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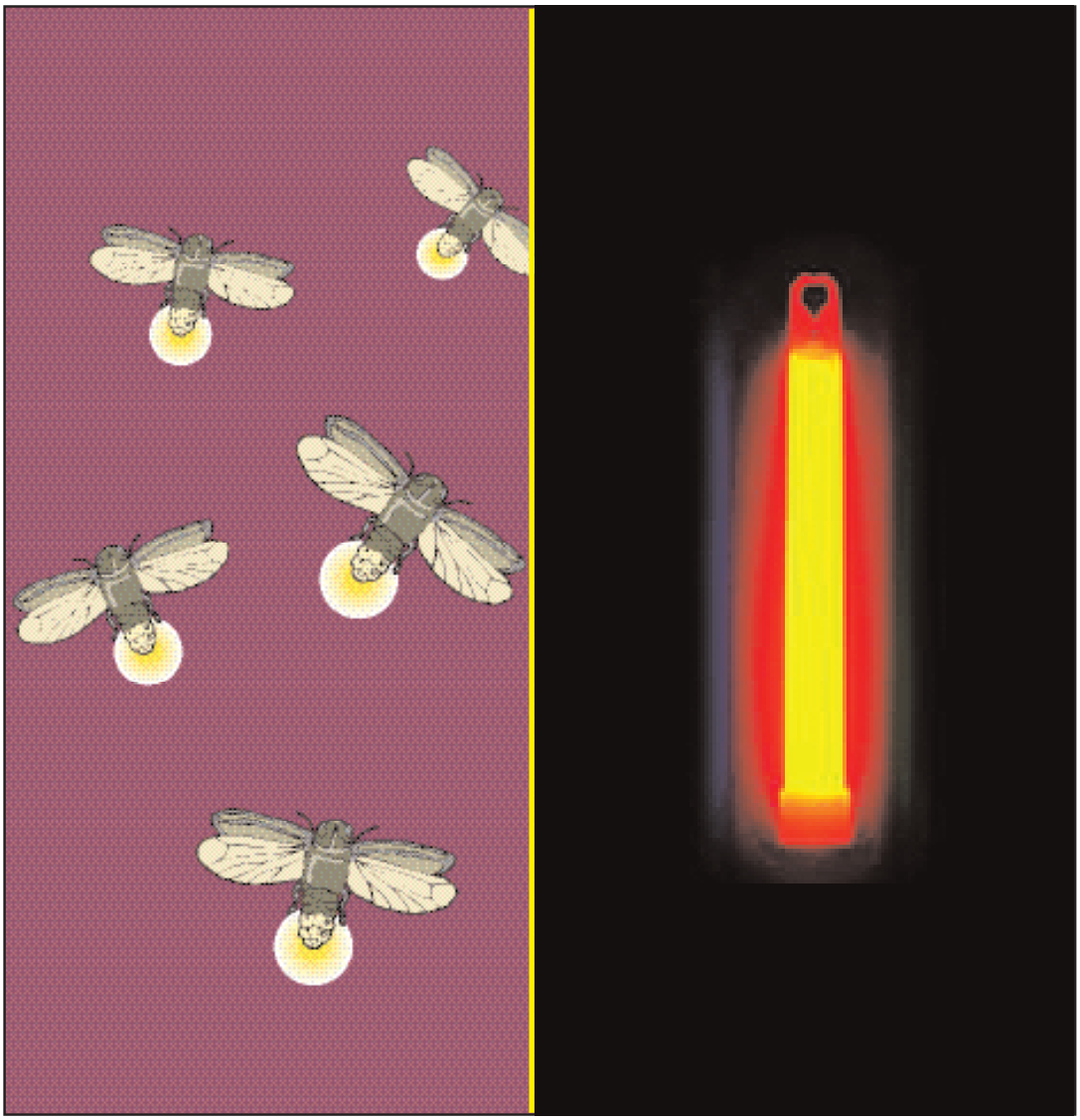
ATP and Firefly Bioluminescence

TEACHER'S MANUAL WITH STUDENT GUIDE



CAROLINA

World-Class Support for Science & Math

ATP and Firefly Bioluminescence

Teacher's Manual

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Student Guide (Photocopy Masters)

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ATP and Firefly Bioluminescence

Overview

The *ATP and Firefly Bioluminescence* kit introduces students to ATP as an energy source for biochemical reactions and familiarizes them with the basic characteristics of enzymes.

Objectives

Student will

- observe the function of enzymes.
- observe the effect of pH changes on reaction rate.
- observe the effect of temperature changes on reaction rate.
- identify a source of chemical energy.

Content Standards

This kit is appropriate for high school students and addresses the following National Science Education Standards:

Grades 9–12
Life Science

- Matter, energy, and organization in living systems

Physical Science

- Chemical reactions
- Interactions of energy and matter

Time Required

Preparation	20 minutes*
Activity	45 minutes
Discussion	15 minutes

***Before beginning this activity, remove the sealed Firefly/ATP pouches (each containing one dropper bottle of ATP powder and two vials of powdered firefly lantern) from the refrigerator and allow them to warm to room temperature.**

Materials

The materials in this kit are sufficient for 32 students working in eight groups of 4.

