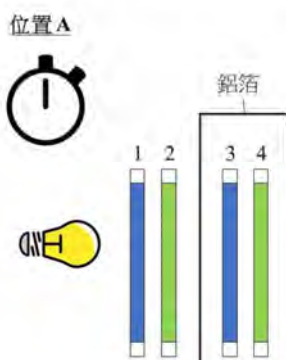
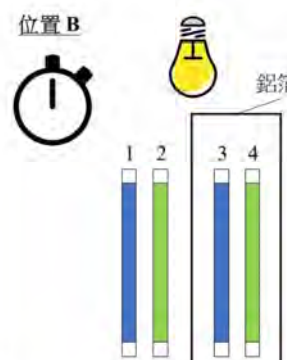

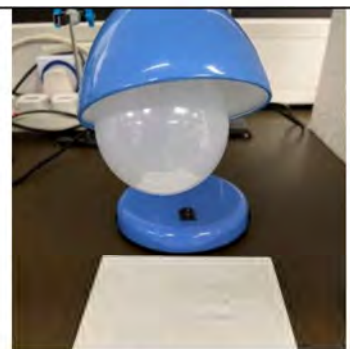
#### 材料和儀器：

- 你收到以下材料和儀器：

DCPIP 溶液	小麥草葉綠體提取物	毛細管
檯燈	鋁箔	計時器
不同顏色的 LED 燈泡 (紅色、綠色、藍色、白色)	尺子	冰浴
自動移液器吸頭	自動移液器	白色瓷磚
相機		

### 參考問題

1. 你的老師建議你使用以下裝置，但你不確定應該將光源放在哪個位置(見下面的圖表和照片)：

位置 A (燈是水平放置的)	位置 B (燈垂直放置在毛細管上方)																									
																										
																										
	<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 25%;"></th> <th style="width: 12.5%;">毛細管 1</th> <th style="width: 12.5%;">毛細管 2</th> <th style="width: 12.5%;">毛細管 3</th> <th style="width: 12.5%;">毛細管 4</th> </tr> </thead> <tbody> <tr> <td>葉綠體提取物</td> <td style="text-align: center;">✓</td> <td style="text-align: center;">✓</td> <td style="text-align: center;">✓</td> <td style="text-align: center;">✓</td> </tr> <tr> <td>DCPIP</td> <td style="text-align: center;">✓</td> <td></td> <td style="text-align: center;">✓</td> <td></td> </tr> <tr> <td>蒸餾水</td> <td></td> <td style="text-align: center;">✓</td> <td></td> <td style="text-align: center;">✓</td> </tr> <tr> <td>光</td> <td style="text-align: center;">✓</td> <td></td> <td></td> <td></td> </tr> </tbody> </table>		毛細管 1	毛細管 2	毛細管 3	毛細管 4	葉綠體提取物	✓	✓	✓	✓	DCPIP	✓		✓		蒸餾水		✓		✓	光	✓			
	毛細管 1	毛細管 2	毛細管 3	毛細管 4																						
葉綠體提取物	✓	✓	✓	✓																						
DCPIP	✓		✓																							
蒸餾水		✓		✓																						
光	✓																									

你會把光源放在哪個位置(A 或 B)? 為什麼?

位置 (將'✓'填在合適格內)	原因
<input type="checkbox"/> A <input type="checkbox"/> B	

2. 一心聲稱 DCPIP 溶液會被葉綠體提取物中的其他物質還原。

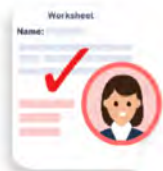
哪個試管(1、2、3、4)可驗證他的說法? 為什麼?

3. 建議一種可以修改裝置以減少測量誤差的方法。解釋為什麼此項修改可以減少測量誤差。

減少測量誤差的方法	解釋為什麼這樣可以減少測量誤差

**Notes for teachers**

- It is suggested that chloroplast extract is prepared for students. If appropriate, students may be asked to perform chloroplast extraction. See the *Supplementary Resource* for the relevant procedures.
- Each group is asked to collect data on two light colours (i.e., white and red, blue and red, white and green, and blue and green). Class data are shared.
- 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.
- The *Supplementary Resource* section contains the list of materials.
- Scan the QR code to view the process of the experiment.

**Task 4**

- Read the following procedures to carry out the investigation.

**Procedure**

1. Place a table lamp with a light bulb 5 cm above a white tile (do not turn it on yet).
2. Use a micropipette to transfer 50  $\mu\text{L}$  of chloroplast extract into a capillary tube (reference tube).
3. Place the capillary tube under the table lamp.
4. Transfer 50  $\mu\text{L}$  of chloroplast extract with DCPIP into a capillary tube.
5. Repeat *Step 4* three times (i.e., 4 tubes containing chloroplast extract with DCPIP).
6. Wrap one capillary tube with aluminium foil (control tube).
7. Place the four tubes next to the control tube under the table lamp (see *Figure 1*).
8. Turn on the lamp (white), and start the timer.
9. Record the time ( $t$ ) taken for the colour of each tube to match the colour of the reference tube in the table below. (As the colour of the tube contents is difficult to see under the coloured lights, the remote is used to switch the coloured bulb to 'white' for 1 second every 60 second to check the colour matching.)
10. Repeat the above steps for the other colour (red) of light bulb by switching the controller.
11. Record the time for *each* of the experimental tubes (tubes 1, 2, and 3) to change the colour.
12. Calculate the average time for colour change and the average rate of colour change ( $1/\text{average time for colour change}$ ). (If no colour change occurs after 20 minutes, record '>1200' and enter the rate of colour change as '0.00'.)
13. Report your group data in this *Google Sheet* by scanning the QR code.

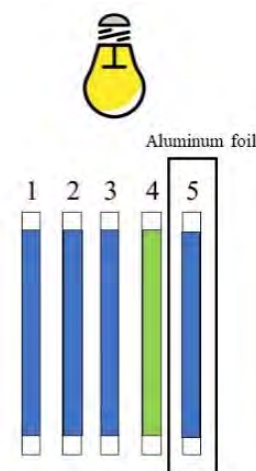


Figure 1



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



#### 任務 4

- 閱讀以下實驗步驟以進行探究:

#### 實驗步驟

1. 將一盞檯燈的燈泡置於白色瓷磚 5 厘米上方(暫不打開)。
2. 使用微量移液器將 50  $\mu\text{L}$  的葉綠體提取液轉移到毛細管中(參考管)。
3. 將毛細管放在檯燈下。
4. 將 50  $\mu\text{L}$  含有 DCPIP 的葉綠體提取液轉移到一支毛細管中。
5. 重複步驟 4 三次(即有 4 支含有 DCPIP 葉綠體提取液的毛細管)。
6. 用鋁箔包裹一支毛細管(對照管)。
7. 將四支毛細管放在對照管旁,置於檯燈下(如圖 1 所示)。
8. 打開燈(白色)並啟動計時器。

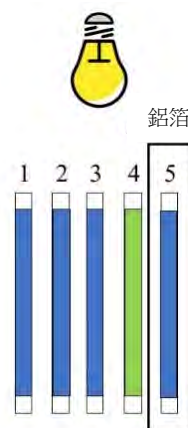


圖 1

9. 記錄每支管子的顏色與參考管匹配所需的時間( $t$ ),填寫於下表中。

(由於在不同色光下很難看清管子內容物的顏色,因此每 60 秒切換到白色燈光 1 秒,以檢查顏色是否匹配)

10. 通過切換色彩控制器,重複上述步驟,使用其他顏色(紅色)燈泡。
11. 記錄每支實驗管(管 1、管 2、管 3)顏色變化所需的時間。
12. 計算顏色變化的平均時間和平均變色速率( $1/\text{顏色變化平均時間}$ )。

(如果 20 分鐘內沒有發生顏色變化,請記錄為 “>1200”, 變色速率輸入為 “0.00”。)

13. 掃描二維碼,在此 *Google Sheet* 報告你小組的數據。



掃描二維碼以獲取 *Google Sheet* 的副本。



Teacher Notes 2



**Notes for teachers**

- The following are possible questions that teachers can use to guide students in thinking about or assessing their scientific inquiry skills related to data analysis and interpretation.
- Student work samples are shown below to illustrate possible student thinking to Q.2.

**Task 5**

**Possible questions**

1. Which of the following claim(s) is/are supported by *the data your group obtained*? (Put a '✓' into the appropriate box(es).)

(a) The rate of photosynthesis of the cat grass is lowest under green light.	<input type="checkbox"/>
(b) The rate of photosynthesis of the cat grass is higher under blue light than red light.	<input type="checkbox"/>
(c) The rate of photosynthesis of the cat grass is higher under white light than red light.	<input type="checkbox"/>
(d) The rate of photosynthesis of the cat grass is highest under blue light.	<input type="checkbox"/>
(e) None of the above.	<input type="checkbox"/>

2. David found that the rate of photosynthesis of the cat grass is highest under white light than under the other light colours he tested (e.g., green and red). He claims that the growth of the cat grass will be highest when grown under white light.

Do you agree with this claim? Why?

- Agree   
 Disagree

Explanation:

3. Propose *one* meaningful investigative question that relates to your experimental data.



**Notes for teachers**

- Q.1 assesses students' understanding of making valid claims based on available data and evidence.
- Q.2 assesses students' understanding of the generalisability of their conclusion.
- Q.3 assesses students' ability to generate a new investigation question that extends the present investigation.

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

### Sample 1

4. David found that the rate of photosynthesis of the cat grass is highest under white light than other light colours he tested (e.g., green, red). He claims that the growth of the cat grass will be highest when grown under white light. Do you agree with this claim? Why?

Agree

Disagree

Explanation:

The white light combined all of the colours, so it contains <sup>all</sup> different wave lengths which the cat grass can absorb the optimum wave length for its growth

### Sample 2

4. David found that the rate of photosynthesis of the cat grass is highest under white light than other light colours he tested (e.g., green, red). He claims that the growth of the cat grass will be highest when grown under white light. Do you agree with this claim? Why?

Agree

Disagree

These ATP can be used in Calvin cycle. More light intensity increase the rate of photosynthesis.

It implies that the cat grass absorbs more <sup>white</sup> light energy <sup>during</sup> photochemical reaction.

Excited electron ~~released~~ pass through electron transport chain

Explanation:

During photochemical reaction, the



#### About the samples

- Both samples wrongly stated that the evidence supports the claim about the growth of the cat grass.
- The students seemed to use biological facts they know to answer the questions rather than assess the generalisability of their results by attending to the relationship between photosynthesis and plant growth.

### Sample 3

4. David found that the rate of photosynthesis of the cat grass is highest under white light than other light colours he tested (e.g., green, red). He claims that the growth of the cat grass will be highest when grown under white light. Do you agree with this claim? Why?

Agree

Disagree

Explanation:

The growth rate of the cat grass is not only depending on the colour of the light supply, e.g. light intensity: a green or red light with a higher light intensity will have a higher rate of photosynthesis than a white light with lower light intensity.

5. Propose one meaningful investigative question that relates to your experimental data.

### Sample 4

4. David found that the rate of photosynthesis of the cat grass is highest under white light than other light colours he tested (e.g., green, red). He claims that the growth of the cat grass will be highest when grown under white light. Do you agree with this claim? Why?

Agree

Disagree

Explanation:

The growth rate may be affected by many other factors such as the temperature and oxygen concentration.

### Sample 5

4. David found that the rate of photosynthesis of the cat grass is highest under white light than other light colours he tested (e.g., green, red). He claims that the growth of the cat grass will be highest when grown under white light. Do you agree with this claim? Why?

Agree

Disagree

Explanation:

Other factors also may lead the growth of cat grass for example, the rate of respiration will be different.



#### About the samples

- All the samples correctly stated that plant growth can also be affected by other factors.
- However, the factors identified are not entirely scientifically accurate. For example, Sample 2 identified oxygen concentration as a factor that can affect plant growth.

**任務 5**

**參考問題**

1. 你小組所獲得的數據支持以下哪一項說法?  
(在下列方格加上‘✓’號以選出你的答案。)

(a) 貓草的光合速率在綠光下最低。	<input type="checkbox"/>
(b) 貓草的光合速率在藍光下高於紅光。	<input type="checkbox"/>
(c) 貓草的光合速率在白光下高於紅光。	<input type="checkbox"/>
(d) 貓草的光合速率在藍光下最高。	<input type="checkbox"/>
(e) 數據皆不能支持以上的說法。	<input type="checkbox"/>

2. 大衛發現, 貓草在白光下的光合速率高於其他測試的光顏色(如綠光、紅光)。他聲稱, 當貓草在白光下生長時, 生長量將最高。你是否同意這一說法? 為什麼?

- 同意  
 不同意

解釋:

3. 提出一個與你的實驗數據相關的研究問題。



## Supplementary Resources

### Possible Modifications

#### 1. Effect of darkness on decolourised DCPIP in chloroplast extract

- When a chloroplast extract containing DCPIP is exposed to light, the DCPIP becomes decolourised. If this decolourised extract is then placed in darkness, the blue colour of the DCPIP will gradually return.
- Teachers may ask students to explain the reason for these observations.



Scan the QR code to see a video.

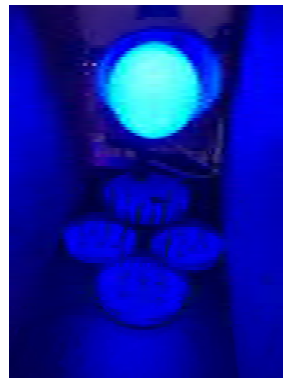
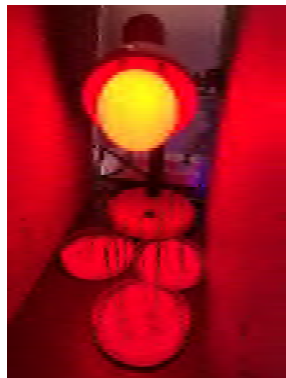
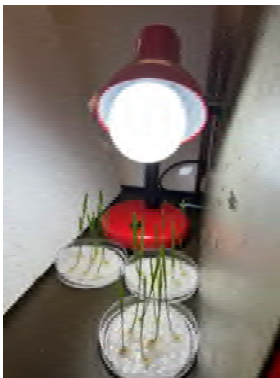


#### 2. Investigating the mode of action of herbicides

- This set-up can also be used to investigate the mode of action of herbicides (see *Photosynthesis Inhibitor Investigation*).

#### 3. Investigating the effect of different wavelengths of light on the growth of cat grass

- The effect of different wavelengths of light on the growth of cat grass can be studied by growing cat grass seeds on moist cotton wool.
- Surface-sterilise the seeds using 20% bleach for 20 minutes and grow the seeds on moist cotton wool.
- Cat grass normally germinates within 1–2 days. Visible morphological differences between different light treatments can be seen within a week.



### 1. Materials for Task 2

#### *Materials for each group*

<ul style="list-style-type: none"> <li>• DCPIP solution</li> </ul>	<ul style="list-style-type: none"> <li>• Wheatgrass chloroplast extract</li> </ul>	<ul style="list-style-type: none"> <li>• Capillary tubes</li> </ul>
<ul style="list-style-type: none"> <li>• Table lamp</li> </ul>	<ul style="list-style-type: none"> <li>• Aluminium foil</li> </ul>	<ul style="list-style-type: none"> <li>• Timer</li> </ul>
<ul style="list-style-type: none"> <li>• LED light bulb (White)</li> </ul>	<ul style="list-style-type: none"> <li>• Ruler</li> </ul>	<ul style="list-style-type: none"> <li>• Ice-bath</li> </ul>
<ul style="list-style-type: none"> <li>• Autopipette tip</li> </ul>	<ul style="list-style-type: none"> <li>• Autopipette</li> </ul>	<ul style="list-style-type: none"> <li>• White tile</li> </ul>
<ul style="list-style-type: none"> <li>• Camera</li> </ul>		

		
Table lamp	LED light bulb (RGB)	Capillary tube

### 2. Materials for Task 4

#### *Chemicals to be prepared*

- *Extraction Buffer* (250 mL) (Dissolve 2.7 g of hydrated disodium hydrogen phosphate, 1.0 g of anhydrous potassium dihydrogen phosphate, 33 g of sucrose and 0.25 g of potassium chloride in 250 mL of distilled water. Adjust pH to 7.5. Store at 4°C refrigerator.)
- *DCPIP solution* (100 mL) (Dissolve 0.1 g of DCPIP and 0.4 g of potassium chloride in 100 mL of distilled water. *Note:* DCPIP solution should be freshly prepared prior to use.)



