HOMESCHOOL SCIENCE LAB

OBSERVING FIREFLY LUCIFERASE BIOLUMINESCENCE PRINCIPLES

Exploring the chemical reaction that produces light in fireflies.

Name: _____

Date: _____

Age/Grade: _____

BACKGROUND

Fireflies produce light through a chemical reaction called bioluminescence. The enzyme luciferase catalyzes a reaction between luciferin (a light-producing molecule), oxygen (O_2), and ATP (energy) adenosine triphosphate) to produce oxyluciferin, AMP, carbon dioxide, and light.

$$\text{Luciferin} + \text{ATP} + O_2 \xrightarrow{\text{luciferase}} \text{Oxyluciferin} + \text{AMP} + CO_2 + \text{(light)}$$

Firefly (Photinus sp.)

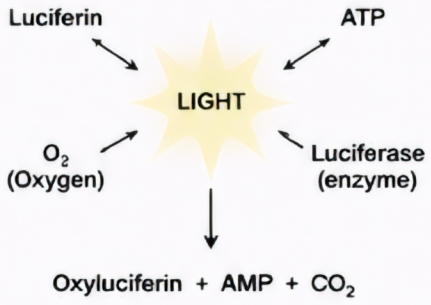
MATERIALS

- Firefly luciferase enzyme (lyophilized powder or liquid)
- D-luciferin
- ATP (disodium salt)
- Magnesium chloride ($MgCl_2$)
- Buffer solution (provided or prepared)
- Distilled water
- 6 test tubes with caps
- Test tube rack
- Pipettes or droppers
- Luminometer or light meter (or camera in a dark room)
- Timer
- Notebook

Note: Store luciferase, luciferin, and ATP on ice or in the refrigerator until use.

EXPERIMENT OVERVIEW

You will mix luciferase with luciferin, ATP, oxygen, and other components to produce light. You will also test how different factors affect the brightness of the bioluminescent reaction.



SAFETY

- This experiment uses small quantities of non-hazardous chemicals for educational purposes.
- Avoid touching your face and wash hands after the experiment.
- Do not ingest any materials.
- Follow adult supervision for proper handling and cleanup.

TIP

Bioluminescence is easiest to see in a dark room. Allow your eyes a few minutes to adjust to the dark for best results.

PROCEDURE

1. Label 6 test tubes 1–6.
2. Add the following solutions to each test tube as indicated in tha table.
3. Vortex or gently mix each tube.
4. Incubate for 2–5 minutes (room temperature).
5. Measure light output using a luminometer or observe visually in a dark room.
6. Record your results in the table.
7. Compare the brightness of each test tube and answer the questions.

Tube #	Luciferase (µL)	Luciferin (µL)	ATP (µL)	$MgCl_2$ (µL)	Buffer (µL)	Water (µL)	Condition Tested	Relative Light Output (very dim – very bright or use light meter)
1	50	50	50	10	340	—	Complete reaction (control)	
2	0	50	50	10	340	—	No luciferase	
3	50	0	50	10	340	—	No luciferin	
4	50	50	0	10	340	—	No ATP	
5	50	50	50	0	340	—	No $MgCl_2$	
6	50	50	50	10	—	340	No buffer (uses water)	

Note: Volumes can be adjusted if using different kit instructions. Keep total volume consistent.

ANALYSIS QUESTIONS

1. Which test tube produced the brightest light? _____
2. Which component(s) are essential for the reaction to produce light?

   _____
3. What is the role of luciferase in this reaction?

   _____
4. Why do you think oxygen is necessary?

   _____
5. How does this experiment relate to how fireflies produce light in nature?

   _____

EXTEND YOUR LEARNING

Test how temperature affects the reaction by running the complete reaction (Tube 1) at different temperatures (e.g., ice bath, room temp, warm water bath). What happens to the light output?

_____

_____

_____

Science from nature. Discovery at home.