

EDVOTEK®

AP
BIOLOGY

BIG IDEA 2
INVESTIGATION

6

EDV0-Kit: AP06

Cellular Respiration

See Page 3 for storage instructions.

EXPERIMENT OBJECTIVE:

The objective of this experiment is to apply the gas laws to the function of the respirometer. Students will observe cell respiration of germinating and non-germinating seeds and describe the effects of temperature on the rate of cell respiration.

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All components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

THIS EXPERIMENT DOES NOT CONTAIN HUMAN DNA. None of the experiment components are derived from human sources.

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Experiment Components

- 1 ml pipets
- Glass beads
- Peas
- Potassium Hydroxide solution
- Cork stoppers
- Absorbent cotton
- Non-absorbent cotton
- Plastic vials
- Parafilm®

Store the entire experiment at room temperature.

This experiment is designed for 10 lab groups.

Requirements

- Thermometers
- Trays (at least 14" long)
- Silicon glue
- Ice
- Cork borer
- Tape
- Timers
- Metal washers

Background Information

All life on earth ultimately depends upon the sun for energy. Photosynthesis in plants traps energy from sun by formation of covalent bonds in complex organic compounds, such as glucose. Organisms release this stored energy by the breakdown of glucose, using a set of enzymatic reactions involving many steps. Breakdown of glucose can occur in the presence of oxygen (aerobically), or in the absence of oxygen (anaerobically). Energy released by the breakdown of glucose is stored in the high energy phosphate bonds of adenosine triphosphate, ATP. Aerobic respiration yields the most energy for organisms, with every mole of glucose producing about 36 - 40 moles of ATP. Under anaerobic conditions, only 2 moles of ATP are produced.

Cellular respiration is the breakdown of organic compounds, resulting in the release of energy. The oxidative breakdown of glucose during cellular respiration produces the energy needed for life in living organisms, as given in equation 1:



Carbon dioxide, CO_2 , a by-product of cellular respiration, is required for trapping the energy of the sun by photosynthesis. Photosynthesis results in the formation of glucose, oxygen, and water, as shown in equation 2:



The free energy content in the terminal phosphate bond of ATP is 7.3 kCal per mole of ATP. Assuming the synthesis of 38 moles of ATP per mole of glucose broken down during aerobic respiration, a total of 277.4 kCal of energy is stored in the high energy bonds of ATP. This represents about 40.4% of the total free energy released during the breakdown of one mole of glucose. The rest of the energy is lost as heat. Some of this is used to maintain a constant body temperature.

This experiment is designed to measure oxygen consumed by either germinating or non-germinating pea seeds. To measure consumed oxygen, the ideal gas law (equation 3) will be utilized:

$$3. \quad PV = nRT$$

- P = the pressure of the gas
- R = the gas constant (a fixed value)
- V = the volume of the gas
- T = the temperature of the gas
- n = the number of molecules of the gas

In equation 3, R is always constant. If the pressure of the system (P), and the temperature of the system (T) remain constant, the volume occupied by the gas is directly proportional to the number of molecules of the gas.

During this experiment, a respirometer will be used to measure the volume of oxygen, and therefore the number of molecules of oxygen, consumed by the pea seeds during cellular respiration. The CO_2 released through respiration (see equation 1) is removed from the system by potassium hydroxide (KOH), as shown in equation 4. Therefore, the amount of CO_2 gas released does not factor into the analysis and measurement of the amount of oxygen consumed.



Background Information



The number of oxygen molecules consumed during respiration by the pea seeds will be directly related to the decrease in volume occupied by gas within the respirometer. The water in the pipet will move toward the region of lower pressure, which is created within the respirometer due to oxygen consumption. This assumes constant volume and pressure of the system. The control vial containing the glass beads will be used to measure any change in water volume due to alterations of temperature and pressure. The data will be corrected to reflect these external influences on the respirometer.

Experiment Overview and General Instructions

EXPERIMENT OBJECTIVE:

The objective of this experiment is to apply the gas laws to the function of the respirometer. Students will observe cell respiration of germinating and non-germinating seeds and describe the effects of temperature on the rate of cell respiration.

