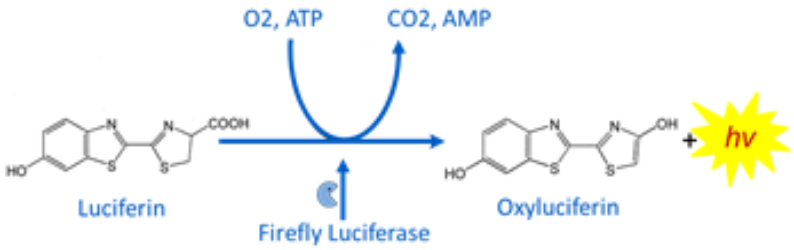
Luciferase Reporter Assay Kit

LUCI-001-100

Introduction

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-Mg^{2+} and oxygen to generate CO_2, AMP, PP_i, 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. The Kit is designed for simple and efficient quantitation of firefly luciferase reporter enzyme activity from cultured cells with high sensitivity and linearity.

Storage condition

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.

Advantages/Features

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

Kit content

- ✓ Luciferase Assay Substrate: 10 ml
- ✓ 5X Cell Lysis Buffer: 4ml*
- ✓ Luciferase (control): 10 µg

**Prepare 1X lysis reagent by adding 4 volumes of water to 1 volume of 5X lysis reagent*

Product use limitation

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

Assay Procedure

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

Preparation of Cell Extract

1. Aspirate the growth medium from cells. Wash cells with PBS.
2. Add 50 µl of Lysis Buffer 1X. Incubate at room temperature for 10 minutes.

Standard curve

3. Program the luminometer for the appropriate delay and measurement times.
4. Produce a standard curve of light emission versus enzyme concentration. It is important to determine the linear range of light detection for your luminometer before performing an experiment, because luminometers can experience signal saturation at high light intensities. 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. The final amounts of Luciferase are 100, 50, 25, 12.5, 6.25 y 3.12 ng and 0 ng/well.

For a 384- Well Solid White Plate add 10 µl of each concentration to the Wells.

Assay Protocol

5. Transfer 20 µl of cell extracts containing Luciferase to the corresponding well of a 96-Well Solid Plate (white) with lid.
6. Add 100 µl/well of substrate solution in each well. 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

7. Measure the light emitted can be read with a luminometer, scintillation counter, or detected with photographic film. Based on the standard curve, gene expression can be quantified.

Plot the Standard Curve

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 de concentration of the enzyme in the cell extracts.