#### **Extraction of chloroplasts (~30 mL)**

1. Weigh 4 g spinach leaves/ 6 g cat grass leaves.
2. Cut the leaves into small pieces using a pair of scissors.
3. Add 40 cm<sup>3</sup> of ice-cold *Extraction Buffer* solution.
4. Add a spoonful of sand.
5. Grind the leaves using a mortar and pestle.
6. Filter the leaf extract using muslin cloth to remove leaf debris
7. Store the filtrate on an ice bath.



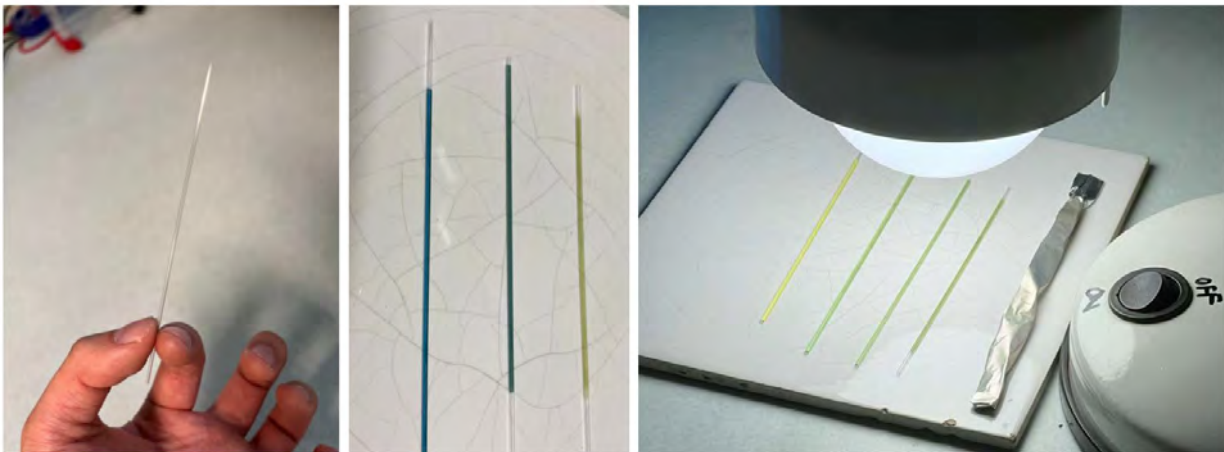
### Trial run

1. Add 1 mL of leaf extract to a 1.5 mL tube.
2. Add 0.15 mL 0.1% DCPIP solution to a 1.5 mL tube.
3. Trial run to see if the time for the white light to change colour is within 5 minutes. Adjust the volume of 0.25 mL 0.1% DCPIP if needed.
4. Prepare the chloroplast extract for each group
  - 1 mL chloroplast extract + distilled water
  - 1 mL chloroplast extract with DCPIP (with aluminium) + DCPIP (with the optimised volume)

### Materials for each group

• *1 mL Chloroplast extract in 1.5 mL tube	• Table lamp with colour controller	• Capillary tubes X 10
• *1 mL Chloroplast extract with DCPIP in 1.5 ml tube (aluminium foil)	• LED light bulb of different colours (Red, Green, Blue, White)	• White tile
• Ice bath	• Ruler	• Timer
• Autopipette (P-200)	• Autopipette tip (P-200)	• Aluminium foil

\* on ice bath



### References

Spencer, R. (2018). Pitch perfect: Investigating the effects of different wavelengths of light on the rate of photosynthesis and grass growth. *School Science Review*, 100(371), 15–20.



# **Yeast Respirometer Investigation**

---



# Yeast Respirometer Investigation

## Overview

- The *Yeast Respirometer Investigation* is related to the use of sugar substitutes in breadmaking. Students investigate the effects of different types of sugar substitutes on the rate of yeast fermentation.
- Students are given the opportunity to design and carry out experiments in which they identify significant assumptions, consider limitations in measurement, and evaluate different experimental designs (i.e., within- and between-subject designs).

## Teaching Plan

*Prerequisite knowledge (scientific ideas)*

- Alcoholic fermentation process
- Alcoholic fermentation as an enzyme-catalysed reaction

Lesson	Lesson sequence	Duration (mins)	Resources
<b>Stage 1 Preparing for the investigation</b>			
<ul style="list-style-type: none"> <li>• The investigation is set in a decision-making context (<b>Decision-making Task</b>).</li> <li>• The investigation is situated in an authentic daily-life context related to the use of sugar substitutes for breadmaking (<b>Contextualisation</b>).</li> <li>• Students have the opportunities to design their own respirometers and trial run their designs (<i>Trial Run</i>).</li> <li>• Students evaluate own and other set-ups in terms of their feasibility and accuracy (<i>Self &amp; Peer Evaluation</i>).</li> <li>• Students read information to better understand the working principles of different set-ups (<i>Reading Materials</i>).</li> </ul>			
1	<ul style="list-style-type: none"> <li>• The teacher introduces the investigation context to students in <i>Worksheet 1</i>.</li> <li>• The teacher provides materials for students to design and trial run their set-ups.</li> </ul>	40	<i>Worksheet 1</i> , Student Samples 1
Before Lesson 2	<ul style="list-style-type: none"> <li>• The teacher distributes <i>Worksheet 2</i> for students to complete at home and be familiar with the working principles of different set-ups.</li> </ul>		<i>Worksheet 2</i>
<b>Stage 2 Designing the investigation</b>			
<ul style="list-style-type: none"> <li>• The teacher shows students the microscale respirometer.</li> </ul>			
2	<ul style="list-style-type: none"> <li>• The teacher provides feedback on students' responses in <i>Worksheet 2</i> in class.</li> <li>• The teacher shows students the microscale respirometer and asks them to explain how the set-up can be used for investigating the effect of sugar substitutes.</li> </ul>	40	<i>Worksheet 3</i>
3	<ul style="list-style-type: none"> <li>• The teacher discusses with the students some questions related to the experimental design.</li> <li>• Teacher provides students with laboratory manual for preparation at home.</li> </ul>	40	Teacher Notes 1
<b>Stage 3 Carrying out the investigation</b>			
<ul style="list-style-type: none"> <li>• Students watch pre-recorded video that show the procedures of how to set up the respirometers (<i>Video with Guidance on Procedures</i>).</li> <li>• The teacher performs demonstration to show how to assemble to microscale set-up (<i>Teacher Demonstration</i>).</li> <li>• Students use microscale instrumentation that reduces the time of the experiments (<b>Microscale Instrumentation</b>).</li> <li>• Students collect more complex data sets by setting up replicates (<b>Complex Data Set</b>).</li> <li>• Students use cameras to record data (<i>Digital Tool</i>).</li> </ul>			

4	<ul style="list-style-type: none"> <li>The teacher performs a demonstration of how to assemble the microscale respirometer.</li> <li>Teacher asks questions to help students connect their lab experience and related ideas/scientific inquiry skills.</li> <li>Students carry out the investigation.</li> </ul>	40	Laboratory Manual
<b>Stage 4 Explaining and evaluating data</b> <ul style="list-style-type: none"> <li>Students evaluate the validity of using two parameters to measure the fermentation rate of the yeast (i.e. the number of bubbles produced in a fixed period, and the time for the colour of the indicator to change from green to yellow).</li> <li>Students use data to make informed decisions on whether and how to use sugar substitutes to produce breads with similar textures and appearances.</li> </ul>			
Before Lesson 5	<ul style="list-style-type: none"> <li>Students complete data reporting and analysis at home.</li> <li>Teacher collects and marks student responses.</li> </ul>		Teacher Notes 2
5	<ul style="list-style-type: none"> <li>Teacher provides feedback on students' performance related to data reporting and analysis.</li> </ul>	40	Teacher Notes 2

### Important Notes

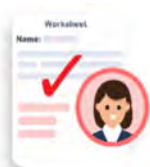
- Students are *not* required to explain why yeasts can use sugar substitutes for fermentation. Rather, they are expected to use data to make decisions about which sugar substitute(s) to replace refined sugar.



# Instructional Materials

## Stage 1 Preparing for the investigation

### Student Worksheet 1



#### Notes for teachers

- Teachers can distribute *Worksheet 1* and instruct students to design experimental set-ups to measure the rate of yeast fermentation.
- Teachers can provide students with concrete materials for their trial runs to see if their set-ups are feasible. See the *Supplementary Resource* section for the list of materials.
- The *Supplementary Resource* section provides examples of possible set-ups.

#### Task 1

- Read the scenario and complete the questions that follow:

#### Scenario

Adam is weight conscious. He has recently replaced refined sugars (e.g., sucrose) with sugar substitutes (i.e., calorie-free sweeteners). He wondered if sugar substitutes can be used to replace sucrose in breadmaking. He would like to investigate the effects of different sugar substitutes on the rate of yeast fermentation.



Refined sugar    Sugar substitute 1    Sugar substitute 2    Sugar substitute 3

To perform the investigation, he must first assemble a set-up that allows him to measure the fermentation rate of yeast. The following shows a list of apparatuses and materials that he can find in the science laboratory:

Yeast	Test tube	Syringe	Phenolphthalein
Balloon	Boiling tube	Syringe cap	Straw
String	Rubber tubing	Paraffin oil	Glass bottle
Boiled sugar substitutes 1, 2, 3	Plastic dropper	Dropper bottle	Sodium hydroxide solution
Boiled distilled water	Timer	Ruler	Water
Boiled sucrose solution	Electronic balance	Measuring cylinder	Petri dish

- Based on the materials and apparatuses given, draw *at least two* set-ups that your group thinks Adam can use to measure the fermentation rate of yeast.

Notes:

- Your group can trial run your set-ups.
- Be prepared to show your peers the set-ups.

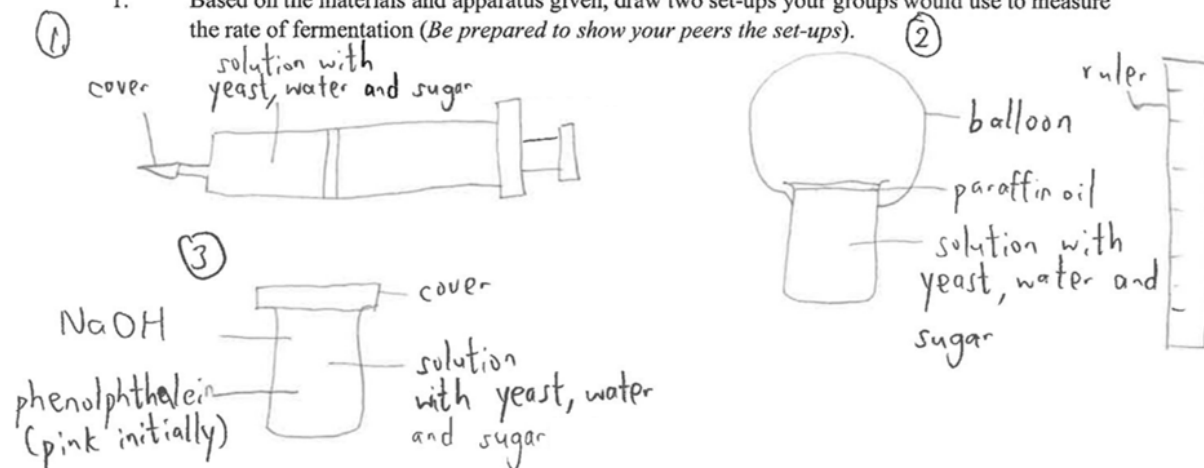
Students had opportunities to try out their designs.

- Briefly explain how you will use the set-ups to investigate the effect of the three sugar substitutes on the rate of fermentation.

## Student Samples 1 (Worksheet 1)

**Directions:**

- Based on the materials and apparatus given, draw two set-ups your groups would use to measure the rate of fermentation (*Be prepared to show your peers the set-ups*).



- Briefly explain how you will use the set-ups to investigate the effect of the three sugar substitutes on the rate of fermentation.

- measure the volume of  $\text{CO}_2$  produced per <sup>unit of</sup> time
- measure the <sup>increase in</sup> height of balloon per unit time
- measure the time for the disappearance of the pink colour ( $\text{CO}_2$  neutralize  $\text{NaOH}$ )

### Notes for teachers

- The student sample shows the drawing of some set-ups designed by students.
- Teachers can distribute *Worksheet 2*, which prompts students to analyse the principles of different set-ups after they have explained their set-ups.
- Students' responses in *Worksheet 2* can be collected using a *Google Form*.
- Teachers can read *Appendix 3* from Chan et al. (2021) for the possible set-ups and their working principles.
- Scan the QR code to access *Appendix 3*.



**任務 1**

- 閱讀以下資訊並回答以下的問題。

**情境**

亞當最近因為關注體重，將精製糖(如蔗糖)換成了代糖(即無熱量甜味劑)。他想知道代糖是否可以用來替代麵包製作所用的蔗糖。他希望研究不同代糖對酵母發酵速率的影響。



為了進行這項探究，他需要先組裝一個裝置來測量酵母的發酵速率。以下顯示了他在實驗室中可以找到的設備和材料清單：

酵母菌	試管	注射器	酚酞
氣球	沸騰管	注射器蓋	吸管
繩	橡膠管	石蠟油	玻璃瓶
經煮沸代糖溶液 1, 2, 3	膠滴管	滴瓶	氫氧化鈉
經煮沸蒸餾水	計時器	尺子	水
經煮沸蔗糖溶液	電子秤	量筒	培養皿

- 運用提供的材料和儀器,繪製 **至少兩種**你們認為亞當可以使用來測量酵母發酵速率的裝置。  
*注意事項:*
  - 你的小組可以嘗試運行這些裝置。
  - 請準備好向你的同學展示這些裝置。
- 簡要說明你將如何使用這些裝置來探究三種代糖對發酵速率的影響。

**Task 2**

- Adam found the following information.

**Source 1: Experimental set-ups for investigating the rate of yeast fermentation**

Adam found two experimental set-ups that can be used to measure the rate of yeast fermentation:

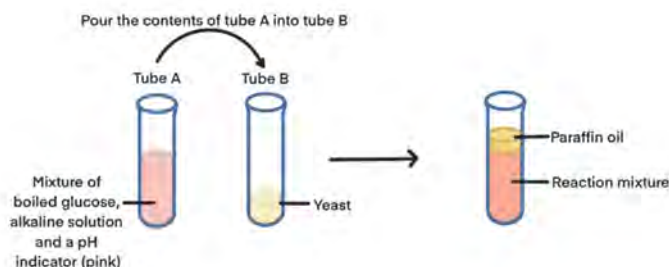
**Set-up 1**

- This set-up uses an airtight syringe containing yeast mixed with boiled sugar solution. To measure the rate of yeast fermentation, the initial position of the plunger at the beginning of the experiment is recorded. Readings are then taken at specific time intervals.



**Set-up 2**

- This set-up involves two tubes. Tube A contains a mixture of boiled glucose solution and an alkaline solution containing a pH indicator (pink under alkaline pH values and colourless when the pH decreases to 7 or lower). Tube B contains yeast. The two tubes are mixed, and a layer of paraffin oil is added to the reaction mixture. The time taken for the disappearance of the pink colour in the reaction mixture can be used to indicate the rate of yeast fermentation.



Answer the following questions about the working principles of the two set-ups Adam has given to you:

- Write a word equation for yeast fermentation.
- Based on your answer in (a),
  - explain why experimental *Set-up 1* can be used to measure the yeast fermentation rate. (*Hints:* Consider the products formed in yeast fermentation. What will happen to the position of the plunger after the experiment starts? Why? How can the fermentation *rate* be determined?)
  - explain why experimental *Set-up 2* can be used to measure the yeast fermentation rate. (*Hints:* Consider the products formed in yeast fermentation and determine if they have an effect on the pH of the reaction mixture. What is the relationship between the time taken for the disappearance of the pink colour in the reaction mixture and the rate of yeast fermentation?)
- Adam reminded you that the boiled glucose solution should be cooled before mixing with the yeast when using both set-ups. Explain why this step is necessary.
  - Suggest another precaution you will need to take when assembling *Set-up 1*.