LABORATORY SAFETY GUIDELINES

1. Wear gloves and goggles while working in the laboratory.
2. Exercise caution when working in the laboratory – you will be using equipment that can be dangerous if used incorrectly.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Always wash hands thoroughly with soap and water after working in the laboratory.
5. If you are unsure of something, ASK YOUR INSTRUCTOR!



LABORATORY NOTEBOOKS

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you will be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before Starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

MATERIALS FOR THE EXPERIMENT

Each student group should receive the following:

- 3 Cork/pipet assemblies
- 3 Vials
- Absorbent cotton
- KOH solution
- Non-absorbent cotton
- 100 ml graduated cylinder
- 25 non-germinated peas
- Small weights to hold vials in water
- Glass beads
- Tape
- Thermometer
- 25 germinated peas
- Tray
- Silicone glue
- Timer
- Parafilm®

Taking Experimental Measurements

1. Set up an ice bath at 10° C and a room temperature bath as assigned by the instructor. This should be done prior to beginning the other steps in order to allow enough time for the water baths to equilibrate to the required temperature. To attain and keep the 10° C temperature, add ice.
2. Label 3 vials (respirometers) A, B, and C.
 - Place a circle of absorbent cotton (approximately the size of a nickel) into the bottom of each vial.
 - Carefully soak with 1- 2 ml of 15% KOH solution, not allowing any KOH solution to touch the side walls of the vials.
 - Place a circle of nonabsorbent cotton into the vials directly on top of the KOH/cotton circle in the vial. This will keep the KOH solution from touching peas during the experiment.
3. Determine the volume of germinated peas.
 - Fill a 100 ml graduated cylinder with 50 ml of water.
 - Add 25 germinated peas to the cylinder and measure the increase in water volume. The difference represents the volume of the peas.
Pea volume = _____ ml.
 - Place the peas on a paper towel. These germinated peas will be used in Vial A.
4. Determine the volume of non-germinated peas.
 - Fill a 100 ml graduated cylinder with 50 ml water.
 - Add 25 non-germinating peas.
 - Add glass beads to raise the volume to equal that obtained with swollen germinated peas.
 - Remove the peas and beads. Place them on a paper towel. The non-germinated peas and beads will be used in respirometer B.
5. Determine the volume of glass beads.
 - Fill the 100 ml graduated cylinder with 50 ml water.
 - Add glass beads to raise the volume so it equals the volume of germinating peas as determined previously in Step 3.
 - Place glass beads on a paper towel, to be used in Vial C.

Be sure that the amounts of KOH and cotton are the same for each vial.

