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## Firefly Luciferase Substrate

(For Research Use Only)

Catalog number LUC015, LUC100

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### Product description

Signosis has developed Firefly Luciferase Substrate for detecting firefly luciferase activity in our luciferase reporter product lines, including stable cell lines, TF reporter plasmids, and our Bac-In reporter systems, as well as third-parties firefly reporters in the market. This affordable and cost-effective reagent produces a strong light signal that is comparable to competitors' substrates.

### Material provided

One bottle of Firefly Luciferase substrate.

- LUC015 – 15 ml of Firefly Luciferase Substrate, enough for 300 reactions
- LUC100 – 100 ml of Firefly Luciferase Substrate, enough for 2000 reactions

### Handling upon arrival

Immediately aliquot substrate to appropriate volumes (1-10 mL) and store at -80°C. **Do not store long term at -20°C or 4°C. Substrate can be temporarily stored at 4°C for short-term use.**



**IMPORTANT:** Avoid multiple freeze-thaw cycles as it will cause a decrease in substrate sensitivity. It is recommended to make aliquots of substrate for one-time use only.

Make sure the substrate is **fully thawed** (no ice) and thoroughly mixed before using.

### Materials required but not provided

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

- 96-well white plate -- Greiner Bio-One P/N 655098
- Cell lysis buffer -- Signosis P/N LS-001

### Assay procedure

The following procedure is designed for 96-well luciferase detection.

1. The day before performing the assay, trypsinize the cells and seed each well of a 96-well **white-wall** plate with  $1 \times 10^4$  cells in 100µl.
2. Incubate the plate in a humidified incubator at 37°C with 5% CO<sub>2</sub> overnight.
3. After the experiment, remove the media by aspiration and add 100µl of PBS to each well.
4. Remove PBS by aspiration and add 20 µl of 1x lysis buffer to each well (to prepare 1x lysis buffer, add one volume of 5x lysis buffer to four volume of distilled water).
5. Incubate cells in lysis buffer for 15 minutes at room temperature.
6. Thaw luciferase substrate 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.**
7. Add 50 µl of luciferase substrate to each well and gently pipette up and down.
8. Immediately read the plate in a luminometer, with setting at 10 seconds integration.