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



**任務**

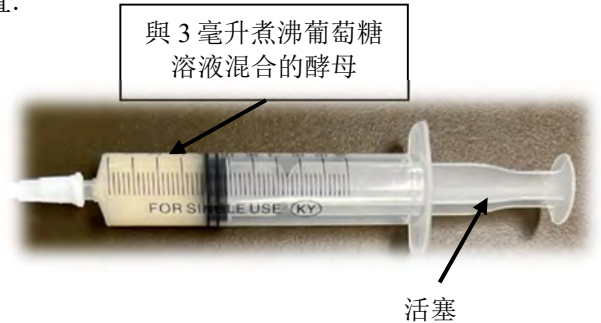
- 亞當找到了以下的資料。

**資料 1: 研究酵母發酵速率的實驗設置**

亞當找到了兩種可用於測量酵母發酵速率的實驗裝置:

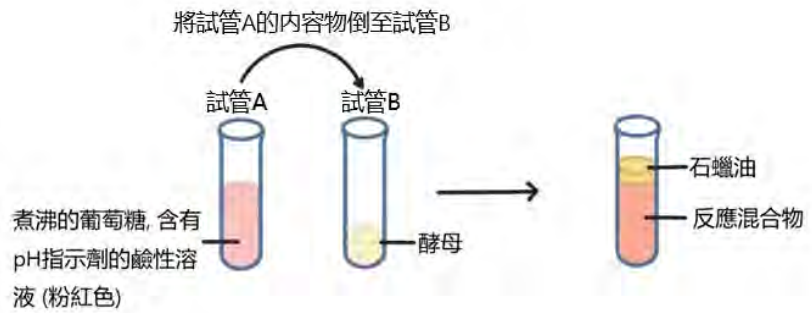
**實驗裝置 1:**

- 這個設置使用了一個氣密注射器，其中裝有與煮沸的糖溶液混合的酵母。為了測量酵母發酵速率，需在實驗開始時記錄活塞的初始位置。然後在特定時間間隔內進行讀數。



**實驗裝置 2:**

- 這個設置包括兩個試管。試管 A 含有煮沸的葡萄糖溶液和含有 pH 指示劑的鹼性溶液(在鹼性 pH 下呈粉紅色，在 pH 降到 7 或更低時無色)。試管 B 含有酵母。將兩個試管混合，並在反應混合液上加一層石蠟油。反應混合液中粉紅色消失的時間可用於表示酵母發酵的速率。



回答關於亞當提供的兩個實驗裝置所涉及工作原理的問題:

- 寫出酵母發酵的文字方程式。
- 根據(a)中的答案:
  - 解釋為什麼 *實驗裝置 1* 可用於測量酵母發酵速率。  
(提示:考慮酵母發酵過程中生成的產品。實驗開始後，活塞會發生什麼變化?為什麼?如何確定發酵速率?)
  - 解釋為什麼 *實驗裝置 2* 可用於測量酵母發酵速率。  
(提示:考慮酵母發酵產生的產品對反應混合液的 pH 有何影響。反應混合液中粉紅色消失的所需時間與酵母發酵速率有什麼關係?)
- 亞當提醒你，在使用這兩種裝置時，煮沸的葡萄糖溶液應先冷卻後再與酵母混合。解釋為什麼需要這一步驟。
  - 建議在組裝 *實驗裝置 1* 時需要採取的另一個預防措施。



掃描二維碼以獲取 *Google Form* 的副本。



## Student Worksheet 3

### Notes for teachers



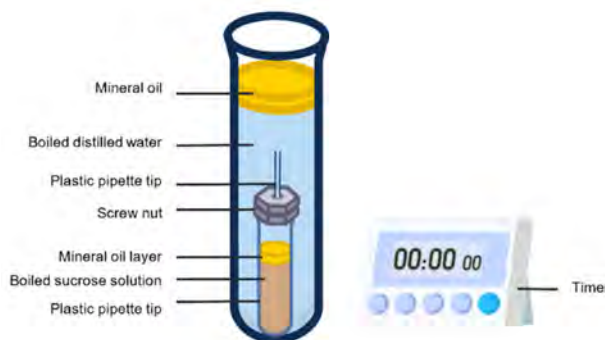
- Teachers can provide feedback on student responses in *Worksheet 2*.
- Teachers can show students the microscale respirometer and ask them to think about how the set-up can be used to measure the rate of yeast fermentation.
- See Chan (2016) for more information about the set-up.
- Teachers may show a video to the students. Scan this QR code to access the video.



### Task 3

#### Possible questions

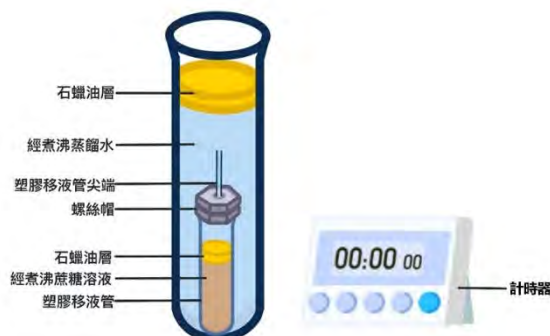
- Andrew suggested using the following experimental set-up to measure the dependent variable. To ensure that the apparatus is functioning properly, he recommends first testing it with the boiled sucrose solution.
  - Predict *one* observable change during the experiment.
  - Describe how you would use the experimental set-up to compare the yeast fermentation rate among sugar substitutes (1, 2, 3).  
(*Hint:* In your answer, please include [1] how to manipulate the independent variable, [2] how to measure the dependent variable, and [3] the relationship between the dependent variable and the measurement method.)



### 學生工作紙 (三)

#### 任務 3

- 回答以下問題。
  - 小智建議你使用以下的實驗裝置來測量因變量。為了確保實驗裝置正常運作，小智建議你先使用經煮沸的蔗糖溶液進行測試。
    - 試預測實驗過程中一項可觀察變化。
    - 描述你如何運用上述的實驗裝置來比較酵母菌在使用哪種代糖(1、2、3)時具有最佳的發酵速率。  
(提示：請在你的回答中：[1]自變量的處理方法，[2]因變量的量度方法，以及[3]因變量和量度方法之間的關聯。)





**Notes for teachers**

- After introducing the microscale respirometer, the following shows the main investigation context for students to work on.
- Some questions can be used by teachers to guide students in thinking about or assessing the scientific inquiry skills related to experimental designs.
- Some student work samples are shown below to illustrate possible student thinking to some questions.

**Task 3**

**Scenario**

Some people who try to lose weight eat less food containing refined sugar. However, the breadmaking process uses a lot of refined sugar (i.e., sucrose). In recent years, it has become more common to use sugar substitutes (zero-calorie sweeteners) to replace refined sugar to reduce calorie intake.

Can sugar substitutes replace refined sugar in making bread? If so, which type of sugar substitute allows the yeast to ferment the best?

Here are some of the materials and apparatuses in the science laboratory:

Yeast	Boiling tube	Plastic dropper	Paraffin oil
Boiled sucrose solution	Boiled distilled water	Timer	Screw nuts
Boiled sugar substitute 1	Boiled sugar substitute 2	Boiled sugar substitute 3	Universal indicator

Using your biological knowledge of yeast fermentation, design a valid and reliable experiment to carry answer the investigation question.

## Possible questions

1. Adam and his friends are discussing the possible significant assumptions of this design.



Amy

A significant assumption is that the fermentation rate is higher for sugar substitute 1.

Is it the assumption that the carbon dioxide produced during fermentation does not dissolve in the sugar substitute solution?



Adam



Carlos

I would say that the rate of carbon dioxide produced by the yeast indicates the rate of fermentation is one of the significant assumptions.

I have one more idea, which is the sugar substitute solution should be boiled before the experiment.



Betty

Which person(s) do you agree with? Put a “✓” into the appropriate box(es) below.  
(Hints: There can be more than one assumption.)

- Amy
- Adam
- Carlos
- Betty

This question format enables the identification of distractors.



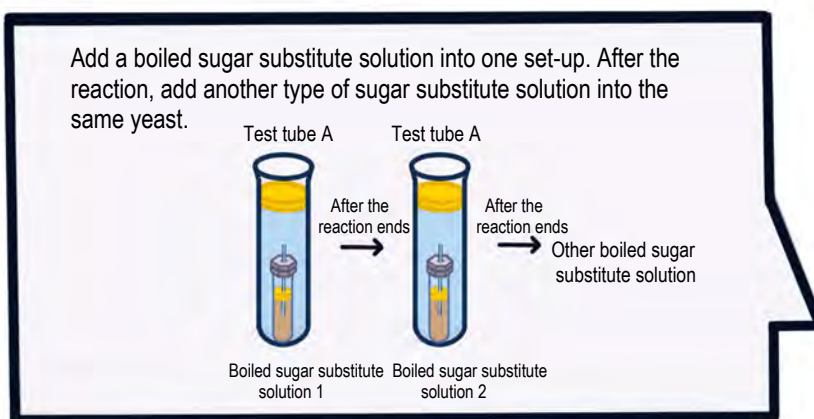
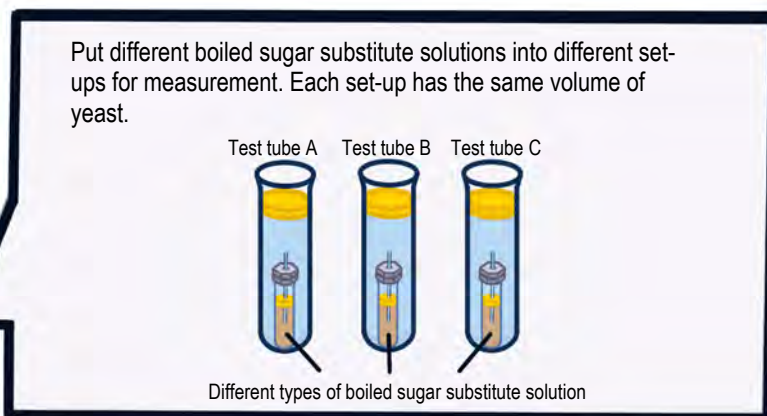
### Notes for teachers

- Q.1 assesses students' ability to identify significant multiple assumptions from the choices. Distractors include precautionary steps and predicted results.

2. Tom and Mary are discussing two experimental designs to achieve the aim of this experiment:



Tom



Mary

Which design would you choose? Why?

(Please put a '✓' in the box of the experimental design you choose.)

- Tom's design
- Mary's design

Explanation:



#### Notes for teachers

- Q.2 assesses students' ability to analyse alternative designs (i.e. within- and between-subject designs) in terms of generating valid and reliable data.

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

### Sample 1

- 小明的設計
- 小美的設計

解釋:

在實驗當中，我們需要以秒錶量度發酵作用的速率，我認為小美的設計，可以令我們更加標準地量度反應的速率，而小明的設計，實驗者需要等待於每個裝置的顏色變化，若令在其量度的反應速率產生誤差，降低了實驗的準確度。而且

等待於量度其中一種糖的反應速率，以及它的顏色變化。

### Sample 2

- 小明的設計
- 小美的設計

解釋:

將不同的代糖溶液放在單獨的試管中，同時進行實驗，以便直觀對比各代糖溶液中酒精發酵的速率；可以更準確量度因變量。

### Sample 3

- 小明的設計
- 小美的設計

解釋:

因小美的設計是在一個裝置加入<sup>一種</sup>經煮沸代糖溶液，在反應完後再加入另一種經煮沸代糖溶液至相同的酵母菌中。我認為有機會上一個的反應物還殘留在裝置中，會影響下一個代糖的測試結果。而小明的設計把不同的代糖溶液放在不同的試管，可以最大可能確保實驗的結果較少誤差。



#### About the samples

- In Sample 1, the better design was not chosen.
- In Sample 2, the better design was chosen but the student did not explain why the design allows for the collection of more valid data. The student provided reasons not related to the validity of the data (i.e. less time).
- In Sample 3, not only was the better set-up chosen but also the carry-over effect was explained. Other effects include the death of the yeast or the change in the pH of the solution as a result of the previous treatment.

任務 3

情境

部分關注體重的人士會儘量減少食用精製糖 (refined sugars) 製成的食物，而製造麵包的過程中會使用蔗糖 (精製糖)。近年來，代糖 (零卡甜味劑) 的使用越來越流行，以取代精製糖，以減少熱量攝取。

代糖是否能夠取代精製糖用於製造麵包? 如果可以的話，**酵母菌在使用哪種代糖時具有最佳的發酵速率?**

為了進行這次的研究，我們需要建立一個能夠測量酵母菌發酵速率的實驗裝置。以下是在科學實驗室中可能使用到的相關設備和物料：

酵母菌	試管	塑膠移液管	石蠟油
經煮沸蔗糖溶液	經煮沸蒸餾水	計時器	螺絲帽
經煮沸代糖溶液 1	經煮沸代糖溶液 2	經煮沸代糖溶液 3	通用指示劑

運用你對酵母菌發酵的生物學知識，設計一個有效且可靠的實驗，以回答探究問題。

參考問題

1. 亞當和他的朋友正在討論這個實驗設計的可能重要假設。



小芬

重要假設是“代糖 1 的發酵速度比較快”。

假設是“在發酵過程中產生的二氧化碳並不會溶解在代糖溶液中”嗎?



亞當

我認為其中一項重要假設是“酵母菌產生的氣體的速率代表發酵的速率”。



小智

我還有一個主意，我認為應該要在實驗前煮沸代糖溶液。



一心

你同意哪個人(或人們)的觀點? 請在合適的方框內加上✓號。  
(提示: 可能會有多於一個假設。)

- 小芬
- 亞當
- 小智
- 一心

3. 小明和小美正在討論兩種不同的實驗設計，以達到這次探究實驗的目標：你會選擇那一種實驗設計？為甚麼？（請在你選擇的實驗設計的方框內加上✓號。）



將不同種類經煮沸的代糖溶液放入不同的裝置中以作量度。  
每個實驗設置中使用相同體積的酵母菌。

試管A      試管B      試管C

不同種類經煮沸的代糖溶液

在一個裝置中加入經煮沸的代糖溶液。在反應完結後  
再加入另一種經煮沸代糖溶液至相同的酵母菌中

試管A      試管A

經煮沸的代糖溶液1      經煮沸的代糖溶液2

其他代糖溶液



- 小明的設計
- 小美的設計

解釋:

## Laboratory Manual

**Notes for teachers**

- Teachers can distribute the manual for students to read and prepare before the investigation.
- The pre-recorded video provides the steps for assembling the microscale respirometer.
- Teachers can perform a demonstration to show students how to set up a microscale respirometer and highlight the critical steps.
- Teachers can ask questions to check if students fully understand the procedures (e.g., how many respirometers do we need to set up?).
- The *Supplementary Resource* section contains the list of materials.
- Scan the QR code to view the process of the experiment.

**Task 4**

- Read the following procedures to carry out the investigation.

**Procedure**

1. Measure 15 mL of boiled distilled water containing a universal indicator using a measuring cylinder. Transfer it to a 25-mL boiling tube.
2. Expel 3 mL of air from a 3-mL plastic pipette.
3. Use the 3-mL plastic dropper to suck up 1 mL of yeast extract solution.
4. Invert the dropper to allow the liquid to flow into the bulb portion.
5. Expel the air from the 3-mL plastic dropper containing the yeast extract.
6. Use the 3-mL plastic dropper to suck up 1 mL of the boiled sucrose solution, sugar substitute 1, sugar substitute 2, sugar substitute 3, or distilled water.
7. Invert the dropper to allow the liquid to flow into the bulb portion.
8. Gently squeeze the bulb of the pipette to mix the yeast extract and sugar/sugar substitute/distilled water solution.
9. Expel the air from the 3-mL plastic dropper.
10. Use the 3 mL plastic dropper to suck up 400  $\mu$ L of paraffin oil.
11. Invert the dropper to allow the liquid to flow into the bulb portion.
12. Secure two screw nuts onto the neck of the pipette.
13. Use forceps to place the entire set-up into the test tube, ensuring that the dropper is fully submerged in the water.
14. Add a layer of paraffin oil.
15. Start a timer and record the time required for the universal indicator to change from green to yellow.
16. Allow the set-up to reach equilibrium for 2 minutes and then record the number of bubbles generated within 15 minutes.
17. Repeat *Steps 1 to 16* one more time.



