SuperLight Luciferase Reporter Gene Assay Kits

1. Description

The SuperLight Luciferase Reporter Gene Assay is based on the quantitation of luciferase expression in mammalian cells, yeast or E. coli cells, using luciferin and ATP as substrates. The reaction results in light production which can be conveniently measured by a luminometer.

Reaction scheme:

\[
\text{luciferase} \quad \text{ATP} + \text{D-luciferin} + \text{O}_2 \quad \rightarrow \quad \text{oxyluciferin} + \text{AMP} + \text{PP}_i + \text{CO}_2 + \text{light}
\]

This bioluminescent reporter gene assay is extremely sensitive and is especially suitable for quantifying luciferase expression in recombinant cells. This ultra-sensitive, homogeneous cell-based assay only requires adding a single reagent to the cells and measuring the light intensity after a short incubation (>2 minutes). Assays can be performed in tubes, cuvettes or multi-well plates. All kit components are compatible with culture media and with all liquid handling systems. With an extended luminescence emission kinetics (half-life 55 min), the SuperLight luciferase assays are especially suitable for high-throughput screening in 96-well, 384-well and 1536-well plates. In addition, the reagent provided in the kits has been formulated for maximum sensitivity, reproducibility and long shelf-life. Applications for this kit include gene regulation studies and high-throughput screening of gene modulators. Key features of this kit include:

Key features:

- **High sensitivity and wide detection range**: Detection of as little as 2 fg luciferase and as few as 4 cells. Plus, the emitted light is linear over seven orders of magnitude.
- **Compatible with routine laboratory and HTS formats**: Assays can be performed in tubes or microplates, on LJL Analyst, Berthold Luminometer, Top-Count, MicroBeta counters, chemiluminescent image plate readers (CLIPR/LeadSeeker). Assay reagents compatible with all liquid handling systems.
- **Fast and convenient**: Homogeneous “mix-and-measure” assay allows detection of luciferase levels within 10 minutes. The optimally combined reagent system allows a single addition step and simultaneous cell lysis and detection.
- **Robust and amenable to HTS**: Z’ factors of 0.6 to 0.8 are observed in 96-well and 384-well plates. Can be readily automated on HTS liquid handling systems.

2. Kit Contents (reconstituted reagent at 2x concentration)

<table>
<thead>
<tr>
<th>Catalog No.</th>
<th>Size (assays)</th>
<th>Reagent</th>
<th>Assay Buffer</th>
<th>Price S$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLLU-200</td>
<td>200</td>
<td>solid</td>
<td>20 mL</td>
<td>300</td>
</tr>
<tr>
<td>SLLU-500</td>
<td>500</td>
<td>solid</td>
<td>50 mL</td>
<td>500</td>
</tr>
<tr>
<td>SLLU-01K</td>
<td>1,000</td>
<td>solid</td>
<td>100 mL</td>
<td>850</td>
</tr>
<tr>
<td>SLLU-HTS</td>
<td>&gt; 5,000</td>
<td>solid</td>
<td>customized</td>
<td>enquire</td>
</tr>
</tbody>
</table>

Storage conditions. The SuperLight Luciferase Assay Reagent is light sensitive and should be stored in the provided amber tube for light protection. The reagent is stable at -20°C for up to one year. The Assay Buffer is stable for up to one year when stored at 2-8°C. After reconstitution in assay buffer, the Reagent is stable for up to 4 weeks at -20°C.

The protocol and Material Safety Data Sheet (MSDS) can be downloaded online at www.i-dna.biz.

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Manufactured by Bioassay Systems Inc., USA
3. General procedure for 96-well plate assay

The SuperLight Luciferase Reporter Gene Assay is based on the bioluminescence generation during the luciferin/luciferase reaction. The reconstituted reagent has been optimized to combine cell lysis and detection into a single step.

