Luciferase Reporter Gene Assay Kit

Catalog Number KA1640

200 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

- ✓ Gene Regulation: gene expression level, characterization of promoter activity, modulation of gene expression by receptors, transcription factors and small molecules.
- ✓ Drug Discovery: high-throughput screen for gene modulators.

Key Features

- ✓ High sensitivity and wide detection range: detection of as little of 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.

Principle of the Assay

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

Reaction scheme:

$$ATP + D\text{-}luciferin + O_2 \xrightarrow{Luciferase} oxyluciferin + AMP + PPi + CO_2 + light$$

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 step (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 40 min), the Luciferase Reporter Gene Assay Kit 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.

General Information

Materials Supplied

List of component

Component	Amount
Reagent	Solid
Assay Buffer	20 mL

Storage Instruction

Store the Reagent in the provided amber tube at -20°C and the Assay Buffer at 2-8°C. Shelf life: 12 months after receipt.

Materials Required but Not Supplied

Clear bottom 96/384-well plates (e.g. Corning Costar) and plate reader.

Precautions for Use

Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents.

Assay Protocol

The Luciferase Reporter Gene Assay Kit is based on the bioluminescence generated during the luciferin/luciferase reaction. The reconstituted reagent has been optimized to combine cell lysis and detection into one single step. Phenol red in culture media does not interfere in this assay. All data in the Technical Notes were obtained in media containing phenol red.

Reagent Preparation

Fresh reconstitution of the Reagent in Assay Buffer is recommended, although the reconstituted Reagent may be stable for up to 4 weeks when stored at -20 °C.

Assay Procedure

✓ Procedure using 96-well plate:

1. 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.
2. 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.
3. 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 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.
4. 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.
5. 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.

✓ Procedure using 384-well plate:

1. 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.
2. 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.
3. Reconstitute the Reagent using the same procedure as for the 96-well assay.
4. 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.
5. 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.

✓ General Considerations

- Incubation time. Both the luciferin/luciferase reaction and cell lysis are fast, so incubation for 2 to 10 minutes following reagent addition is generally enough for mammalian cells (e.g. HEK293, CHO).
- 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 optimal number of cells per well be determined 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.

Data Analysis

Calculation of Results

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.

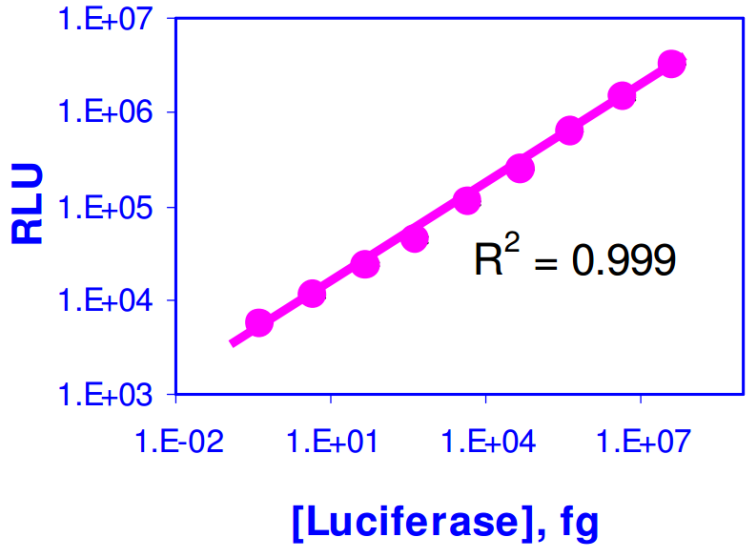

Figure 1. Linearity of the Luciferase Reporter Gene Assay Kit in 384- well plate. The detection limit estimated from the blank controls was 2 fg. Light intensity was linear from 2 fg up to 46 ng.

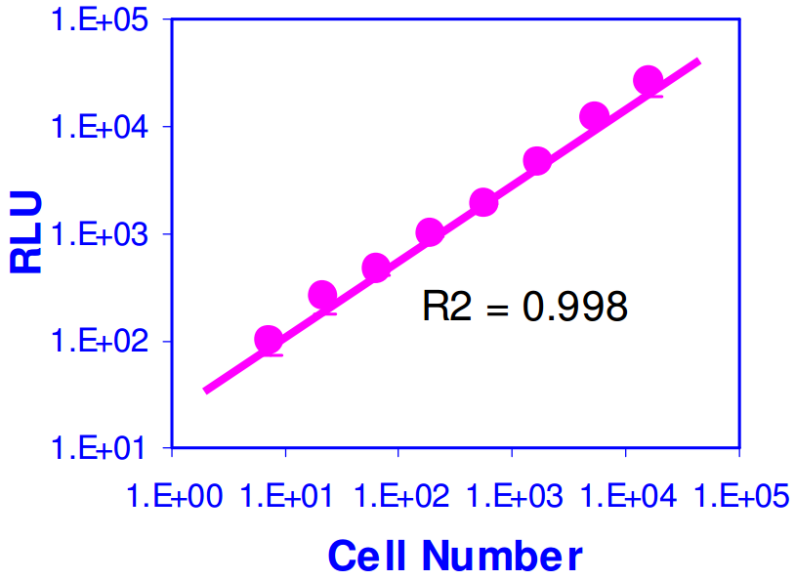

Figure 2. Linear relationship between emitted light and number of HEK293 cells transiently transfected with a CRE-luciferase reporter construct in a 384-well plate assay. Detection limit: 4 cells.

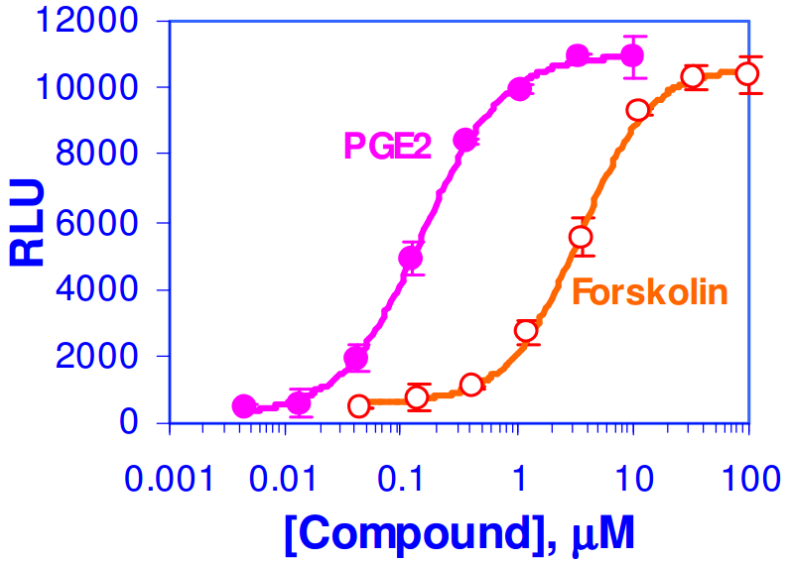

Figure 3. Up-regulation of CRE-dependent luciferase expression by prostaglandin E2 (PGE2) and adenylyl cyclase activator forskolin in HEK293 cells transiently transfected with a CRE-luciferase construct. EC_{50} = 0.15 µM for PGE2 and 3.5 µM for forskolin.