Scan this QR code to see how to assemble the experimental set-up.

#### 任務 4

- 閱讀以下實驗步驟以進行探究:

#### 實驗步驟

1. 使用量筒量取 15 mL 經煮沸的蒸餾水(含有通用指示劑)，並倒入 25 mL 的試管中。
2. 小心擠出 3 mL 塑膠移液管內的空氣。
3. 使用 3 mL 塑膠移液管吸取 1 mL 酵母液。
4. 將移液管倒置，讓液體流入球形部分。
5. 小心擠出 3 mL 塑膠移液管(含有酵母液)內的空氣。
6. 使用 3 mL 塑膠移液管吸取 1 mL 經煮沸的蔗糖溶液/代糖 1/代糖 2/代糖 3/蒸餾水。
7. 將移液管倒置，讓液體流入球形部分。
8. 輕輕擠壓移液管球形部分，將酵母萃取液和糖溶液/代糖溶液/蒸餾水混合。
9. 小心擠出 3 mL 塑膠移液管內的空氣。
10. 使用 3 mL 塑膠移液管吸取 400  $\mu$ L 石蠟油。
11. 將移液管倒置，讓液體流入球形部分。
12. 將兩個螺絲帽扣在移液管頸部。
13. 使用鉗子將整個設置放入試管，直至移液管完全浸沒在水中。
14. 加入一層石蠟油。
15. 啟動計時器，記錄通用指示劑從綠色轉為黃色所需的時間。
16. 等待兩分鐘讓設置達到平衡，然後記錄在 15 分鐘內生成的氣泡數量的數量。
17. 重複步驟 1-16 一次。



掃描二維條碼以查看如何組裝實驗設置。

## Teacher Notes 2

**Notes for teachers**

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

**Task 5****Possible questions**

1. According to the data you collected, which of the following parameters can more accurately measure the yeast fermentation rate? Explain your answer.  
(Put a '✓' in the appropriate box.)
  - Time taken for the universal indicator to change from green to yellow
  - Number of bubbles produced within 15 minutes
2. Adam wants to use sugar substitutes instead of sucrose to make bread. He hopes his bread will be as fluffy as when using sucrose. Based on the data you collected, answer the following two questions:
  - (a) Which sugar substitute should Adam use? Explain your answer.  
(Put a '✓' into the appropriate box.)
    - Sugar substitute 1
    - Sugar substitute 2
    - Sugar substitute 3
  - (b) Propose a method to make the fermentation rate using the selected sugar substitute similar to the rate using sucrose.  
(*Hint: Consider the factors that affect the yeast fermentation rate.*)
3. Adam found that yeast can use one of the sugar substitutes. He would like to determine the optimum temperature at which the yeast ferments the sugar substitute. How would you modify the experimental design of this investigation to achieve this goal?

**Notes for teachers**

- Q.1 assesses students' ability to assess the appropriateness and accuracy of the methods to determine the dependent variable based on their data.
- Q.2 assesses students' ability to make informed decisions based on their data.
- Q.3 assesses students' ability to modify the experimental designs for answering a new investigation question.

The following are some examples of students' responses to Q.1:

### Sample 1

2. 根據你所收集的數據，以下哪項指標能更準確量度酵母發酵的速率？試解釋你的答案。  
(在下列方格 ✓ 以選出你的答案)
- 通用指示劑由綠色轉為黃色所需要的時間  
 十五分鐘內所產生的氣泡數目

因為試管內的通用指示劑，受醱母所發酵釋出二氧化碳及氣泡，以二氧化碳是酸性物質，使通用指示劑由綠色轉為黃色，若釋放大量二氧化碳在通用指示劑裏，使通用指示劑變得更清晰的黃色。

### Sample 2

2. 根據你所收集的數據，以下哪項指標能更準確量度酵母發酵的速率？試解釋你的答案。  
(在下列方格 ✓ 以選出你的答案)
- 通用指示劑由綠色轉為黃色所需要的時間  
 十五分鐘內所產生的氣泡數目

醱母十五分鐘內所產生的氣泡數目較為明顯及最快能見到醱母與醱的速率。而綠色轉為黃色的色差中，不能確保是否已完全轉為黃色。

### Sample 3

2. 根據你所收集的數據，以下哪項指標能更準確量度酵母發酵的速率？試解釋你的答案。  
(在下列方格 ✓ 以選出你的答案)
- 通用指示劑由綠色轉為黃色所需要的時間  
 十五分鐘內所產生的氣泡數目

因為數氣泡數目是最容易反映 $\text{CO}_2$ 釋出的量，如果使用指示劑變色時間為指標，不同人看顏色變化的定義不同，例如怎樣才算黃色，或者淺黃色算不算等爭議，根據顏色是主觀的，每人對色覺的敏感度不一，因此數氣泡數目能最大程度減少 $\text{CO}_2$ 釋出需時及數量之誤差，更能顯示實驗的準確度。



#### About the samples

- In Sample 1, the right measurement parameter was not chosen, and invalid reasons were provided.
- In Samples 2 and 3, the right parameter was chosen. Sample 3 further explained why relying on visual inspection by naked eyes as a measurement method is an important limitation (i.e. subjective judgment of colour change).

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

**Sample 1:**

4. 如果以其中一種實驗中使用的代糖取替蔗糖以製造麵包，在相若的發酵時間下，令麵包的鬆軟程度像使用蔗糖那樣。請根據你所收集的數據回答以下兩題。

(a) 你可以使用哪一種代糖？請解釋你的答案。  
(在下列方格 ✓ 以選出你的答案)

- 代糖 1 號
- 代糖 2 號
- 代糖 3 號

它的反應速率最接近蔗糖，說明  
在使用了號代糖製造麵包時，鬆軟  
程度最接近蔗糖麵包。

(b) 提出一項方法，使使用該代糖的發酵速率達到與使用蔗糖相近的水平。  
(提示：考慮影響酵母發酵速率的因素)

使用該代糖時，增加酵母菌的份量，  
調整至它們的酵母發酵速率接近一致

**Sample 2**

(a) 你可以使用哪一種代糖？請解釋你的答案。  
(在下列方格 ✓ 以選出你的答案)

- 代糖 1 號
- 代糖 2 號
- 代糖 3 號

代糖 3 號的發酵程度最快，它產生的氣泡時間較其他代糖快，缺  
氧呼吸的速率也快可以最大程度令麵包較鬆軟，代糖 3 號在實  
驗過程中產生最多的氣泡有 29 個而代糖 2 號有 16 個，代糖 1 號有  
7 個。蔗糖就有 29 個，因此代糖 3 號與蔗糖的發酵速率  
相若，能與蔗糖一樣令麵包鬆軟程度一樣。

(b) 提出一項方法，使使用該代糖的發酵速率達到與使用蔗糖相近的水平。  
(提示：考慮影響酵母發酵速率的因素)

根據實驗代糖 3 的發酵速率比蔗糖的發酵速率快。這有機  
會是因為溫度是代糖 3 的最適溫度，酶的活躍度最多，發  
酵速率最快，而也有機會是因份量有差別。可以把溫度  
下降或上升減低酶的活躍度也平衡代糖與蔗糖的發  
酵速率。

### Sample 3

4. 如果以其中一種實驗中使用的代糖取替蔗糖以製造麵包，在相若的發酵時間下，令麵包的鬆軟程度像使用蔗糖那樣。請根據你所收集的數據回答以下兩題。

(a) 你可以使用哪一種代糖？請解釋你的答案。

(在下列方格 ✓ 以選出你的答案)

- 代糖 1 號  
 代糖 2 號  
 代糖 3 號

因為根據本實驗結果的記錄中，蔗糖溶液的試管中的通用指示劑由綠色轉為黃色所需要的時間為約11分27秒，而代糖3號所需時間為約11分43秒，時間差距相當接近，但其餘的糖溶液所需時間為約13分鐘，及沒有結果。由此可見，代糖3號是除了蔗糖溶液外使酵母發酵效率最好的溶液，因此產生較多的乙醇和二氧化碳，所以代糖3號溶液的氣泡釋能出量和蔗糖溶液差不多為最高，在15分鐘內均產生了約32個氣泡，而比其他糖溶液的氣泡為3個或23個，因為較多氣泡能使麵更鬆軟，所以代糖3號是最佳之選。

(b) 提出一項方法，使使用該代糖的發酵速率達到與使用蔗糖相近的水平。

(提示：考慮影響酵母發酵速率的因素)

煮沸該代糖溶液，如煮沸該代糖溶液能去除溶液當中的氧氣，減少酵母發酵時的阻礙因素，使酵母能在乾淨沒有太多雜質的環境下發酵，增加酵母發酵時的速率，因此達到與使用蔗糖一樣的水平。



#### About the samples

- In all three samples, correct decisions concerning the type of sugar substitute were made based on the data collected. However, Sample 1 did not refer to the data collected to justify their decisions. Sample 3 is detailed in comparing all the data collected for decision-making.
- Sample 3 did not make use of biological principles in terms of the factors that can speed up the yeast fermentation rate to make the necessary modifications. Sample 1 identified the method while Sample 2 showed some issues with the use of data.

**任務 5****參考問題**

1. 根據你所收集的數據，以下哪項指標能更準確量度酵母發酵的速率？試解釋你的答案。  
(在下列方格加上✓號以選出你的答案)
  - 通用指示劑由綠色轉為黃色所需要的時間
  - 十五分鐘內所產生的氣泡數目
2. 亞當想在烘焙麵包時用代糖取代蔗糖。他希望他的麵包像用蔗糖一樣蓬鬆。根據你的數據，
  - (a) 亞當應該使用哪一種代糖？  
(在下列方格加上✓號以選出你的答案)
    - 代糖 1 號
    - 代糖 2 號
    - 代糖 3 號
  - (b) 提出一項方法，使使用該代糖的發酵速率達到與使用蔗糖相近的水平。  
(提示：考慮影響酵母發酵速率的因素)
3. 亞當發現酵母可以利用其中一種代糖。他想找出酵母的最佳溫度。你將如何修改這項探究實驗設計來實現這一目標？



## Supplementary Resources

### Possible Modifications

#### 1. Investigating yeast bead fermentation

- A syringe can be used to set up a yeast respirometer conveniently.
- If yeast beads are used, the reaction mixtures can be collected, and the solution can be used for titration against an alkaline solution containing a pH indicator.



#### Notes for teachers

- Teachers can use the following procedures.
- The procedures for making yeast beads can be found in *Yeast Bead Invertase/Catalase Investigation*.
- Scan the QR code to view a video that shows the whole experiment.
- Read the *Technician Notes* section for the materials required for this experiment.
- A video showing how to set up a yeast respirometer using a syringe is available via the QR code alongside the procedure.



#### 1. Prepare the yeast beads.



#### 2. Set up the respirometers.



#### 3. Perform a titration to determine the number of drops of solution for colour change of the alkaline solution.



## Procedure

### Setting up the respirometer

1. Remove the plunger from a 10-mL syringe.
2. Cap the syringe using a plastic syringe cap.
3. Use a pair of forceps to transfer 50 yeast beads to the syringe.
4. Pipette 2.5 mL of distilled water into the syringe.
5. Place the syringe with the distilled water vertically on the rack.
6. Repeat *Steps 1 to 5* with the sucrose solution and sugar substitutes (1, 2).
7. Insert the plunger in the syringe with distilled water.
8. Invert the syringe so that the yeast beads sink to the bottom of the syringe.
9. Remove the plastic cap from the syringe.
10. Gently tap the syringe to remove air bubbles.
11. Gently press the plunger to push the distilled water up to the top of the syringe.
12. Cap the plastic syringe to ensure that it is airtight.
13. Repeat *Steps 7–12* with the sucrose solution and sugar substitutes (1, 2).
14. Start the timer when all the respirometers are set.
15. Record the initial position of all plungers at  $t = 0$  minute.
16. After 20 minutes, the final positions of all plungers are recorded.

A video showing some mistakes in experimental procedures can sensitise students to avoid similar mistakes in their own experiments.



Scan this QR code to see how to assemble the experimental set-up.

### Determining the volume of sugar solutions for neutralisation of the alkaline solution

1. Pipette 1 mL of alkaline solution with a pH indicator into eight 5-mL conical flasks.
2. Use a dropper to withdraw all the solutions from each syringe to a 10-mL beaker.
3. Add each solution dropwise to the conical flask containing alkaline solution on a white tile. Gently shake the conical flask after adding each drop. Keep adding sugar solution until the blue colour changes to green.
4. Record the exact number of drops of sugar solution that was added. Write  $>20$  if the colour has not changed after adding 20 drops.
5. Repeat *Step 3* and *Step 4* one more time and calculate the average result.



#### Notes for teachers

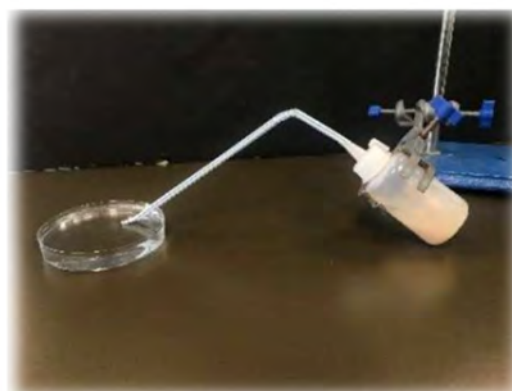
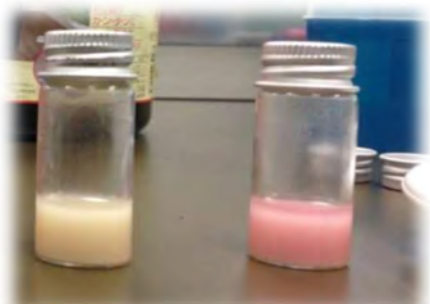
- Teachers can ask the technician to perform a trial run to adjust the alkalinity of the alkaline solution. This will be done by adjusting the volume of the sodium carbonate solution added. The goal is to find the alkaline solution composition where the positive control (i.e., sucrose solution) requires less than 10 drops to change the colour of the alkaline solution.

### Materials for Task 1

15% Yeast (activated)	Test tube	Syringe	Phenolphthalein
Balloon	Boiling tube	Syringe cap	Straw
String	Rubber tubing	Paraffin oil	Glass bottle
10% Sucrose solution	Plastic dropper	Dropper bottle	0.1 M Sodium hydroxide solution
Boiled distilled water	Timer	Ruler	Water
Straw	Electronic balance	Measuring cylinder	Petri dish

\* Containers of varying sizes can be provided to students.

#### *Possible set-ups*



## Materials for Task 4

### Chemicals to be prepared

- Boiled water (containing universal indicator) (Boil tap water and add appropriate volume of universal indicator [i.e., when the solution is sufficiently green]).
- 10% Sugar solution (boiled)/Distilled water (boiled) (Dissolve 10 g in 100 mL sugar solution. Boil the sugar solution/distilled water. Adjust pH to 7.4.)
- 15% Yeast extract (1 mL in 1.5 mL tube) (to be prepared on that day) (Add 15 g yeast in 100 mL distilled water. Add a spoonful of sugar. Stir on a magnetic stirrer. Wait for 30 minutes to activate the yeast. Keep stirring. Aliquot just before the experiment.)

### Materials for each group

• 25 mL Measuring cylinder	• >150 mL Boiled distilled water (containing universal indicator)	• 1 mL Boiled 10% sucrose solution/sugar substitute 1/sugar substitute 2/sugar substitute 3/distilled water in 1.5-mL tube X 2
• Boiling tubes X 10	• 1 mL 15% Yeast extract in 1.5-mL tube X 10	• 400 µL Paraffin oil X 10
• Boiling tubes rack	• 3 mL Plastic dropper (tip = 3.5 cm) X 10	• Paraffin oil in dropper bottle
• Timer	• Screw nut X 20	• Camera (supplied by students)

\* Volume depends on the size of the boiling tubes.