Included in the kit:

- 4 sealed Firefly/ATP pouches, each containing:
  - 2 vials of powdered firefly lantern
  - 1 dropper bottle of ATP powder
- 2 dropper bottles of 0.1 M acetic acid
- 2 dropper bottles of 0.1 M sodium hydroxide
- 2 dropper bottles of sodium chloride solution
- 4 pipets
- 3 petri dishes
- buffer pH 8 Chemvelope
- Teacher's Manual with reproducible Student Guide

Needed, but not supplied:

- 2 clamps for holding test tubes
- 2 test tubes (for heating firefly lantern)
- ice bath or lab-only refrigerator
- hot water bath
- 4 beakers or cups (to hold buffer solution at each station)

Note: Upon receipt, refrigerate the sealed pouches until you are ready to use the kit. Before beginning this activity, remove the sealed pouches of ATP powder and powdered firefly lantern from the refrigerator and allow them to warm to room temperature.

Safety Information

Stress prudent laboratory safety practices. Ensure that you and your students follow precautions and use personal protective equipment when appropriate. Know and follow school and district guidelines for lab safety, proper laboratory materials usage, and disposal of laboratory wastes. Review pertinent portions of any Material Safety Data Sheets (MSDS) with students prior to beginning the activity.

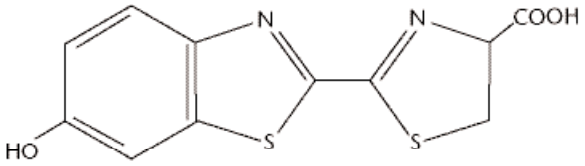
Background

Chemical Reaction

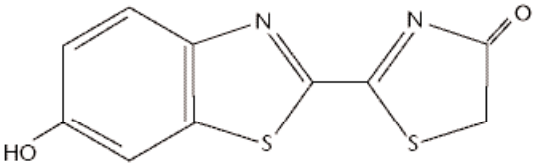
Bioluminescence is the light produced from a chemical reaction within a living organism. In firefly bioluminescence, chemical energy from ATP is converted to light by an enzyme-catalyzed reaction whose reactants are luciferin, adenosine triphosphate (ATP), and oxygen (O_2). The enzyme luciferase catalyzes the reaction. The products are oxidized luciferin ("oxyluciferin"), adenosine monophosphate (AMP), inorganic pyrophosphate (PP_i), carbon dioxide (CO_2), and light.

$$\text{luciferin} + \text{ATP} + O_2 \xrightarrow{\text{luciferase}} \text{oxyluciferin} + \text{AMP} + PP_i + CO_2 + \text{light}$$

The following illustrations show the structure of luciferin and oxyluciferin.



Luciferin



Oxyluciferin

pH Effect

Due to their ionic properties, enzymes are characteristically sensitive to changes in electrolyte concentrations, particularly pH changes. The pH optimum for the activity of firefly luciferase is 7.8. When the students change the pH of the experimental solution, the luminescence can be quenched and restored as the pH is adjusted. Some color changes may also be noted.

Temperature Effects

Temperature changes influence enzymatic reactions in much the same way they influence chemical reactions—reaction rates increase as temperature increases. The reaction rate of an enzymatic reaction increases with increasing temperature until about 50°C, when the temperature is sufficient to denature the enzyme. Reaction rates above this temperature decrease drastically. Exposure of luciferase to high heat (65°C or greater) for as little as 60 seconds denatures the enzyme and destroys its catalytic ability.

Cooling an enzyme typically does not inactivate it, but does slow the reaction rate. Cooling firefly luciferase in a refrigerator or in ice extends the duration of light production but diminishes the brightness of the light.

Salt Effect

The addition of sodium chloride solution to a vial of glowing lanterns quenches the light almost immediately. Although it is not known how a firefly controls the flashing of its lantern, chloride ions may be involved.

Student Misconceptions

Students may believe that enzymes cause a reaction to occur. In reality, enzymes lower the activation energy, which causes the reaction to occur more rapidly.

Students may think that the enzyme (luciferase) itself is what is glowing. Review the chemical equation and emphasize that the ATP and the luciferin from the firefly lantern are reacting with oxygen to produce the glow. The enzyme only causes the reaction to happen more quickly.

Students often believe that ATP is energy. ATP is not energy; rather, it is an energy-storing molecule. Energy is stored in the bonds between the phosphate groups in ATP. When the bonds are broken, the energy is released.

Preparation

1. Refrigerate the sealed Firefly/ATP pouches (containing ATP and powdered firefly lantern) until you are ready to conduct the classroom activities.
2. Photocopy the Student Guide at the end of this manual for each student.
3. Mix the buffer solution according to the instructions on its container. Equally distribute the buffer solution between four beakers or cups.
   Note: Once mixed, the buffer solution is good for three weeks.
4. Before beginning the activity, allow the refrigerated components warm to room temperature.
5. If desired, pre-assign the class into eight groups of four students each. During the activity, two groups (i.e., eight students) will work at each station. Groups at the same station will perform the same activity and will share some (but not all) materials.
6. Set up four workstations, each capable of supporting two groups of four students. Equip the stations as follows:

Station 1. pH Effect

2 vials of powdered firefly lantern
dropper bottle of ATP powder
buffer solution
pipet
2 dropper bottles of 0.1 M acetic acid
2 dropper bottles of 0.1 M sodium hydroxide
petri dish

Station 2. Temperature Effect—Cold

2 vials of powdered firefly lantern
dropper bottle of ATP powder
buffer solution
pipet
ice bath (or access to a lab-only refrigerator)
petri dish