A. Plate and culture cells (80 µL) in white opaque 96-well tissue culture plates. Typical culture medium contains DMEM, 10% fetal bovine serum and antibiotics (penicillin/ streptomycin, gentamycin, etc). Amino acids and other nutrients can be added to the culture medium. Assays can be performed on either adherent cells or cells in suspension. The cells can be either stably or transiently transfected with the luciferase gene. Culture volume can vary from 50 to 100 µL, although 80 µL is used in this protocol. Blank control wells containing no cells should also be prepared.

B. Add test compounds and controls to cells. Mix well and incubate for the cells desired period of time. Incubation time for gene regulation studies can be from several hours up to 3 days. It is recommended that assays be run in duplicate or triplicate. A volume of 20 µL compounds in PBS or culture medium is recommended.

C. Reconstitute the Reagent. First equilibrate the Reagent and Assay Buffer to room temperature. Then simply combine the Assay Buffer and Reagent by pipetting a small volume (e.g. 1 mL) buffer to the Reagent tube. Vortex briefly and pipet the reconstituted solution to the Assay Buffer bottle. Repeat this step to transfer all of the Reagent to the Assay Buffer bottle. Mix by inversion until the Reagent is thoroughly dissolved. After this is done, mark the bottle label as Reconstituted Reagent.

D. Add 100 µL (per 80 µL of cell culture) of the reconstituted Reagent to each well and mix well with the cells. Incubate for 2 minutes at room temperature. The volume of the reagent can be adjusted depending on the volume of cell culture. Store the reconstituted reagent at -20 °C.

E. Measure luminescence on a luminometer. The integration time can be 1 sec to 2 min depending on the luciferase expression level and instrument sensitivity. For most luminometers (Berthold Luminometer, LJI Analyst, Top Count, MicroBeta Counters, CLIPR and LeadSeeker), integration 1 to 5 sec is appropriate.

4. General procedure for 384-well plate assay

A. Plate and culture cells (25 µL) in white opaque 384-well tissue culture plates. Culture volume can vary from 20 to 50 µL, although 25 µL is used in this protocol. Set up blank control wells containing no cells.

B. Add test compounds and controls to cells. Mix well and incubate for the cells desired period of time. A volume of 5 µL compounds in PBS or culture medium is recommended.

C. Reconstitute the Reagent. First equilibrate the Reagent and Assay Buffer to room temperature. Then simply combine the Assay Buffer and Reagent by pipetting a small volume (e.g. 1 mL) buffer to the Reagent tube. Vortex briefly and pipet the reconstituted solution to the Assay Buffer bottle. Repeat this step to transfer all of the Reagent to the Assay Buffer bottle. Mix by inversion until the Reagent is thoroughly dissolved. After this is done, mark the bottle label as Reconstituted Reagent.
D. Add 30 µL (per 25 µL of cell culture) of the reconstituted Reagent per well and mix well with the cells. Incubate for 2 minutes at room temperature. The volume of the reagent can be adjusted depending on the volume of cell culture.

E. Measure luminescence on a luminometer. The integration time can be 1 sec to 2 min depending on the luciferase expression level and instrument sensitivity. For most luminometers (Berthold Luminometer, LJL Analyst, Top Count, MicroBeta Counters, CLIPR and LeadSeeker), integration 1 to 5 sec is appropriate.

5. General considerations

**Incubation time.** Both the luciferin/luciferase reaction and cell lysis are fast, so incubation for 2 to 10 minutes is generally enough.

**Cell number.** The optimized reporter gene assay reagent is very sensitive to luciferase (detection limit 2 fg) and exhibits linearity over seven orders of magnitude. As few as 4 cells can be determined and a linear response is still observed with as many as 80,000 cells per 96-well. For assay optimization, it is recommended that the number of cells per well be determined and the optimal cell number be established by serial dilution of cells. Cells can be adherent or in suspension cultures.

**Cell lysis and mixing.** For the sake of convenience, the addition of 1 volume of reconstituted reagent to 1 volume of cells allows a sufficient mixing. No additional mixing is required since the specially formulated buffer instantly lyses mammalian cells.

