Signosis
Innovative Plate Assay Solutions

Dual Luciferase (Firefly & Renilla) Assay System (For Research Use Only)

Catalog number FRLUC010, FRLUC050

Product description

Signosis' Dual Luciferase (Firefly & Renilla) Assay System provides a sensitive, easy-to-use solution for measurement of Firefly and Renilla luciferase activities. This all-in-one kit provides optimized Firefly and Renilla substrates together with a high-performance lysis buffer for efficient cell lysis and rapid detection of both Firefly and Renilla Luciferase activity from a single sample.

The Dual Luciferase Assay System is ideal for use with Signosis' luciferase reporter product lines, including stable cell lines, transcription factor (TF) reporter plasmids, and Bac-In reporter systems, as well as third-parties dual reporters in the market. Delivering strong and reliable light signals comparable to leading alternatives, this cost-effective kit provides researchers with an affordable choice for dual luciferase assays without compromising performance.

Principle

The Firefly luciferase signal is measured first by adding the Firefly Substrate Reagent to the samples. In this reaction, Firefly luciferase catalyzes the oxidation of D-luciferin in the presence of ATP and magnesium, producing a bright yellow luminescence. The reaction has a high quantum yield, and both luciferase and luciferin are minimally toxic, which has contributed to Firefly luciferase becoming one of the most widely used reporter systems.
Renilla luciferase, in contrast, is ATP-independent. It catalyzes the oxidation of coelenterazine to coelenteramide in the presence of oxygen, generating a blue luminescence. When the Renilla Substrate Reagent is added, it simultaneously quenches the Firefly signal while enabling accurate measurement of the Renilla signal.
Both luciferases have relatively short half-lives compared to fluorescent proteins, making them well suited for dynamic studies. Importantly, the accuracy of Firefly luciferase reporter assays can be further enhanced by normalization to a co-reported control, such as Renilla luciferase, measured within the same sample.

Material provided

- 1 bottle of Firefly Substrate Reagent
- 1 bottle of Renilla Substrate Reagent
- 1 bottle of lysis buffer

Handling upon arrival

For long term storage, it is highly recommended to aliquot and store the luciferase substrates in -80°C.

IMPORTANT: Avoid multiple freeze-thaw cycles as it will cause a decrease in substrate sensitivity.

Materials required but not provided

(May be substituted with a comparable third-party product)

- 96-well white plate -- *Greiner Bio-One P/N 655098*

Assay procedure

The following procedure is designed for 96-well luciferase detection. Please adjust to different plating conditions as necessary.

Preparation: To prepare 1x lysis buffer, add one volume of 5x lysis buffer to four volume of distilled water.

Example: Add 100 µl of 5x lysis buffer to 400 µl of distilled water to create 500 µl of distilled water.

1. Remove media from cell samples.
2. Wash cells with 100 µl of PBS
3. Add 20 µl of 1x lysis buffer to each well.
4. Incubate cells in lysis buffer for 15 minutes at room temperature.
5. Thaw luciferase substrates reagents at room temperature prior to use. Perform the assay when the substrate reaches room temperature.
   NOTE: Do not thaw the substrate at temperature above 30°C.
6. Add 50 µl of Firefly Substrate Reagent to each well and gently pipette up and down.
7. Immediately read the plate in a luminometer, with setting at 10 seconds integration. This is your Firefly signal data.
8. After reading Firefly signal, add Renilla Substrate Reagent to the wells and gently pipette up and down.
9. Immediately read the plate in a luminometer, with setting at 10 seconds integration. This is your Renilla signal data.