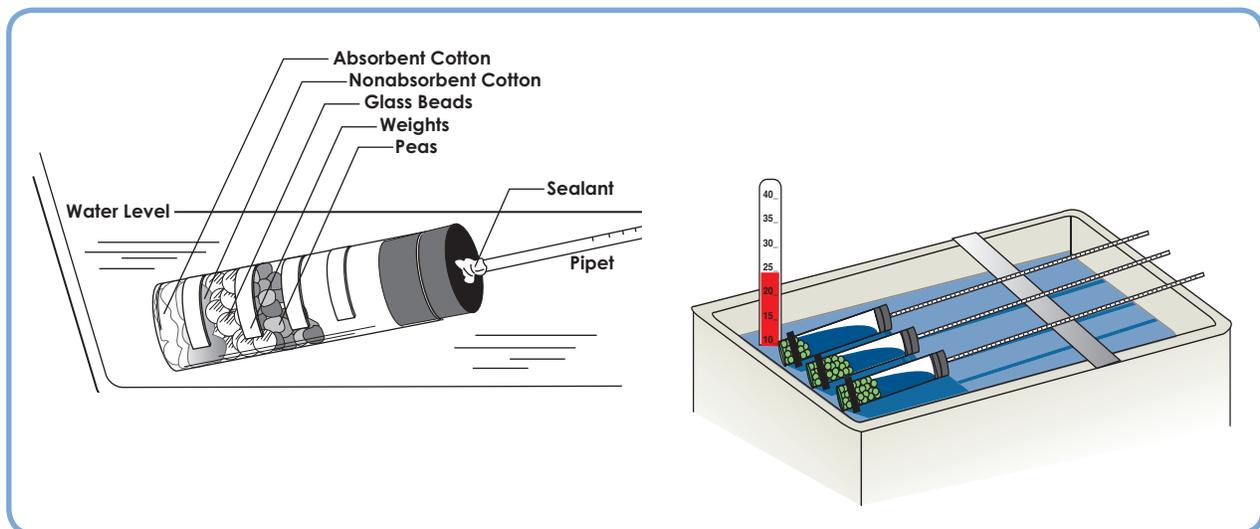
Taking Experimental Measurements

- Place the germinating peas into Vial A. Insert the cork/pipet assembly into the vial.
- Place the non-germinated peas + glass beads into Vial B. Insert the cork/pipet assembly into the vial.
- Place the glass beads into Vial C. Insert the cork/pipet assembly into the vial.
- Wrap Parafilm® or plastic wrap tightly around the seams (cork and tube) to seal any potential leak.
- Make sure the ice water bath has equilibrated to 10° C. Record the temperature of the room temperature bath.
- Place a piece of masking or lab tape over the water bath to suspend pipet tips out of the water during the equilibration phase.
- Place Vials A, B & C into the room temperature waterbath. Place them with the calibrated side of the pipet facing up to allow measurements to be taken.
- Allow the respirometers to equilibrate in the water baths for 8 minutes.
- After the equilibration phase, immediately submerge each respirometer.

Water in the water bath will enter the pipets and travel a short distance. As respiration occurs inside the vials, oxygen is consumed and the pressure drops. Over time, as pressure drops, additional water from the water bath enters the pipets. Make sure the vials do not fill up with water. If it does, there is a leak which must be corrected. Reassemble the faulty respirometer.

- Empty any water** from the respirometers and equilibrate them in the 10° C ice bath (same as step #13). Repeat step 14 for the 10° C bath.

NOTE:
Be sure to remove **ALL WATER** from the respirometers before transitioning to 10° C.

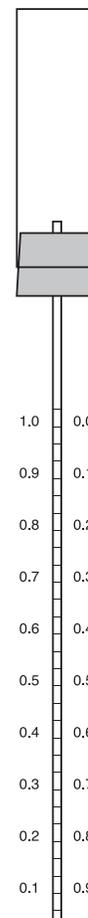


Taking Experimental Measurements

16. Arrange the vials so you can read the volume markings on each pipet. Place lead donuts or other weighted objects on the vials to keep vials submerged.
17. Record the starting temperature. Maintain temperature by adding ice or water as necessary during the experiment.
18. Record the starting point ("Time 0") volume of each pipet. Take readings of the volume of water in each pipet every 5 minutes for 20 minutes. Record these values in the tables on the following page.
19. Collect class data for Vials A, B, and C for both temperature water baths.
20. Correct volumes measured for changes in environmental variables at both temperatures.
 - Vial C measures volume changes due to environmental variables. For Vial C, the environmental effects are calculated by subtracting the volume at given time (T_x) from the starting volume at starting time (T_0). This can be written as:
Vial C volume at T_x - Vial C volume at T_0 .
 - Environmental effects must be applied to data collected for Vial A as shown below:
**[Vial A volume at T_x - Vial A volume at T_0] -
[Vial C volume at T_x - Vial C volume at T_0]**
 - Environmental effects must be applied to data collected for Vial B as shown below:
**[Vial B volume at T_x - Vial B volume at T_0] -
[Vial C volume at T_x - Vial C volume at T_0].**

When reading the pipets, use the inverse markings on the pipet (i.e. starting with 0.1 ml rather than 0.9 ml) at the pipet tip.

Use these markings (on the left.)