## Materials for Yeast Bead Syringe

### Materials for each group

• 10 mL Syringe X 5	• Syringe cap X 5	• Autopipette (P-1000)
• 5 mL Conical flask X 10	• Forceps X 5	• Autopipette tip (P-1000)
• >8 mL *Alkaline solution with a pH indicator	• >2.5 mL Boiled sugar substitute solution 1, 2	• >2.5 mL Boiled distilled water
• Plastic dropper X5	• >2.5 mL Boiled sucrose solution	• Timer
• 25 mL Beaker X 5		

\*40 mL 0.04% Bromothymol blue + 10 mL 2% Na<sub>2</sub>CO<sub>3</sub> + 150 mL distilled water.

# Materials for making yeast beads can be found in *Yeast Bead Invertase/Catalase Investigation*.

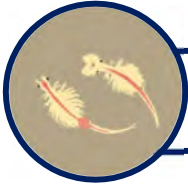
## References

- Chan, K. K. H. (2016). A simple micro-scale set-up for investigating yeast respiration in high school biology classrooms. *The American Biology Teacher*, 78(9), 669–675.
- Chan, K. K. H., West-Pratt, J., Ng, R. C. K. (2021). Using yeast fermentation as a context for meaningful learning of procedural understanding. *The American Biology Teacher*, 83(1), 26–32.



# **Brine Shrimp Investigation**

---



# Brine Shrimp Investigation

## Overview

- This *Brine Shrimp Investigation* is about examining the behaviours of brine shrimps (Millar, et al., 1994).
- Students design experimental set-ups to investigate the preference of brine shrimps for light colours.
- Students are given the opportunity to design and carry out experiments in which they generate inquiry questions, establish sampling strategies, set up replicates, consider the importance of larger sample sizes, use multiple controls, and construct explanations.

## Teaching Plan & Key Features

Lesson	Lesson sequence	Duration (mins)	Resources
<b>Stage 1 Preparing for the investigation</b>			
<ul style="list-style-type: none"> <li>• Students observe and generate questions about brine shrimps to drive inquiry (<i>See-Think-Wonder thinking routine, Driving Question Board</i>).</li> <li>• Students read information about brine shrimps (<i>Reading Materials</i>).</li> </ul>			
1	<ul style="list-style-type: none"> <li>• The teacher allows students to observe adult brine shrimps and brine shrimp larvae.</li> <li>• The teacher invites students to ask questions about the brine shrimps.</li> <li>• Students read background information on brine shrimps.</li> <li>• The teacher introduces the investigation question based on the questions proposed by the students in the <i>Driving Question Board</i>.</li> </ul>	40	<i>Worksheet 1</i>
<b>Stage 2 Designing the investigation</b>			
<ul style="list-style-type: none"> <li>• Students publish their experimental designs for peer feedback (<i>Mini Whiteboard</i>).</li> <li>• Students evaluate their experimental set-ups and those of their peers (<i>Gallery Walk</i>).</li> <li>• Students providing feedback to their peers (<i>Self &amp; Peer Evaluation</i>).</li> </ul>			
2	<ul style="list-style-type: none"> <li>• Students are given the opportunity to see the materials and apparatuses and design their own experimental set-ups in the investigation.</li> <li>• Students share their experimental designs on <i>mini whiteboards</i>.</li> <li>• Students evaluate their experimental set-ups and those of their peers in a <i>gallery walk</i> activity.</li> <li>• The teacher provides feedback on students' experimental designs.</li> <li>• The teacher introduces the main investigation scenario and instructs students to design their set-ups.</li> </ul>	40	<i>Worksheet 2, Student Samples 1, Worksheet 3</i>
3	<ul style="list-style-type: none"> <li>• Students evaluate their experimental set-ups and those of their peers.</li> <li>• The teacher provides feedback on students' experimental designs.</li> </ul>	40	Teacher Notes 1, Student Samples 2, <i>Worksheet 4</i>
<b>Stage 3 Carrying out the investigation</b>			
<ul style="list-style-type: none"> <li>• Students collect more complex data sets by setting up replicates (<b>Complex Data Set</b>).</li> <li>• Students use cameras to record data (<i>Digital Tool</i>).</li> </ul>			
4	<ul style="list-style-type: none"> <li>• The teacher provides students with the laboratory manual.</li> <li>• Teacher asks questions to help students connect their lab experience and related ideas/scientific inquiry skills</li> <li>• Students carry out the investigation.</li> </ul>	40	Laboratory Manual

**Stage 4 Explaining and evaluating data**

- Students propose explanations to account for their data based on additional information about the brine shrimps (**Explanation Construction Task**).
- Students reflect on their learning experiences using the reflection templates (*Reflection Cards*).

Before Lesson 5	<ul style="list-style-type: none"><li>• Students complete data reporting and analysis at home.</li><li>• The teacher collects and marks student responses.</li></ul>		Teacher Notes 2
5	<ul style="list-style-type: none"><li>• The teacher provides feedback on students' performance related to data reporting and analysis.</li><li>• Students reflect on their learning and share their reflections.</li></ul>	40	Teacher Notes 2, <i>Worksheet 5</i> , Student Samples 3

**Important Notes**

- This investigation involves the use of live organisms. Students should handle brine shrimps with care and respect their lives.

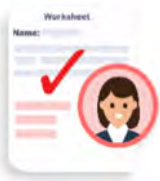


# Instructional Materials

## Stage 1 Preparing for the investigation

### Student Worksheet 1

#### Notes for teachers







- Teachers distribute *Worksheet 1* and invite students to observe adult brine shrimps and brine shrimp larvae.
- The *Supplementary Resource* section contains the list of materials.
- Teachers use the *See-Think-Wonder* thinking routine to encourage students to make careful observations and propose scientific questions.
- More information about thinking routines can be found on the website: <https://pz.harvard.edu/thinking-routines>
- Teachers may project the microscope image of brine shrimp larvae. Scan the QR code below to see an example.
- Student questions may be posted on the *Driving Question Board*.
- After collecting student questions, teachers can distribute the reading material about brine shrimps and focus students on the inquiry question, ‘Do brine shrimps prefer to live in the light or the dark?’



#### Task 1

- Examine the brine shrimps given. Answer the following questions:
  - What did you notice about the brine shrimps?
  - What do you want to know more about the brine shrimps?

 *Brine shrimps are very delicate animals, and you must take care not to harm them when you handle them.*

See 	Think 	Wonder 
<div data-bbox="229 1648 775 1809" style="border: 1px solid black; background-color: #ffffcc; padding: 10px; margin: 10px auto; width: fit-content;"> <p>“<i>See-Think-Wonder</i>” is a thinking routine that encourages careful observation and thoughtful interpretation. It helps stimulate curiosity and sets the stage for inquiry.</p> </div>		

## Information about Brine Shrimps

### Directions

Read background information about brine shrimps.

Brine shrimps (*Artemia*) are crustaceans and relatives of crayfish, lobsters, and hermit crabs. They are often referred to as ‘sea monkeys’ and are found worldwide in salt lakes. The salinity of salt lake water can exceed 280 g salt/L, whereas that of sea water is 35 g salt/L.

Brine shrimps begin their lives as tiny larva after hatching from tiny cysts. Young shrimp larvae are called *nauplii* (nor-plee-ee). In approximately 4–6 weeks, the shrimps reach their adult size of approximately 1 cm in length. See *Figure 1*.

Brine shrimps have a head, middle (thorax), and tail (abdomen). On the front of the head are two little black eyes. There are also two small antennas that stick out forward. These are sensory structures for feeling the environment ahead. Brine shrimps usually move about on their backs, upside down with their leafy legs uppermost. The 11 pairs of leafy legs are used for swimming along in the water and as gills.

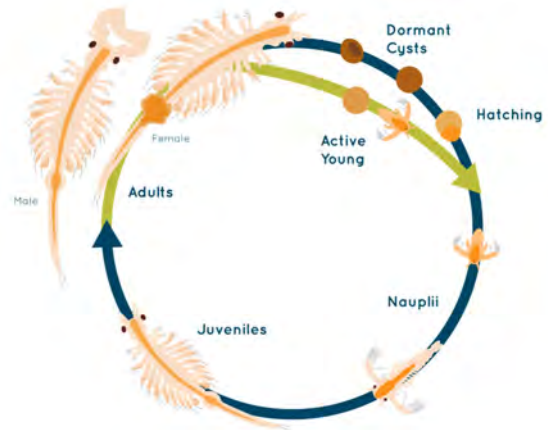


Figure 1

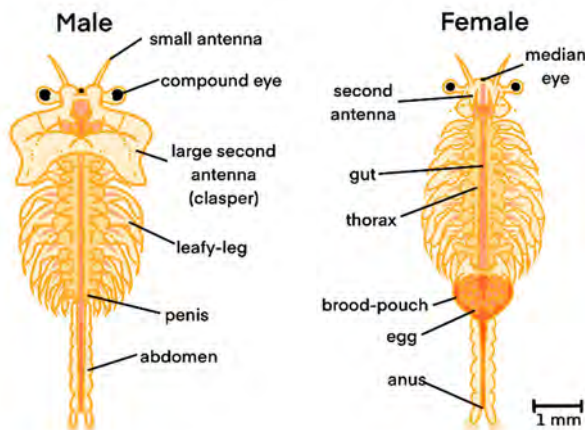


Figure 2

In males, the second antenna develops into large, hooked claspers. Males have a translucent body and are sometimes greenish-blue in colour.

The females are brown/red in colour and have a bundle of eggs in a brood-pouch halfway along their bodies.

See *Figure 2* for diagrams of brine shrimps.

Modified from Dockery and Tomkins (2000); Tomkins (2000).

### References


Dockery, M., & Tomkins, S. (2000). *Brine Shrimp Ecology*. British Ecological Society.




Tomkins, S. (2000). A review of the use of the brine shrimp, *Artemia* spp, for teaching practical biology in schools and colleges. *Journal of Biological Education*, 34(3), 117–122.

Students are encouraged to find answers from the reading materials to the questions they have posed.

**任務 1**

- 仔細觀察提供的豐年蝦，回答以下問題：
  - 根據你的觀察，豐年蝦有什麼特點？
  - 你想了解豐年蝦什麼方面的知識？

 豐年蝦非常纖細脆弱，觸摸時必須小心，以免傷害牠們。

See 	Think 	Wonder 

## 有關豐年蝦的資料

閱讀有關豐年蝦的背景資料：

豐年蝦 (*Artemia*) 是一種甲殼類動物，與龍蝦、螯蝦和寄居蟹有親戚關係。它們通常被稱為「海猴子」，在世界各地的鹽湖中都可以發現它們的身影。鹽湖的水鹽度可以超過每公升 280 克鹽，遠高於海水的鹽度(每公升 35 克鹽)。

豐年蝦的生命從微小的囊蟲孵化開始。年輕的豐年蝦幼蟲稱為卵仔 (*nauplii*)。約在 4–6 週的時間裡，豐年蝦長成約 1 厘米大小的成年個體。請參見圖 1。

豐年蝦的身體分為頭部、中部(胸部)和尾部(腹部)。在頭部的前方有兩隻小黑眼睛和兩個向前伸出的小觸角，這些結構有感應環境的功能。豐年蝦通常是背朝上游泳，腳上有葉狀結構，同時也充當著鰓的功能。這十一對葉狀結構用於在水中游泳。

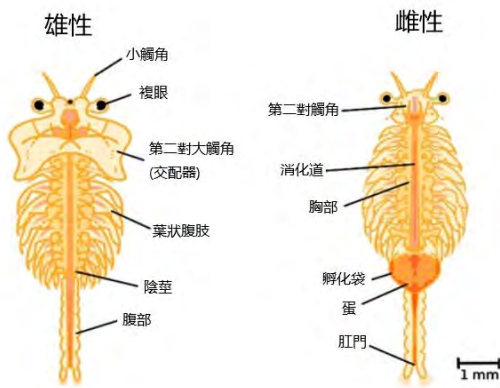


圖 2

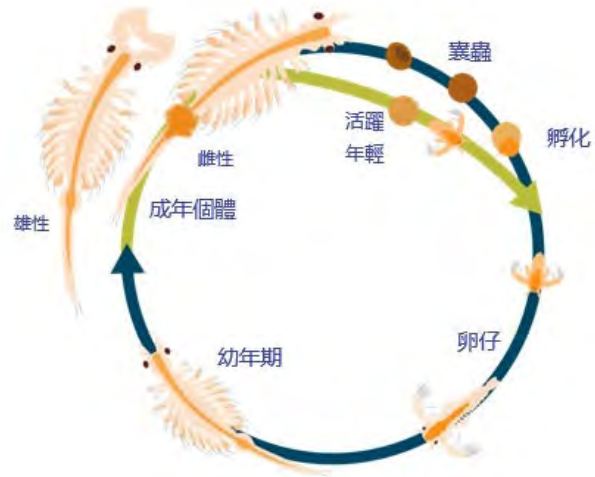


圖 1

雄性豐年蝦的第二對觸角會發展成大而彎曲的交配器。雄性身體半透明，有時會呈現綠藍色。雌性是棕紅色的，身體中部有一束蛋在孵化袋中。請參見圖 2 以了解更多有關豐年蝦的詳細信息。

## Student Worksheet 2



### Notes for teachers

- Teachers distribute *Worksheet 2* and instruct students to design the experimental set-ups.
- Teachers can show students the materials and apparatuses to facilitate their design.
- Teachers can ask students to share their set-ups in small groups and instruct them to draw their experimental designs on *mini whiteboards*.
- Teachers can facilitate a *gallery walk* activity to give students an opportunity to evaluate the set-ups designed by their peers using the strategy *Two Stars and a Wish*.
- Some student work samples are shown below to illustrate possible student thinking.

### Task 2(a)

- Imagine that you are working in a shop that sells brine shrimps. You would like to know more about the behaviours of brine shrimps. In particular, you wondered about the following question:

*Do brine shrimps prefer to live in the light or in the dark?*

- You found the following materials in the laboratory:

<ul style="list-style-type: none"> <li>• 1 beaker of adult brine shrimps</li> <li>• Containers of various shapes (petri dish, measuring cylinder, and water tank)</li> <li>• Salt water</li> <li>• Aluminium foil</li> </ul>	<ul style="list-style-type: none"> <li>• Timer</li> <li>• Camera (mobile phone)</li> <li>• Light source</li> <li>• Dropper for transferring brine shrimps</li> <li>• Any other equipment you need (please specify)</li> </ul>
--	---

- You may want to think about the following questions when designing your experiment:
  - What factor will you change?
  - What factor will you measure?
  - How will you collect the data?
  - How will you reduce the measurement errors?
  - What factors must be controlled?
- What is your experimental design? You may want to use a diagram to show your idea.

### **Task 2(b)**

- Share your ideas with the person next to you.
- Draw your group's experimental set-up on the mini whiteboard.  
(*Note: Please annotate your group's diagram to highlight any important design decisions.*)

Students are allowed express their ideas either through drawings or in written words.


### Mini Whiteboard Template

**Investigation question:**

- *Do brine shrimps prefer to live in the light or the dark?*

**Our experimental set-up:**

*Designed by:*



Students display their drawings, making their thinking visible.



Scan the QR code to get a copy of the *Mini Whiteboard*.



