TECHNICAL MANUAL

Firefly Luciferase Assay Kit (CV0013)

- **SKU CODE:** CV0013
- **SIZE:** 100 assays
- **DETECTION PRINCIPLE:** Luminescent
- **RUO:** Research-Use-Only

Revised: 07/2025

Firefly Luciferase Assay Kit (CV0013)

Please read entire manual carefully before starting experiment.

Table of Contents

1. Key Features

Applications

Detection and quantification of Firefly Luciferase.

Sample type

Reporter enzyme from cultured cells.

Measuring instrument:

Photographic film or scintillation counting

Rapid results:

Results in 15 minutes

2. Storage & Expiry

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.

3. Product Description

Luciferase Assay Kit (from the firefly Photinus pyralis) is an accurate, sensitive and easy method for studying gene reporter 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-Mg^{2+} and oxygen to generate CO_2, 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.

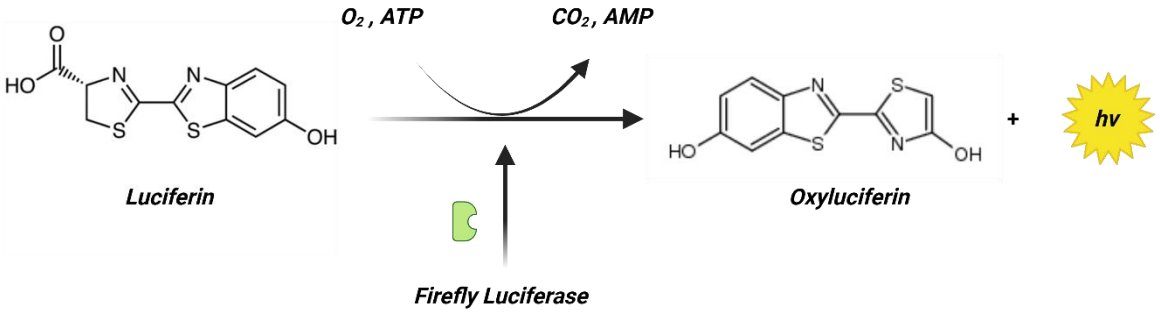

Figure 1. Reaction mechanism of Firefly Luciferase Assay. The enzyme catalyses the oxidation of reduced luciferin in the presence of ATP-Mg^{2+} and oxygen to generate CO_2, AMP, PPi, oxyluciferin and light.

4. Kit Contents

No	Component Name	Quantity
1	Luciferase Assay Substrate	10 mL
2	5X Cell Lysis Buffer*	4 mL
3	Luciferase (control)	10 µg

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

5. Important Notes

1. This assay kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
2. Please read the instructions carefully before beginning the assay. Strict adherence to the protocol is essential for accurate results.
3. Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
4. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

6. 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 µl of Lysis Buffer 1X per well of a 96-well plate. Incubate at room temperature for 10- 15 minutes.

B. Standard Curve

1. 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.

- Dilute Luciferase to 1000 ng/ml in lysis buffer.
- Make serial dilutions to get concentration of 5, 2.5, 1.25, 0.625, 0.312, 0.156 ng/ml and a blank control.
- Add 20 µl of serial dilutions to the wells of a 96-Well Solid White Plate. (For a 384 Well Solid White Plate add 10 µl of each concentration to the Well).

C. Assay Protocol

1. Transfer 20 µl of cell extracts containing Luciferase to the corresponding well of a 96-Well Solid Plate (white) with lid.
2. Add 100 µl/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 µl of the Luciferase substrate solution to 10 µl of the cell lysate or luciferase standard).

3. Program the luminometer for the appropriate delay and measurement times.
4. Measure the light produced.

7. Data Analysis

Plot standard curve and determine the sample concentration.

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

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