Taking Experimental Measurements

MEASUREMENT OF OXYGEN CONSUMPTION BY GERMINATING AND DRY PEAS AT TWO TEMPERATURES

Reading at Room Temperature

| Time X Minutes | "A" Germinating Peas | | | "B" Dry Peas with Beads | | | "C" Beads Only | |
|-------------------|----------------------|-------------|------------------------|-------------------------|-------------|------------------------|-------------------|-------------|
| | Reading at Time X | Difference* | Corrected Difference** | Reading at Time X | Difference* | Corrected Difference** | Reading at Time X | Difference* |
| 0 | | | | | | | | |
| 5 | | | | | | | | |
| 10 | | | | | | | | |
| 15 | | | | | | | | |
| 20 | | | | | | | | |

Reading at 10° C

| Time X Minutes | "A" Germinating Peas | | | "B" Dry Peas with Beads | | | "C" Beads Only | |
|-------------------|----------------------|-------------|------------------------|-------------------------|-------------|------------------------|-------------------|-------------|
| | Reading at Time X | Difference* | Corrected Difference** | Reading at Time X | Difference* | Corrected Difference** | Reading at Time X | Difference* |
| 0 | | | | | | | | |
| 5 | | | | | | | | |
| 10 | | | | | | | | |
| 15 | | | | | | | | |
| 20 | | | | | | | | |

* Difference = (Initial reading at 0 minutes) – (Reading at measured time)

** Corrected Difference = (Initial PEAS Reading at 0 minutes - Initial PEAS Reading at X minutes) –
(Initial BEAD Reading at 0 minutes - Initial BEAD Reading at X minutes)

Experiment Procedure



Experiment Results and Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. Graph your corrected data (difference) for Vials A, B, and C for both temperatures. Place time (in minutes) on the x-axis and volume (ml O₂ consumed) on the y-axis. Data from both temperatures should be plotted on the same graph. Draw the best straight line through the data points.
2. What accounts for the difference in oxygen consumption seen between the germinating and non-germinating seeds?
3. List some of the constant controls in this experiment.
4. Why do the glass beads seem to be using oxygen?
5. Why are the readings corrected using the glass bead values?
6. What is the function of KOH in this experiment?
7. From the slope of the lines, determine the rate of oxygen consumption at 10° C and room temperature for the germinating and non-germinating pea seeds. Determine the slope of the lines over a middle section of each line by dividing the difference in volume reading by the difference in time. Volume (ml O₂ consumed) values are determined from the line.

| Experimental Conditions | Calculations | Rate in ml O ₂ /minute |
|-----------------------------|--------------|-----------------------------------|
| Dry Peas/ 10°C | | |
| Dry Peas/ Room Temp. | | |
| Germinated Peas/ 10°C | | |
| Germinated Peas/ Room Temp. | | |