**任務 2(a)**

- 試想像你是一家豐年蝦售貨店的店員。你想了解更多豐年蝦的行為習性，尤其是以下問題：

豐年蝦喜歡生活在光亮還是黑暗的環境裏呢？

- 你在實驗室找到了以下材料：

<ul style="list-style-type: none"> <li>• 1 個燒杯的豐年蝦</li> <li>• 各種形狀的容器(培養皿、量筒、水箱)</li> <li>• 鹽水</li> <li>• 鋁箔</li> </ul>	<ul style="list-style-type: none"> <li>• 計時器</li> <li>• 相機(手機)</li> <li>• 光源</li> <li>• 用於轉移豐年蝦的滴管</li> <li>• 其他你需要的設備(請具體說明)</li> </ul>
---	--

- 在設計實驗時，你需要考慮以下問題：
  - 你需要改變哪個因素？
  - 量度哪個因素？
  - 你應該如何收集數據？
  - 你應該如何減少測量誤差？
  - 你需要控制哪些因素？
- 你會如何設置實驗裝置？試以手繪圖表達。

**任務 2(b)**

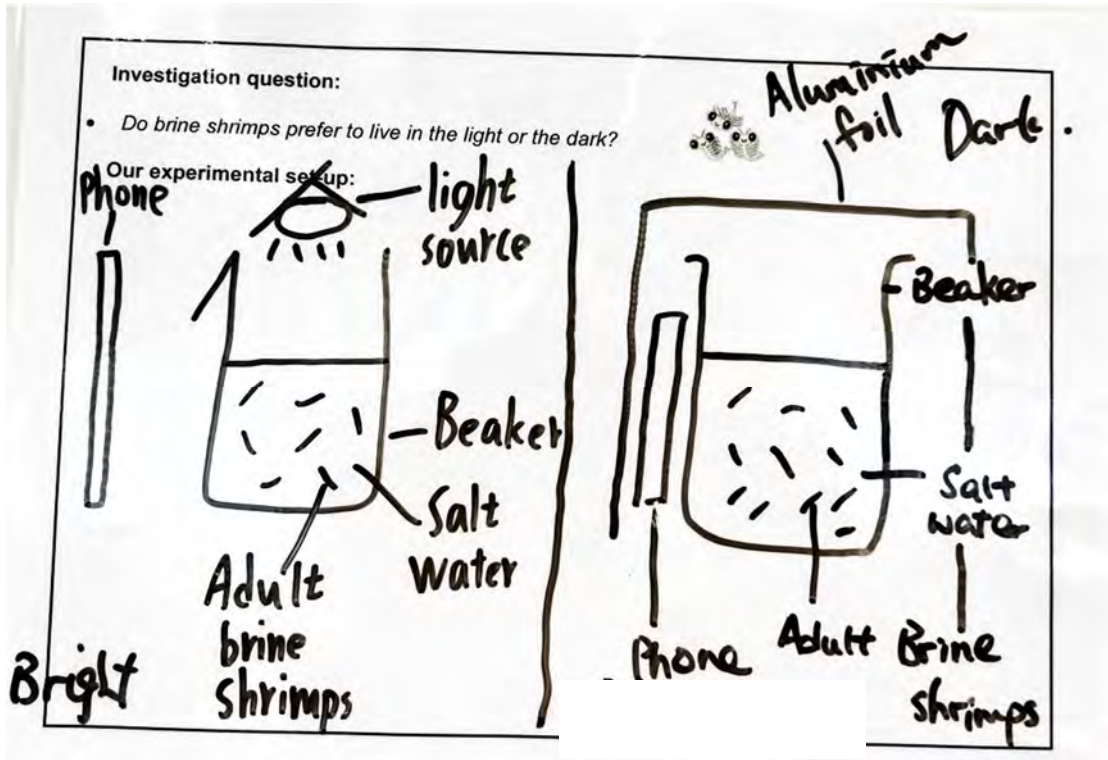
- 與旁邊的組員分享你的想法。
- 在迷你白板上畫出你們小組的實驗設置。  
(在你們小組的手繪圖上添加註釋，標明設計概念。)



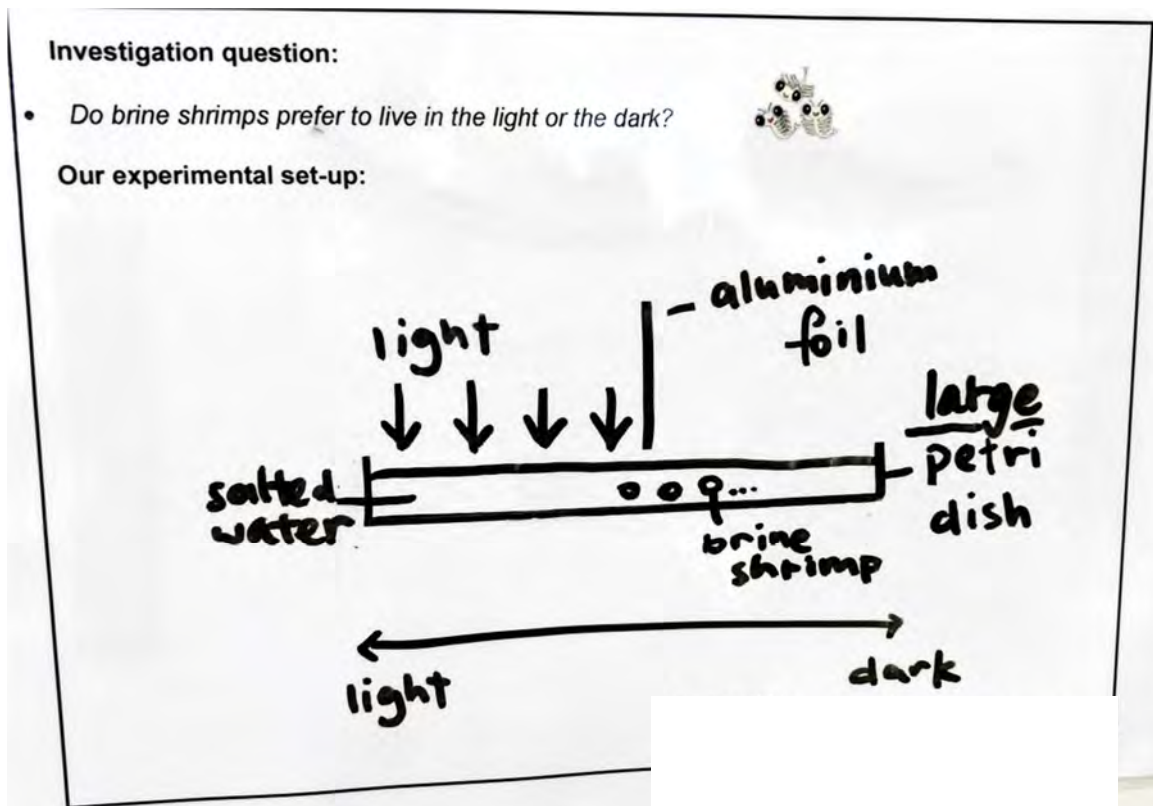
掃描二維碼以獲取迷你白板的副本。



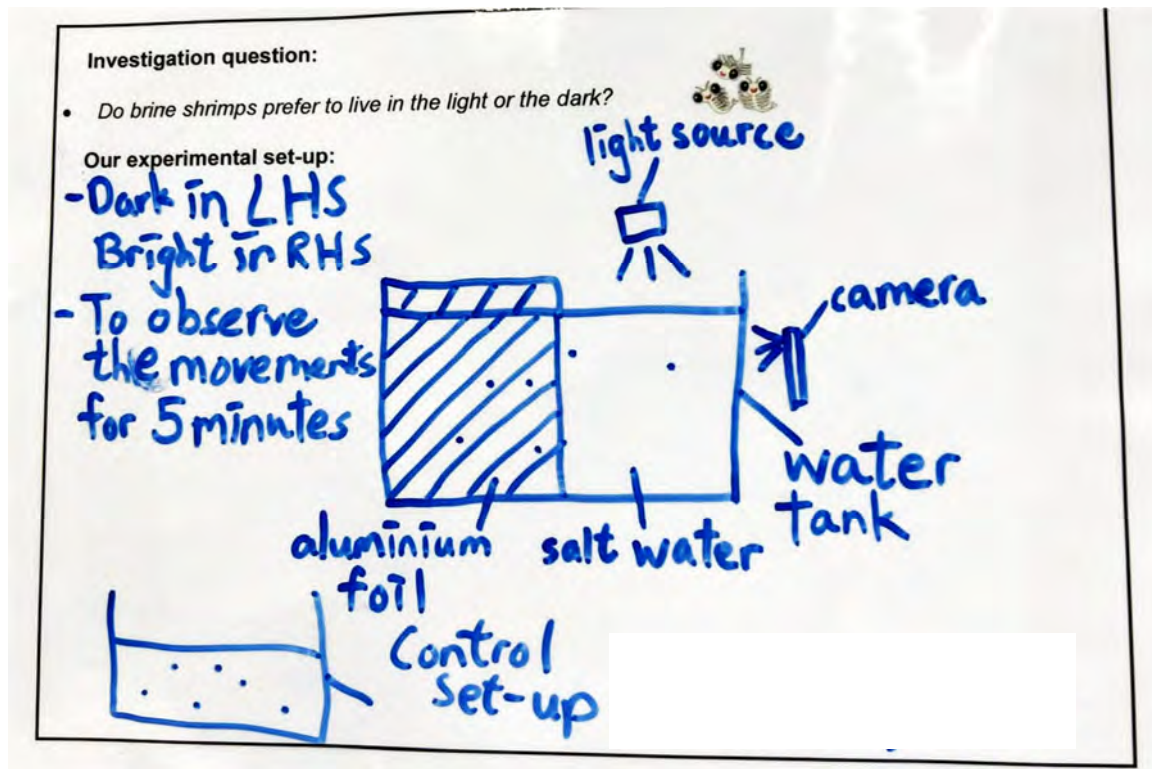
Sample 1



Sample 2

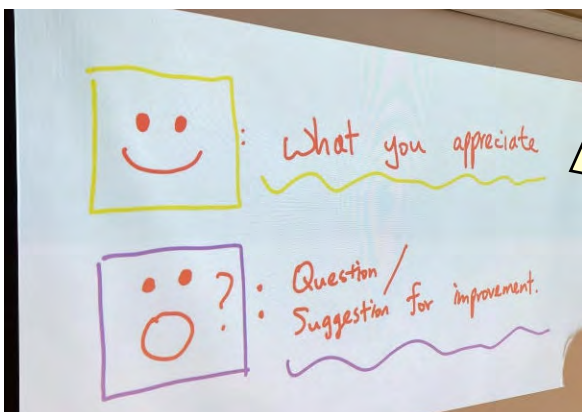


### Sample 3



#### Notes for teachers

- Teachers can distribute *Worksheet 3* and do a *gallery walk* activity for students to evaluate their peers' set-ups.
- Some set-ups on the worksheet are similar to the typical student samples. For example, it is common for students to propose using two containers, one for a light condition and one for a dark condition (Sample 1, Design A on *Worksheet 3*).
- Students may also propose a set-up with light intensity as a continuous independent variable (Sample 2).
- Students may also propose control set-up (Sample 3, Design B on *Worksheet 3*).
- Teachers can press students for their reasoning for why the set-up they choose can produce the most accurate and reliable data.

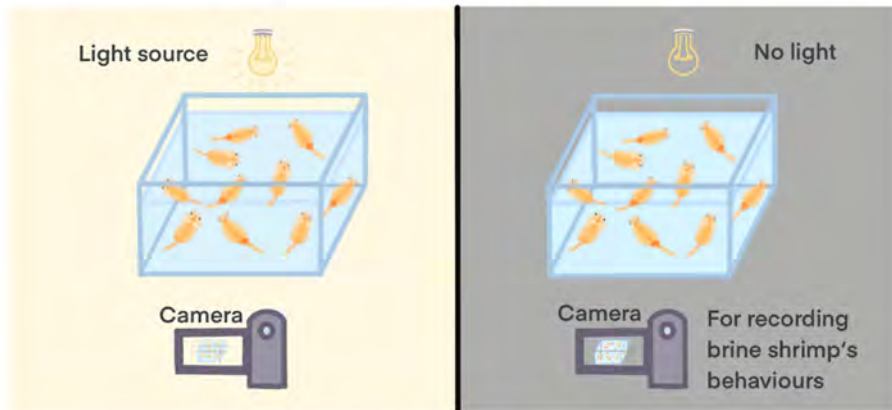


- Teachers can ask students to write comments on individual *Post-it* notes, which they can then post to share feedback on their classmates' experimental designs. Teachers may use the feedback strategy "Two Stars and a Wish" and ask students to provide two positive comments (the "stars") about each experimental design, along with one constructive suggestion for improvement (the "wish").

**Task 3**

- Examine the following experimental designs.

**Design A**



**Design B**



- Which set-up do you think can produce data that can answer the investigation question? Why do you think so?

We think that

- |  |   |
|--|---|
| <input type="checkbox"/> Design A          | <input type="checkbox"/> Design B                 |
| <input type="checkbox"/> My group's design | <input type="checkbox"/> My peers' design (_____) |

can produce data that can answer the investigation question

because



**Notes for teachers**



- The following shows the main investigation context for students to work on.
- Some questions may be used by teachers to guide students in thinking about or assessing the scientific inquiry skills related to experimental designs.
- Student work samples are shown below to illustrate possible student thinking.
- Scan the QR code to get a copy of the *Google Form*.



Teachers can decide whether to give feedback to students and what type of feedback to provide based on their responses in the *Google Form*.

**Task 4**

**Scenario**

You would like to find out whether brine shrimp larvae would prefer to live in different light colours (i.e., red, green, yellow, and blue light). You find the following materials in the school laboratory for your investigation:

Brine shrimp	Camera	Timer
Light source (red, green, yellow, and blue)	Petri dish	Salt water
Plastic dropper (for transferring the brine shrimp larvae)	Aluminium foil	Measuring cylinder (for measuring salt water)

(a) Complete the following table to show your design:

<b>Independent variable (X)</b> (What is X? How to change and manipulate X?)	<b>Dependent variables (Y)</b> (What is Y? How to measure Y?)	<b>Controlled variables</b> (Anything else that likely affects Y?)
<b>Sample</b> (How many individuals?)	<b>Controls</b> (Do you need control? Why?)	<b>Errors</b> (How will you reduce errors?)

(b) Draw your experimental design and annotate your diagram:

**任務 4**

**情境**

你想知道豐年蝦是否更喜歡生活在不同顏色的光(即紅色、綠色、黃色和藍色光)裏。你在學校實驗室裏找到了以下材料來進行研究:


豐年蝦幼蟲	相機	計時器
光源 (紅色、綠色、黃色、藍色)	培養皿	鹽水
塑膠滴管 (用於轉移豐年蝦)	鋁箔	量筒(用於量度鹽水體積)

(a) 完成以下表格，表達你的設計:

自變量 (X) (什麼是 X? 如何改變和處理 X? )	因變量 (Y) (什麼是 Y? 如何量度 Y? )	控制變量 (有沒有其他因素可能影響 Y 的因素?)
樣本 (需要多少數量?)	對照裝置/組 (你是否需要對照組? 為什麼?)	誤差 (你將如何減低誤差?)

