

1. Identification

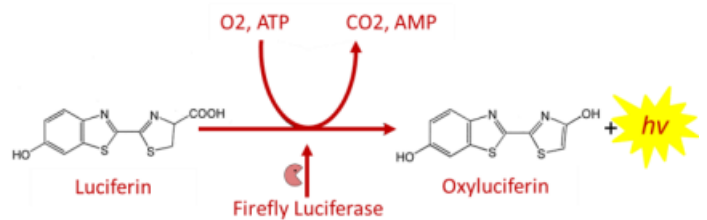
Product name
Firefly Luciferase Assay Kit

1000 assays

Cat. No:
CA135

2. Description

Luciferase from the firefly (*Photinus pyralis*) is the most used photoprotein in molecular biology studies and has been used as a reporter for studying gene regulation and function in transformed cell lines in culture. Firefly luciferase has an apparent molecular weight of 62 kDa, which is active as a monomer and does not require subsequent processing for its activity. The enzyme catalyzes the oxidation of reduced luciferin in the presence of ATP-Mg²⁺ and oxygen to generate CO₂, AMP, PPI, oxyluciferin, and produces a flash of light that is proportional to the quantity of luciferase in the reaction mixture.



The Luciferase Assay Substrate includes coenzyme A, ATP, and luciferin. Including coenzyme A in the reaction enhances the sensitivity of the assay and provides a sustained light reaction (half-life >5 minutes). This eliminates the need for automated luminometer injection of substrate and allows analysis by photographic film or scintillation counting.

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3. Composition & Storage

Item	Quantity
Luciferase Assay Substrate	100 mL
5X Cell Lysis Buffer*	20 mL
Luciferase (control)	50 µg

*Prepare 1X Cell Lysis Buffer by adding 4 volumes of water to 1 volume of 5X Cell Lysis Buffer.

4. Storage

The kit is shipped on gel pack. Store all kit components at -20°C or below, protected from light. The kit is stable at -20°C for three months and at -70°C for up to 1 year from date of receipt.

Avoid repeated freeze-thaw cycles.

5. Applications

- ✓ Detection and quantification of Firefly Luciferase.
- ✓ Reporter enzyme from cultured cells.

6. Features

- ✓ Easy to use.
- ✓ Rapid: results within 15 minutes.
- ✓ Sensitivity
- ✓ Accuracy
- ✓ Ideal for high throughput assays



7. Assay Procedure

Note: The Luciferase Substrate Solution and samples should be at ambient temperature prior to performing a luciferase assay.

The reagent has been validated in a 96-well format. Other format will require scaling and optimization by the end-user.

A. Preparation of Cell Extract

1. Aspirate the growth medium from cells. Wash cells with PBS.
2. Add 50 μ l of Lysis Buffer 1X per well of a 96-well plate. Incubate at room temperature for 10-15 minutes.

B. Standard curve

3. Produce a standard curve of light emission versus enzyme concentration.
To produce a standard curve of light units versus relative enzyme concentration, make serial dilutions of recombinant luciferase (standard) in 1X lysis buffer supplemented with 1mg/ml BSA. The addition of BSA is necessary to ensure that luciferase is not lost from solution by adsorption.
 - a. Dilute Luciferase to 1000 ng/ml in lysis buffer.
 - b. Make serial dilutions to get concentration of 5, 2.5, 1.25, 0.625, 0.312, 0.156 ng/ml and a blank control.
 - c. Add 20 μ l of serial dilutions to the wells of a 96-Well Solid White Plate. (For a 384-Well Solid White Plate add 10 μ l of each concentration to the Well)

C. Assay protocol

4. Transfer 20 μ l of cell extracts containing Luciferase to the corresponding well of a 96-Well Solid Plate (white) with lid.
5. Add 100 μ l/well of substrate solution in each well of cell extract (or luciferase standard). Shake 30 seconds the plate to get homogenize the reaction. (For 384-well plates, add 25 μ l of the Luciferase substrate solution to 10 μ l of the cell lysate or luciferase standard).
6. Program the luminometer for the appropriate delay and measurement times.
7. Measure the light produced.

D. Plot the Standard Curve

E. Determine the Sample Concentration

8. Use the blank control to eliminate the background. Measure the samples.
9. Extrapolate the luminescence data (light units) to Luciferase standard curve to calculate the concentration of the enzyme in the cell extracts.

7. Further information

Product Use Limitations	This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
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