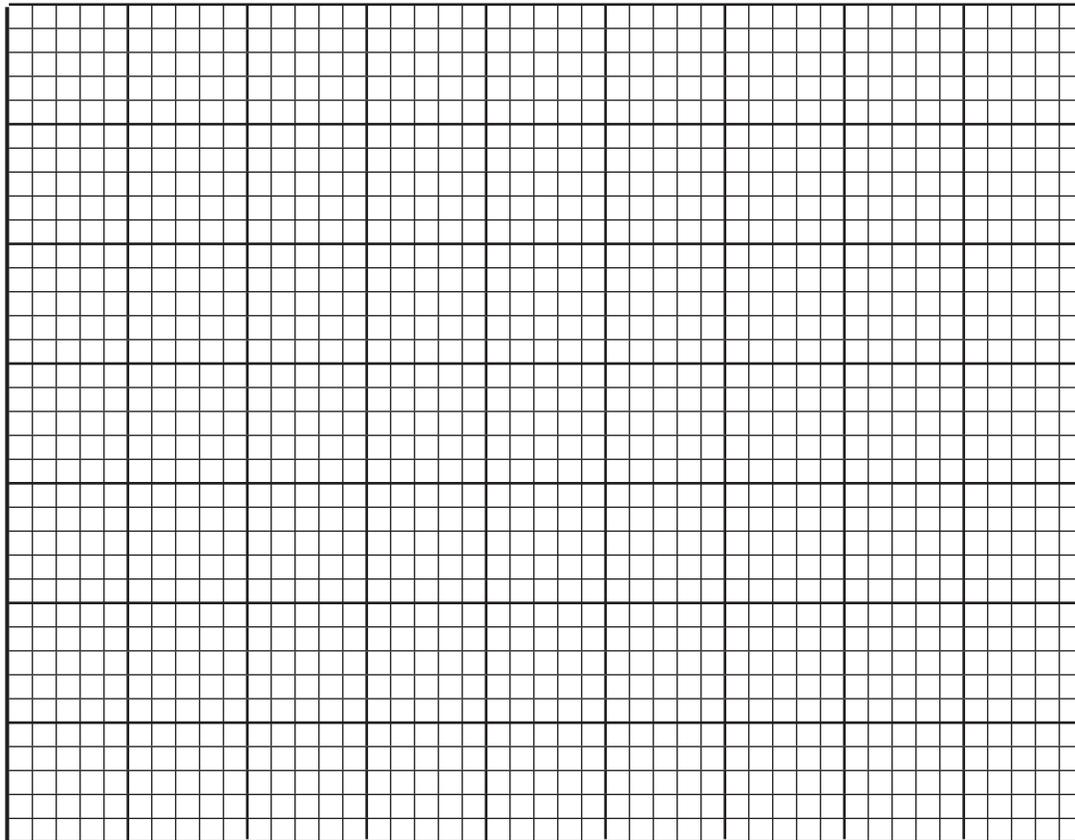
Experiment Results and Study Questions

8. Compare the rate of oxygen consumption at 10° C and room temperature. Why are they different?
9. How do you think the rates of respiration would change in peas that have been germinating for 0, 24, 48, 72, and 96 hours. Why?
10. Write a hypothesis using the same experimental design to compare the rates of respiration in a mouse at both room temperature and at 10° C.
11. Using the same experimental design, write a hypothesis to test the respiration rate of a 15 g reptile and a 15 g mammal at 10° C.
12. What basic cellular process is responsible for the oxygen consumption?

Experiment Results and Study Questions

Graph the results from the Corrected Difference column for the germinated peas and dry peas at both room temperature and at 10° C. Label the horizontal x-axis "Time in Minutes." Label the vertical y-axis "ml O₂ Consumed."

Title: _____



What is the independent variable? _____

What is the dependent variable? _____

What two hypotheses are being tested in this experiment?

Further Investigations

1. Design an experiment(s) to test the respiration rate of a reptile and a mammal of similar weights at two different temperatures.
2. Weigh the peas and find the average oxygen consumed/gram. Divide the milliliter oxygen/hour by the weight in grams.

Instructor's Guide

Notes to the Instructor & Pre-Lab Preparations

Overview of Laboratory Investigations

The “hands-on” laboratory experience is a very important component of science courses. Laboratory experiment activities allow students to identify assumptions, use critical and logical thinking, and consider alternative explanations, as well as help apply themes and concepts to biological processes.

EDVOTEK experiments have been designed to provide students the opportunity to learn very important concepts and techniques used by scientists in laboratories conducting biotechnology research. Some of the experimental procedures may have been modified or adapted to minimize equipment requirements and to emphasize safety in the classroom, but do not compromise the educational experience for the student. The experiments have been tested repeatedly to maximize a successful transition from the laboratory to the classroom setting. Furthermore, the experiments allow teachers and students the flexibility to further modify and adapt procedures for laboratory extensions or alternative inquiry-based investigations.

Organizing and Implementing the Experiment

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances.

If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK web site.

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Safety Data Sheets can be found on our website at: **www.edvotek.com/safety-data-sheets**

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Pre-Lab Preparations

If time does not permit each lab group to perform tests at both temperatures, assign half of the lab groups to perform the 10° C experiment and the other half to perform the room temperature experiment.