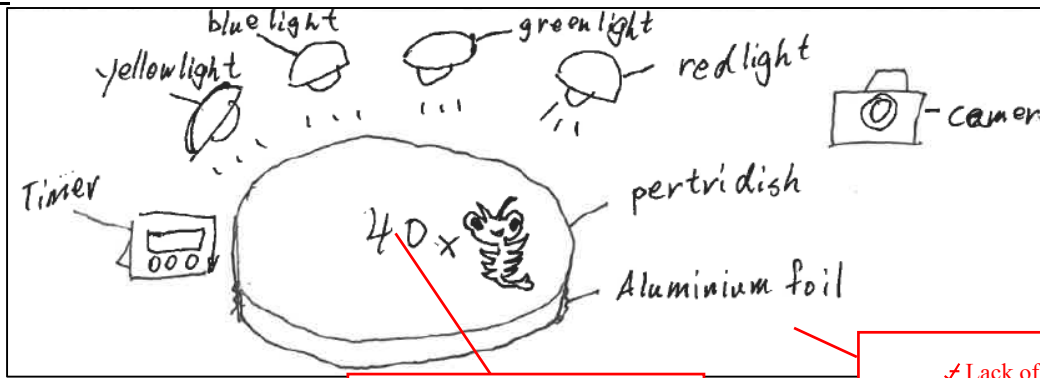
(b) 畫出你的實驗裝置並標註手繪圖:

掃描二維碼以獲取  
*Google Form* 的副本



## Student Samples 2 (Task 4)

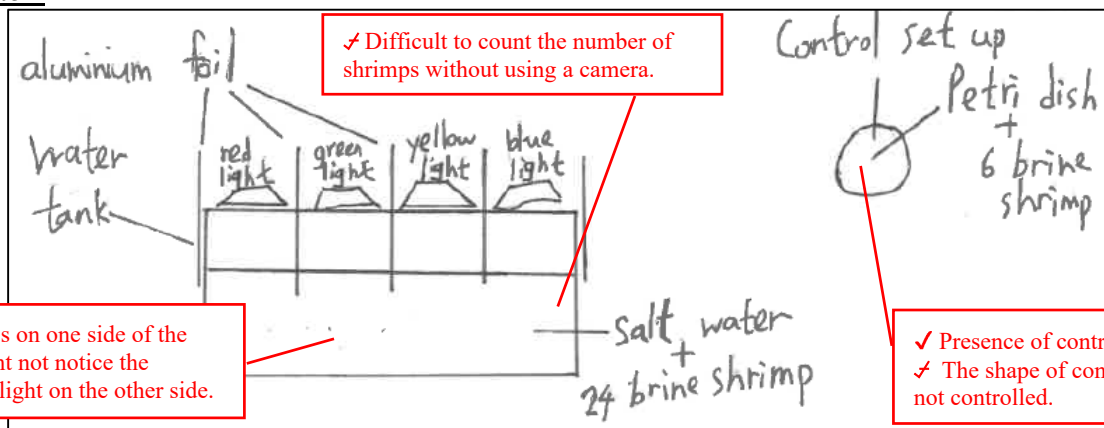
### Sample 1



✓ Large sample size.

✗ Lack of controls.

### Sample 2

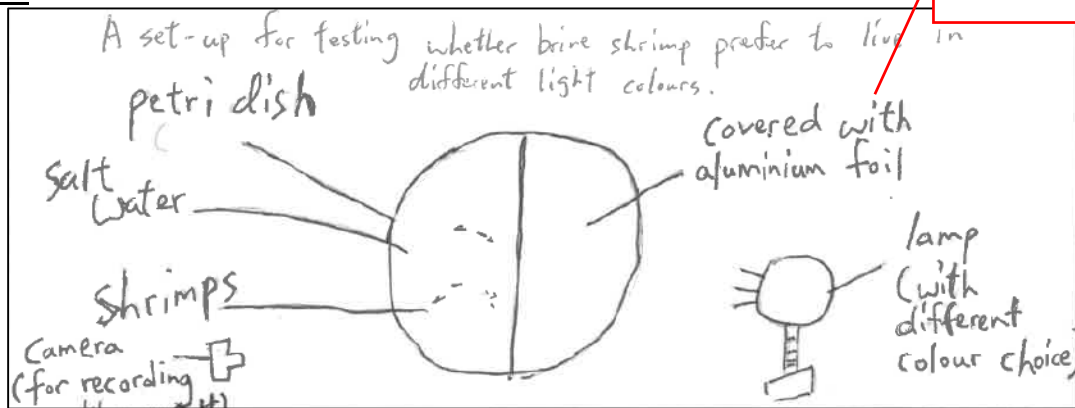


✗ Difficult to count the number of shrimps without using a camera.

✗ Shrimps on one side of the tank might not notice the coloured light on the other side.

✓ Presence of control setup  
✗ The shape of container is not controlled.

### Sample 3



✗ Lack of controls

#### Notes for teachers

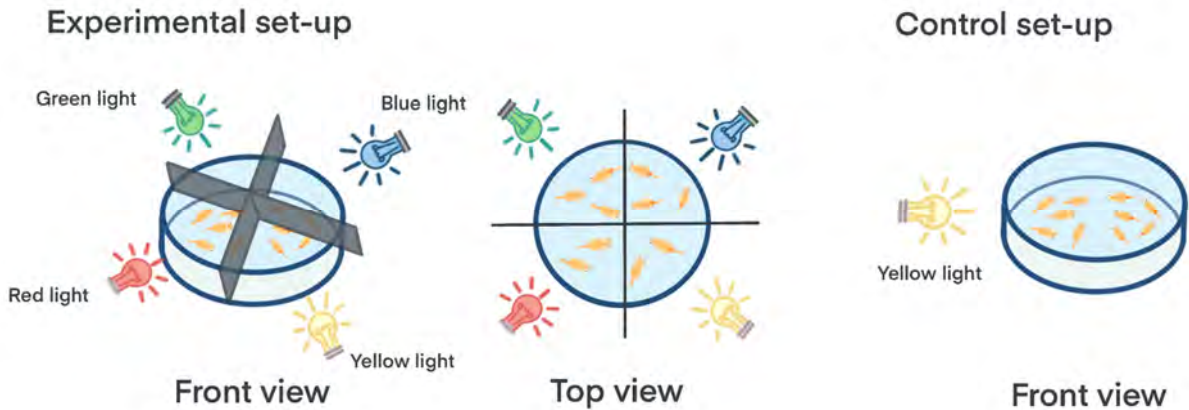


- Teachers can distribute *Worksheet 4* and ask students to evaluate their peers' set-ups and the set-ups on *Worksheet 4*.
- Some set-ups on the worksheet are similar to the typical student samples. For example, it is typical for students to propose Design A (Sample 1).
- Teachers can focus on several scientific inquiry skills such as how to sample the brine shrimps, the concept of controls, and alternative designs.
- Teachers can press students for their reasoning for why the set-up they choose can produce the most accurate and reliable data.

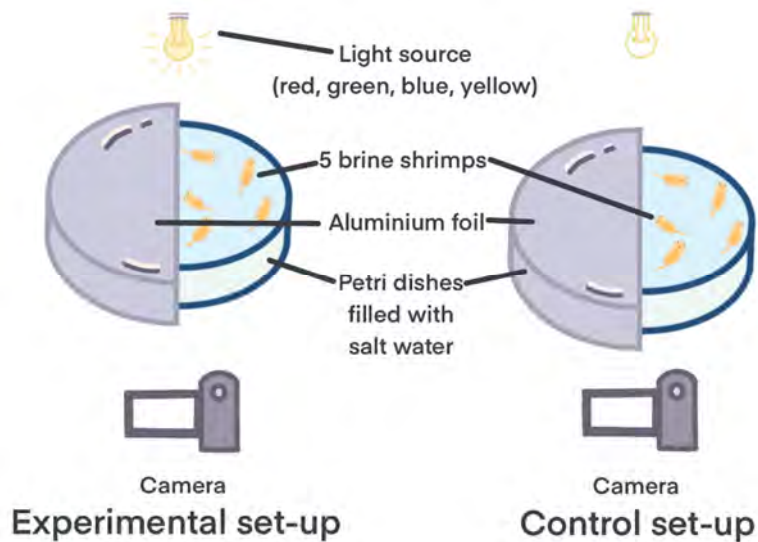
**Task 5**

- Examine the following experimental designs.

**Design A**



**Design B**



- (a) Which one of the following set-ups can generate data that are more accurate and reliable? Why do you think so?

We think that

- Design A                       Design B
- Our group's design

can produce data that are more accurate and reliable *because*

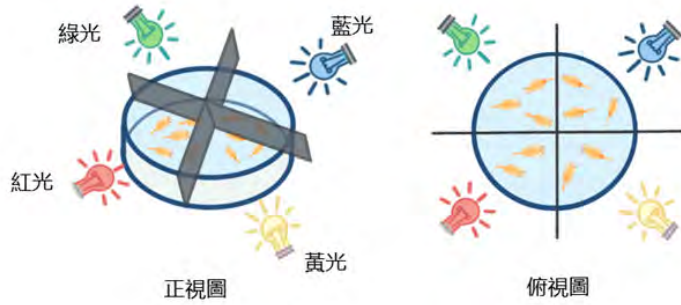
- (b) What else can be improved in the set-up in (a) to generate data that are more accurate and reliable? Why do you think so?

**任務 5**

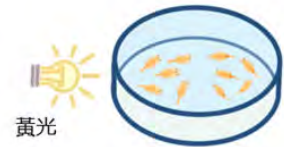
- 仔細研究以下實驗設計：

設計 A

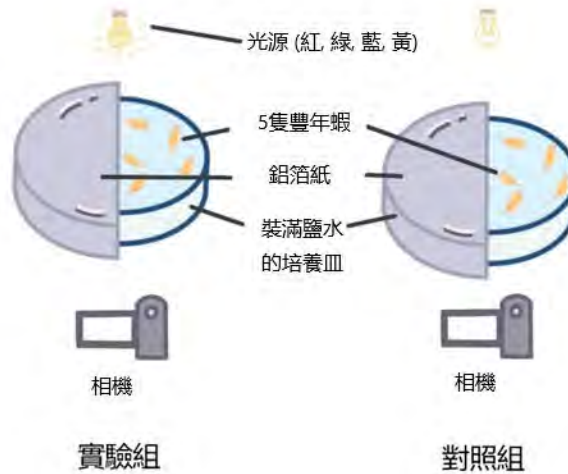
實驗組



對照組



設計 B



- (a) 哪個實驗設置的數據更精確、可靠？為什麼？

我們認為

設計 A  設計 B

我們小組的設計

數據更精確、可靠，因為

- (b) 你認為設置(a)還可以如何改進，令數據更精確、可靠？為什麼？



**Notes for teachers**

- Teachers can distribute the manual for students to read and prepare before the investigation.
- Each group can be assigned to investigate the effect of two light colours. Data can be shared among the class.
- Teachers can ask questions to check if students fully understand the procedures.
- The *Supplementary Resource* section contains the list of materials.

Examples of questions include: How will you position the petri dishes, and why? How will you ensure that a similar number of brine shrimp are used in each petri dish?

**Task 6**

**Procedure**

1. Use a plastic dropper to transfer 3 mL of brine shrimp larvae into 3 petri dishes.
2. Gently swirl the petri dish to evenly distribute the brine shrimp larvae.
3. Cover each petri dish with a lid (half black in colour).
4. Position the table lamp 2 cm above the lid of the petri dishes.
5. Switch on the light (red/green colour).
6. Cover the set-up with a black cloth/plastic bag.
7. Wait for 5 minutes without disturbing the petri dishes.
8. Remove the black cloth/plastic bag. Be careful not to disturb the petri dishes.
9. Switch on the white light, and immediately record the distribution of the brine shrimp larvae.
10. Repeat *Steps 2–9* with blue/yellow light, white light (positive control), and without light (negative control).

*Brine shrimps are very delicate animals, and you must take care not to harm them when you handle them.*

**Results**

Light colour	Distribution of brine shrimp larvae			Preference for this light colour (Strong attraction, weak attraction, no attraction)
	Petri dish 1	Petri dish 2	Petri dish 3	
1	With light  No light	With light  No light	With light  No light	
2	With light  No light	With light  No light	With light  No light	
<b>Control</b>	<b>Distribution of brine shrimp larvae</b>			<b>Function of this control set-up</b>
3 Positive (white light)	With light  No light	With light  No light	With light  No light	
4 Negative (no light)	No light  No light	No light  No light	No light  No light	

### Notes for teachers



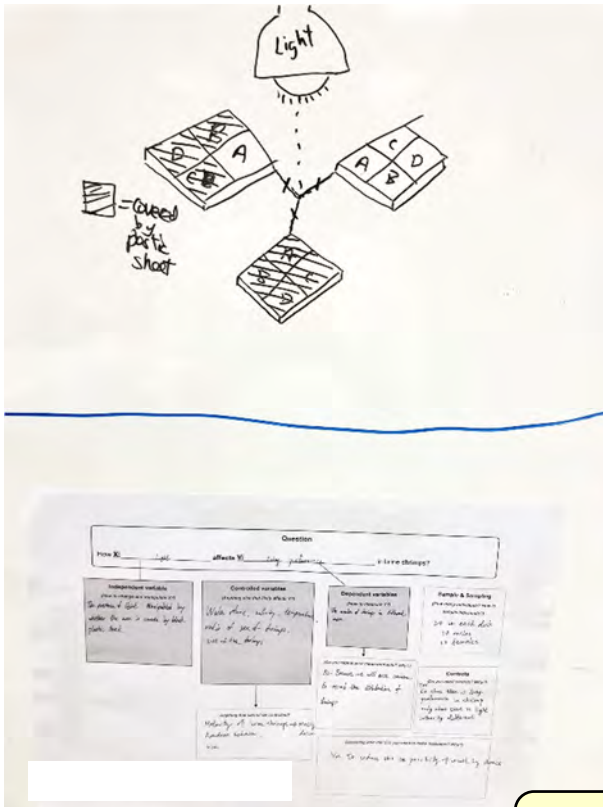
- Brine shrimp larvae are clearly phototaxis. However, teachers may use adult brine shrimps and ask students to use their data as evidence to support their claims about the preference of light/light colours of adult brine shrimps.
- Students may be allowed to design their own set-ups without following a manual. They can be provided with a *Reference Manual* as a basis for creating their own procedures.
- Students can be asked to display their experimental design, data collected, and claims on the *Inquiry Display Board*.



Scan the QR code to view the process of the experiment.



The petri dishes can be painted black by using spray paint.



	(brightest)			(darkest)
	A	B	C	D
Exp.	3, 8, 8	4, 3, 3	1, 2, 4	6, 8, 5
	6, 3, 3	3, 3, 3	7	6
+ve control	4, 7, 3	5, 2, 7	8, 5, 4	2, 6, 6
	4, 6, 7	4, 6, 7	5, 6, 7	4, 6, 7
-ve control	3, 7, 4	6, 3, 3	4, 4, 5	7, 6, 8
	4, 6, 7	4	4, 3, 3	7

- Students display their drawings, experimental designs, and data on the *Inquiry Display Board*.

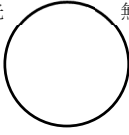
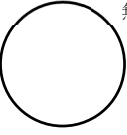
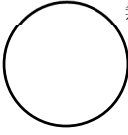
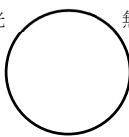
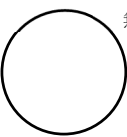
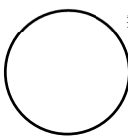
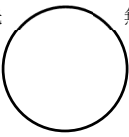
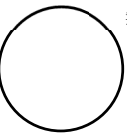
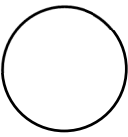
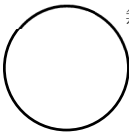
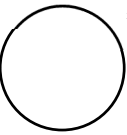
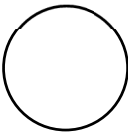
**任務 6**

- 閱讀以下實驗步驟以進行探究:

**實驗步驟**

1. 使用塑膠滴管將 3 mL 豐年蝦幼蟲轉移到 3 個培養皿中。
2. 輕輕搖晃培養皿，令豐年蝦幼蟲均勻分佈。
3. 用蓋子蓋住每個培養皿(一半蓋子塗黑)。
4. 將桌燈放在離培養皿蓋子 15 厘米的位置。
5. 打開燈(紅色/綠色)。
6. 用黑色布/塑袋覆蓋實驗裝置。
7. 不要打亂培養皿的內容物，等待五分鐘。
8. 取下黑色布/塑袋。小心不要打亂培養皿。
9. 打開白光，立即記錄豐年蝦幼蟲的分佈情況。
10. 重複步驟 2–9，用藍光/黃光，白光(陽性對照)和沒有光(陰性對照)代替。

**實驗結果:**

光線顏色	豐年蝦幼蟲分佈			對這種光線顏色的偏好 (吸引程度：強，弱，無)
	培養皿 1	培養皿 2	培養皿 3	
1	有光  無光	有光  無光	有光  無光	
2	有光  無光	有光  無光	有光  無光	
<b>對照</b>	<b>豐年蝦幼蟲分佈</b>			<b>這對照設置的作用</b>
3 陽性 (白光)	有光  無光	有光  無光	有光  無光	
4 陰性 (無光)	無光  無光	無光  無光	無光  無光	



**Notes for teachers**

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

**Task 7**

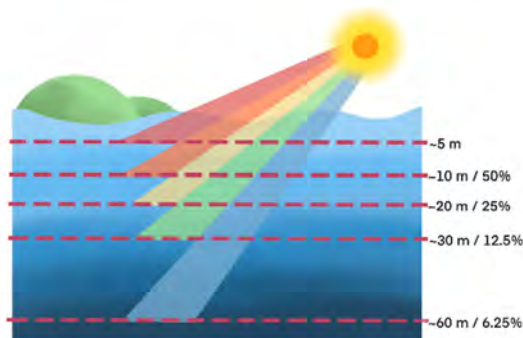
**Possible questions**

1. Complete the following table to show what deduction about the light colour preference of brine shrimps can be made by comparing the results of the following set-ups:

Set-ups	Deduction
1 versus 4	
2 versus 4	

2. You read the following information on a website:

Sunlight is a mixture of all colours of light. However, as sunlight travels through water, red and yellow light are absorbed, leaving only blue and green to be transmitted. In other words, blue light passes through water best, followed by green, yellow, and red lights. Red light is quickly filtered from water as the depth increases and effectively never reaches the deep ocean.



