ONE-STEP LUCIFERASE ASSAY KITS FOR DETECTION OF FIREFLY LUCIFERASE

Principle of the Luciferase Assay

The luciferase assay kit is based upon the bioluminescent measurement of firefly luciferase. This enzyme catalyses the formation of light from ATP and luciferin according to the following reaction:

\[
\text{Luciferase} \quad ATP + \text{Luciferin} + O_2 \rightarrow \text{Oxyluciferin} + \text{AMP} + \text{PPi} + \text{CO}_2 + \text{LIGHT}
\]

\[\text{Mg}^{++}\]

The intensity of light emission is linearly related to the amount of luciferase and is measured using a luminometer. Luciferase is the most widely used genetic reporter in studies on gene expression due to its high sensitivity, dynamic range, and natural absence from mammalian cells. The luciferase assay kit offers the advantages of high sensitivity, consistent reproducibility, and cost-effectiveness along with the added convenience of a one-step assay.

Typically, the luciferase assay kit (with lysis buffer) has been formulated for assaying luciferase activity in cells grown in the 96-well format. It is recommended that for optimum performance, cells are cultured in 100µl volumes in bioluminescence-compatible white-walled, clear-bottomed 96-well plates. Before Luciferase expression can be measured by bioluminescence, the enzyme must first be extracted from the cells. The reconstituted Luciferase assay kit (plus lysis buffer) facilitates the simultaneous extraction and measurement of luciferase from mammalian cells. For optimal convenience, the kit can be used in conjunction with automatic reagent dispensing facilities, however, this is not an essential requirement of this assay, as a multichannel pipette capable of dispensing 100µl may be used for the addition of reconstituted Luciferase Detection Reagent. The luciferase assay kit is intended for the detection of the expression of firefly luciferase and is not suitable for the assay of luciferase from other origins.

The reagent has all the ingredients necessary for lysing cells as well as the luciferin substrate and stabilizers of the luciferase reaction in a single solution unlike other kits where the assay buffer has to be mixed with the luciferin substrate provided in a separate bottle.

Luciferase assay kit: This kit can be conveniently used for measuring luciferase activity in 96-well plates (or in preparations of lysed cells). As seen in Figure, this assay kit compares well with the Promega bright-glow assay system for measurement of luciferase activity. The luciferase assay kit can also be used to measure luciferase activity in prelysed cell extracts. In this case, 5-50 ul of the
cell lysate is mixed with 100 ul of the luciferase kit, mixed well and read immediately in the luminometer.

**PROTOCOL:**

**ASSAY IN 96-WELL FORMAT:** Luciferase assay kits, Aspirate off the supernatent culture media from cells grown in multiwell tissue culture dishes. Add 100 ul of luciferase assay buffer (plus lysis buffer) incubate for at least 10 minutes and read in a lumionometer or microplate reader. Alternately 100 ul of the luciferase assay reagent can be added to cells with upto 50 ul of the culture medium, incubated for 10-15 minutes and assayed for luciferase activit

**ASSAY OF LUCIFERASE ACTIVITY IN CELL LYSATES:** The luciferase kits can also be used to measure luciferase activity in prelysed cells. Mix 5-50 ul of luciferase containg sample or cell lysate with 100 ul of the luciferase assay kit and read immediately in the luminometer. All assay reagents should be close to room temperature at the time of assay.

**STORAGE:** Store reagents at –20 ° C or –70° C