1. To germinate peas:
 - Two days before lab, place 25 seeds/group into a dish and cover with distilled water overnight.
 - The following day, place swollen pea seeds into a moist paper towel and place the towel into a paper bag stored in a drawer overnight.
2. To assemble respirometers:
 - Insert the pipets into the cork. The non-tapered end of the pipet should not extend more than 5 mm beyond the smaller end of the cork.
 - Seal around the hole with silicon glue. **AFTER** students add peas and/or glass beads, have them wrap Parafilm® around the seams (cork and tube, pipet and cork) to seal any potential leaks. Each group requires three respirometers.
3. The day before the lab:
 - Fill 10 trays or pans with water at room temperature.
 - Determine the necessary volume based on the size of trays you have available for water baths.
4. On the lab day, set up a pipetting station for the KOH solution.

MATERIALS FOR THE EXPERIMENT

Each student group should receive the following:

- 3 Cork/pipet assemblies
- 3 Vials
- Absorbent cotton
- KOH solution
- Non-absorbent cotton
- 100 ml graduated cylinder
- 25 non-germinated peas
- Small weights to hold vials in water
- Glass beads
- Tape
- Thermometer
- 25 germinated peas
- Tray
- Silicone glue
- Timer
- Parafilm®

Avoiding Common Pitfalls

1. Assemble respirometers ahead of time to ensure that the silicone glue will dry.
2. Make sure the trays are large enough to contain the assembled respirometers (at least 14" long). A large oblong glass casserole dish (27 cm W x 38 cm L x 6.5 cm D) works well if plastic trays are not available.
3. You can cut the cotton circles for the vials ahead of time or the students can perform this step.
4. Have the students wrap Parafilm® or plastic wrap around the seams of the assembled respirometers (around the seam of the cork and vial and at the area where the pipet enters the cork). This is very important, otherwise the respirometers will leak and the experiment will not work.
5. Depending on the size of the students groups, they may want to each be responsible for taking readings on one respirometer (each group has 3).

Experiment Results and Analysis

MEASUREMENT OF OXYGEN CONSUMPTION BY GERMINATING
AND DRY PEAS AT TWO TEMPERATURES

Reading at Room Temperature (Individual results will vary)

| Time X Minutes | "A" Germinating Peas | | | "B" Dry Peas with Beads | | | "C" Beads Only | |
|-------------------|----------------------|-------------|------------------------|-------------------------|-------------|------------------------|-------------------|-------------|
| | Reading at Time X | Difference* | Corrected Difference** | Reading at Time X | Difference* | Corrected Difference** | Reading at Time X | Difference* |
| 0 | 0.64 | | | 0.65 | 0 | | 0.63 | |
| 5 | 0.55 | 0.09 | 0.08 | 0.64 | 0.01 | 0.00 | 0.62 | 0.01 |
| 10 | 0.44 | 0.20 | 0.18 | 0.62 | 0.03 | 0.01 | 0.61 | 0.02 |
| 15 | 0.33 | 0.31 | 0.29 | 0.61 | 0.04 | 0.02 | 0.61 | 0.02 |
| 20 | 0.22 | 0.42 | 0.40 | 0.61 | 0.04 | 0.02 | 0.61 | 0.02 |

Reading at 10°C (Individual results will vary)

| Time X Minutes | "A" Germinating Peas | | | "B" Dry Peas with Beads | | | "C" Beads Only | |
|-------------------|----------------------|-------------|------------------------|-------------------------|-------------|------------------------|-------------------|-------------|
| | Reading at Time X | Difference* | Corrected Difference** | Reading at Time X | Difference* | Corrected Difference** | Reading at Time X | Difference* |
| 0 | 0.66 | | | 0.64 | | | 0.65 | |
| 5 | 0.61 | 0.05 | 0.04 | 0.63 | 0.01 | 0.00 | 0.64 | 0.01 |
| 10 | 0.58 | 0.08 | 0.06 | 0.62 | 0.02 | 0.00 | 0.63 | 0.02 |
| 15 | 0.56 | 0.10 | 0.09 | 0.61 | 0.03 | 0.00 | 0.62 | 0.03 |
| 20 | 0.53 | 0.13 | 0.10 | 0.60 | 0.04 | 0.01 | 0.62 | 0.03 |