Based on the above information, propose a possible explanation for your experimental results.



**Notes for teachers**

- Q.1 assesses students' ability to make claims based on the data by logical deduction.
- Q.2 assesses students' ability to propose possible explanations based on given information and data.

The following shows some students' responses to Q.2:

**Sample 1**

From my experiment results, more shrimps are attracted to blue and white light. On the other hand, shrimps are less attracted in red light. In my opinion, the speed of light in the water is the reason why it differs.

**Sample 2:**

The attraction is stronger under blue light as blue light can go deeper in water.

**Sample 3:**

The attraction to certain light colours correlates with how deep different colours can pass through water. Brine shrimp larvae may have evolved to be more sensitive to blue light.



**About the samples**

- Sample 1 could not relate the experimental results with the information given. There is a lack of biological explanation.
- Sample 2 could relate the brine shrimp preference for blue light with greater transmittance of light in water but could not give a biological explanation.
- Sample 3 could relate the brine shrimp preference for blue light with greater transmittance of light in water and provide a biological explanation.

**任務 7**

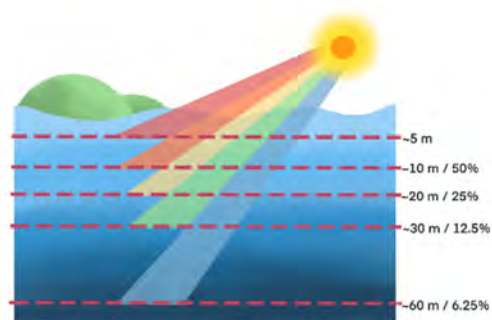
**參考問題**

1. 完成下表,以比較以下實驗裝置的結果,從而得出對豐年蝦幼蟲對不同顏色的光偏好的推斷:

裝置 1 和 4	
裝置 2 和 4	

2. 你在一個網站上讀到以下信息:

陽光是由全部不同顏色混合而成。但當陽光穿過水時，紅色和黃色光便會被吸收，只留下藍色和綠色光繼續傳播。換句話說，藍光在水中傳播得最好，綠光排第二，黃光排第三，最差是紅光。隨著深度的增加，紅光很快就從水中過濾掉，所以紅光實際上從未傳至深海。



根據上述信息，試為你的實驗結果提供一個合理的解釋。

**Notes for teachers**



- Teachers can distribute the mini whiteboards and invite students to reflect on their learning from the whole investigation.
- Teachers can ask students to read other reflections and compare their learning with others.
- Teachers may also ask students to elaborate on their reflections.


**Task 8**

- Reflect on your learning from the *Brine Shrimp Investigation*. What is your most important/impressive learning? Write down your thoughts on the mini whiteboards.

Reflection Card Template

My most important/impressive learning from the

***Brine Shrimp Investigation:***



Students share their reflections with their peers.

Name: \_\_\_\_\_ Class: (   )



Scan the QR code to get a copy of the *Mini Whiteboard*.



學生工作紙 (五)

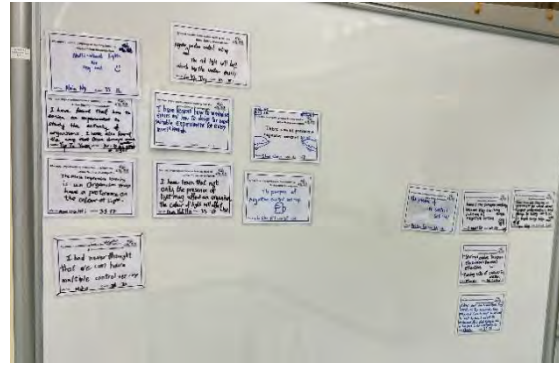
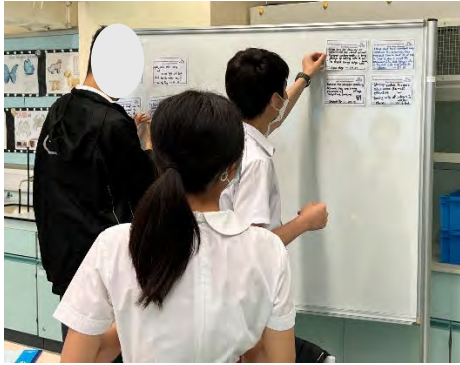
**任務 8**

- 反思你從 *豐年蝦探究* 中的學習。你最重要/最深刻的學習是什麼?將你的想法寫在迷你白板上。



掃描二維碼以獲取 *迷你白板* 的副本。





My most important/impressive learning from the *Brine Shrimp Investigation*:

handle the samepts ~~carefully~~ because they are living things.

positive U.S. negative control

My most important/impressive learning from the *Brine Shrimp Investigation*

There can be positive + negative control set up

Name: \_\_\_\_\_ Class: \_\_\_\_\_

My most important/impressive learning from the *Brine Shrimp Investigation*

I have learn that not: only the presence of light may affect an organism, the colour of light will affect also.

Name: \_\_\_\_\_ Class: \_\_\_\_\_

My most important/impressive learning from the *Brine Shrimp Investigation*

I had never thought that we can have multiple control set-up

Name: \_\_\_\_\_ Class: \_\_\_\_\_



**About the samples**

- As shown above, typically students not only identified the learning of scientific inquiry skills (e.g., concepts of controls) as important learning outcomes but they would also develop affective outcomes related to handling living organisms.



## Supplementary Resources

### Possible Modifications

#### 1. 'Plankton rainbow' demonstration

- The plankton rainbow demonstration can be set up using several glow sticks in multiple colours (blue, green, and red) and/or light sources with different colours.



- Details can be found in Exploratorium (2024).
- Scan the QR code for a video of the plankton rainbow demonstration.

### Technician Notes

#### 1. Materials for Task 1

- Adult brine shrimp in a petri dish
- Juvenile brine shrimp in a petri dish



#### 2. Materials for Task 2

- |  |  |
|--|--|
| <ul style="list-style-type: none"> <li>1 beaker of brine shrimps</li> <li>Containers of various shapes (petri dish, measuring cylinder, water tank)</li> <li>Salt water</li> <li>Aluminium foil</li> </ul> | <ul style="list-style-type: none"> <li>Timer</li> <li>Camera (mobile phone)</li> <li>Light source</li> <li>Plastic dropper for transferring brine shrimps</li> </ul> |
|--|--|

#### 3. Materials for Task 6

##### Materials for each group

<ul style="list-style-type: none"> <li>Petri dish (with half of the lid painted black) X 6</li> </ul>	<ul style="list-style-type: none"> <li>Plastic dropper</li> </ul>	<ul style="list-style-type: none"> <li>Black cloth/plastic bag</li> </ul>
<ul style="list-style-type: none"> <li>Brine shrimp larvae in a beaker</li> </ul>	<ul style="list-style-type: none"> <li>LED light bulb of different colours (Red, Green, Blue, White)</li> </ul>	<ul style="list-style-type: none"> <li>Timer</li> </ul>
<ul style="list-style-type: none"> <li>Ruler</li> </ul>	<ul style="list-style-type: none"> <li>Table lamp</li> </ul>	<ul style="list-style-type: none"> <li>Camera</li> </ul>

### References

- Dockery, M. & Tomkins, S. (2000). *Brine Shrimp Ecology*. British Ecological Society.
- Exploratorium. (2024). Plankton rainbow: Biology & perception science activity. <https://www.exploratorium.edu/snacks/plankton-rainbow>
- Millar, R., Lubben, F., Gott, R. & Duggan, S. (1994). Investigating in the school science laboratory: Conceptual and procedural knowledge and their influence on performance. *Research Papers in Education*, 9(2), 207–248.
- Tomkins, S. (2000). A review of the use of the brine shrimp, *Artemia* spp, for teaching practical biology in schools and colleges. *Journal of Biological Education*, 34(3), 117–122.



# Concluding Remarks

---



# Summary

## Design Principles & Implementation Strategies

- Several design principles and implementation strategies for investigative practical work can be distilled from the nine sets of investigative practical work in this educative curriculum material package.
- In terms of teaching goals,
  - avoid verification-based practical work that simply illustrates scientific content or concepts.
  - purposefully embed opportunities for students to learn different scientific inquiry skills and participate in different forms of investigative practical work throughout secondary school.
- In terms of design and implementation of effective investigative practical work,

Stage	Design principles	Implementation strategies	
<b>1</b> Preparing for the investigation	<ul style="list-style-type: none"> <li>▪ Situate the investigations in meaningful scenarios/contexts relevant to students (<b>Contextualisation</b>)</li> <li>▪ Provide sufficient background information for students to comprehend the context of the investigation</li> <li>▪ Assess students' background knowledge related to the investigation</li> <li>▪ Allow students to raise questions about the investigation or the context</li> </ul>	<ul style="list-style-type: none"> <li>• Reading Materials</li> <li>• Diagnostic Assessment</li> <li>• <i>See-Think-Wonder</i> Chart</li> <li>• Driving Question Board</li> </ul>	Embedding scaffolds and structures for students to express their thinking Orchestrating meaningful dialogues around important scientific thinking Providing substantive feedback to advance student thinking
<b>2</b> Planning and designing the investigation	<ul style="list-style-type: none"> <li>▪ Allow students to design their own set-ups</li> <li>▪ Show students the materials and apparatuses to facilitate their design</li> <li>▪ Allow students to trial run their designs and set-ups</li> <li>▪ Engage students to evaluate and revise their own and others' set-ups/designs</li> </ul>	<ul style="list-style-type: none"> <li>• Investigation Planning Template</li> <li>• Annotated Diagrams</li> <li>• Virtual Laboratory</li> <li>• Mini Trial Run</li> <li>• Mini Whiteboard</li> <li>• Gallery Walk</li> <li>• Self &amp; Peer Evaluation</li> </ul>	
<b>3</b> Carrying out the investigation	<ul style="list-style-type: none"> <li>▪ Remove experimental procedures that require significant procedural demands</li> <li>▪ Allow students to modify procedures in the reference manual</li> <li>▪ Make use of microscale instrumentation (<b>Microscale Instrumentation</b>)</li> <li>▪ Make observations vivid and interesting (e.g., colourful)</li> <li>▪ Engage students in collecting a large set of data (e.g., repeating their measurements, setting up replicates) (<b>Complex Data Set</b>)</li> <li>▪ Perform a demonstration of difficult procedures</li> <li>▪ Provide support for data collection (e.g., data collection sheet, guidance on procedures [e.g., video])</li> <li>▪ Allow students to use digital tools to collect and record data</li> </ul>	<ul style="list-style-type: none"> <li>• Reference Manual</li> <li>• Video with Guidance on Procedures</li> <li>• Teacher Demonstration</li> <li>• Integrated Instruction Sheet</li> <li>• Data Collection Sheet</li> <li>• Digital Tool (e.g., camera for recoding (time-lapse) videos/data)</li> </ul>	
<b>4</b> Analysing, interpreting, evaluating and explaining data	<ul style="list-style-type: none"> <li>▪ Allow students to use digital tools to visualise, represent and analyse data</li> <li>▪ Engage students in analysing and interpreting complex data sets</li> <li>▪ Allow students to compare their data sets with those of other groups</li> <li>▪ Involve students in assessing the quality of their data by critically evaluating their own and their peers' data (or class data)</li> <li>▪ Have students use data to make or evaluate claims and scientific explanations/make decisions/solve problems (<b>Explanation Construction/Decision-making/Problem-solving Task</b>)</li> <li>▪ Promote student reflection on the process of the investigation (e.g., learning from errors, improving the experimental designs)</li> </ul>	<ul style="list-style-type: none"> <li>• Digital Tool (e.g., <i>Google Sheet</i> for recording and manipulating data)</li> <li>• Data-sharing Web Platform</li> <li>• Inquiry Display Board</li> <li>• Reflection Card or Journal</li> </ul>	

- Teachers may find the following design guidelines useful for developing and modifying classroom learning/assessment tasks related to scientific investigations:

<b>Design guidelines</b>	<b>Description</b>
1. Contextualise the investigation task	<ul style="list-style-type: none"> <li>• Situating investigations in authentic and meaningful contexts that activate students' prior knowledge and resources and legitimise the investigations</li> </ul>
2. Avoid verification-based practical work	<ul style="list-style-type: none"> <li>• Engaging students in the meaningful use of data obtained from the investigation to solve problems, make decisions, and explain phenomena, rather than simply verifying scientific concepts they have learnt</li> </ul>
3. Include scientific investigation skills related to experimental design, data analysis, and interpretation as objectives of learning/assessment.	<ul style="list-style-type: none"> <li>• Including scientific investigation skills related to experimental design, data analysis, and interpretation as objectives of learning/assessment in addition to content knowledge</li> </ul>
4. Maintain a certain degree of openness in the investigation task	<ul style="list-style-type: none"> <li>• Providing opportunities for students to make decisions throughout the course of the scientific investigation (e.g., generating inquiry questions, designing their own experiments, modifying standard procedures, processing their data and etc)</li> </ul>
5. Reduce learning noise in experimental procedures	<ul style="list-style-type: none"> <li>• Removing or reducing experimental procedures that require significant procedural demands</li> <li>• Using microscale instrumentation to shorten the duration of the investigation</li> </ul>
6. Involve students in collecting and making sense of more complex data sets using digital tools (when applicable)	<ul style="list-style-type: none"> <li>• Allowing students to collect or work with a larger data set (e.g., by repeating their measurements, setting up replicates, or pooling class data)</li> <li>• Using digital tools to collect, process, and analyse data sets</li> </ul>
7. Embed scaffolds and structures	<ul style="list-style-type: none"> <li>• Embedding various types of scaffoldings and structures, including sensory (e.g., visual aids or graphic organisers), social, and linguistic scaffoldings (e.g., sentence frames or definitions of key words) <i>without</i> watering down the cognitive demands of the task</li> </ul>
8. Use multiple task and question components in assessments	<ul style="list-style-type: none"> <li>• Using multiple task components with at least one or more components for eliciting students' cognitive understandings</li> <li>• Using multiple questions and formats to elicit students' cognitive understandings related to experimental designs, data analysis, and interpretation</li> </ul>
9. Use scoring tools in assessments	<ul style="list-style-type: none"> <li>• Using scoring tools (e.g., rubrics, checklists, or scoring guides) that clearly delineate objectives of assessment and articulate students' levels and progressions in their thinking about experimental design, data analysis, and interpretation for a holistic scoring of student responses</li> </ul>

- The project team hopes that this educational curriculum material package will be a useful resource for teachers in enhancing the teaching and learning of biology through meaningful use of investigative practical work.



# Resources

## Resource Files

- Readers can download the resources of each investigative practical work. These electronic files are also available on the USB flash drives.

### Microscale Amylase Investigation



### Yeast Bead Invertase Investigation



### Yeast Bead Catalase Investigation



### Banana Ripening Investigation



### Lipase Inhibitor Investigation



### Photosynthesis Inhibitor Investigation



### Cat Grass Investigation



### Yeast Respirometer Investigation



### Brine Shrimp Investigation



## Feedback

- The project team welcomes feedback and comments on the educative material package. If you have any questions or feedback about the package, please fill out the *Google Form* (please scan the QR code). The project team will contact you.