6. Data analysis

The light intensity (RLU) is directly proportional to the luciferase concentration. For dose-response studies, the data are plotted against compound concentration and the EC$_{50}$ for gene up-regulator compound and IC$_{50}$ for a gene down regulator compound can be determined by non-linear regression analysis using Prism or other data analysis tools.

7. Literature

**Gene Regulation (receptors, small molecules, transcription factors)**


Characterization of promoter activity (promoter identification, polymorphism, environmental chemicals)


Modulation of gene expression by small molecules


High-throughput screening for gene modulators Reviews


cAMP response element (CRE) dependent


Proto-oncogene MYCN dependent


IL-2 promoter dependent

**Hypoxia-response element (HRE) dependent**


**Cyclooxygenase-2 (COX-2) promoter dependent**


**DPC4 (deleted in pancreatic carcinoma) dependent**


**Zeta-globin promoter dependent**


**NFAT-depedent**


**Miscellaneous**


**Serum response element (SRE) dependent**

SuperLight Luciferase Reporter Gene Assay Kits

Technical Notes

The CellQuanti-Blue assay kit has been specially optimized and formulated to provide a sensitive, convenient and robust assay for cell proliferation and cytotoxicity. Key features of the kits are as follows:

- **Safe.** Non-radioactive assay.

- **High sensitivity and wide detection range:** detection of as little of 2 fg luciferase. The emitted light is linear over seven orders of magnitude. Detection of as few as 4 cells.

- **Compatible with routine laboratory and HTS formats:** assays can be performed in tubes or microplates, on LAL Analyst, Berthold Luminometer, Top-Count, MicroBeta counters and chemiluminescent image plate readers (CLIPR/LeadSeeker). Assay reagents compatible with all liquid handling systems.

- **Saves time.** High-throughput assay in 96-well and 384-well plates allows simultaneous processing of a large number of samples.

- **Homogeneous and convenient.** A single reagent and "mix-incubate-measure" type assay. No wash and reagent transfer steps are involved.

- **Robust and amenable to HTS.** Z’ factors of 0.7 to 0.9 are routinely observed in 96-well and 384-well plates. Can be readily automated on HTS liquid handling systems.
Figure 1. Linearity of the SuperLight Luciferase Assay. Firefly luciferase was diluted in the Assay Buffer in a white opaque 384-well plate. The SuperLight Luciferase Reagent was added and mixed with the enzyme. The detection limit estimated from the blank controls was 2 fg. Light intensity was linear from 2 fg up to 46 ng.

![Graph showing linearity of the SuperLight Luciferase Assay](image1)

\[ R^2 = 0.999 \]

Figure 2. Linear relationship between emitted light and cell number. HEK293 cells were transiently transfected with a CRE-luciferase reporter construct. Cells were treated with 100 µM forskolin to stimulate luciferase expression. Cells were then serially diluted and treated with SuperLight Luciferase Assay Reagent in a white opaque 384-well plate. The light intensity (RLU) was linear (\( R^2 = 0.997 \)) to the cell number. The detection limit estimated from the blank controls was 4 cells.

![Graph showing linear relationship between emitted light and cell number](image2)

\[ R^2 = 0.998 \]

Figure 3. Up-regulation of CRE-dependent luciferase expression by prostaglandin E2 (PGE2) and adenylyl cyclase activator forskolin in HEK293 cells transiently transfected with a CRE-luciferase construct. Cells were treated with PGE2 and forskolin for 18 hours prior to the reporter gene assay. Data represent mean ± SD (n = 3). The curves were fitted using Prism. The EC_{50} for PGE2 and forskolin were 0.15 µM and 3.5 µM, respectively. Hill slopes were 1.3 and 1.4, respectively.

![Graph showing up-regulation of CRE-dependent luciferase expression](image3)

\[ [\text{Compound}], \text{µM} \]

\[ \text{RLU} \]