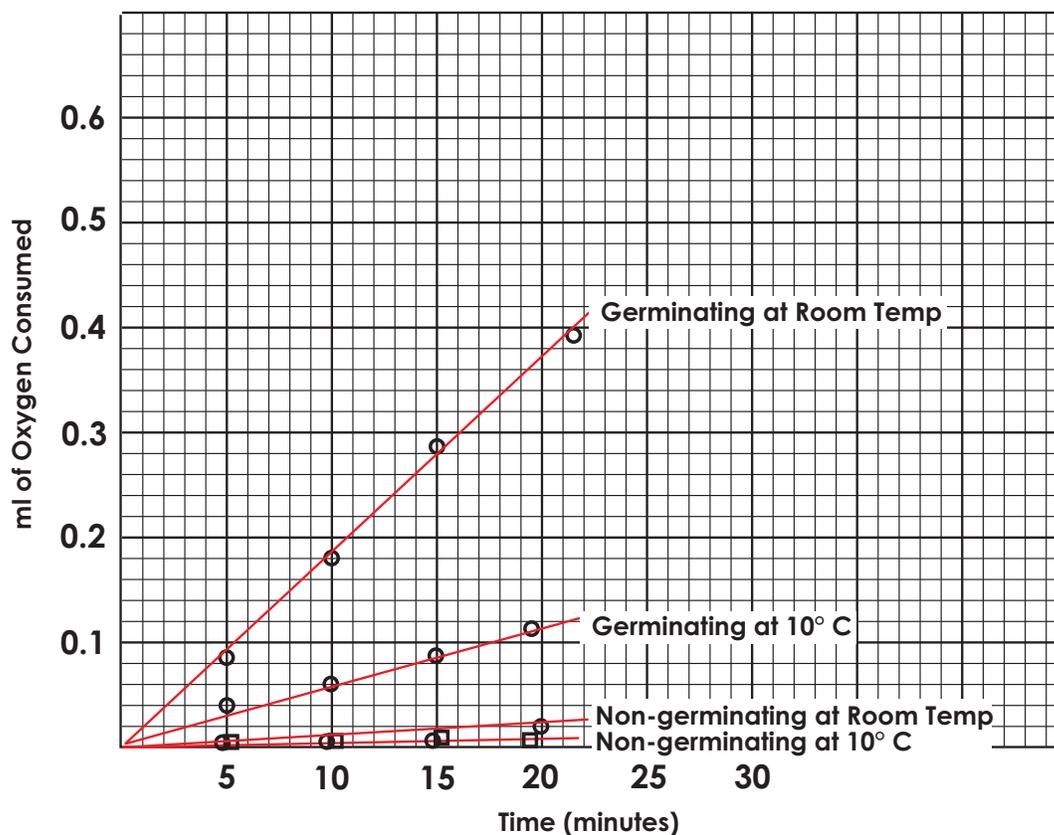
* Difference = (Initial reading at 0 minutes) – (Reading at measured time)

** Corrected Difference = (Initial PEAS Reading at 0 minutes - Initial PEAS Reading at X minutes) –
(Initial BEAD Reading at 0 minutes - Initial BEAD Reading at X minutes)

Experiment Results and Analysis

Graph the results from the Corrected Difference column for the germinating peas and dry peas at both room temperature and at 10° C. Label the horizontal x-axis "Time in Minutes." Label the vertical y-axis "ml O₂ Consumed."

Title: O₂ Consumed at Two Temperatures



What is the independent variable? Time (minutes)

What is the dependent variable? O₂ consumed (ml)

What two hypotheses are being tested in this experiment?

Non-germinating peas use less O₂ than germinating peas.

Peas germinating at colder temperatures use less O₂ than peas germinating at warmer temperatures.

**Please refer to the kit
insert for the Answers to
Study Questions**

**Please refer to the kit
insert for the Answers to
Study Questions**