

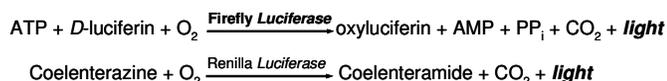
SuperLight™ Dual Luciferase Reporter Gene Assay Kit

Bioluminescent Assay for Dual Reporter Gene Systems

DESCRIPTION

The accuracy of reporter assays can be improved by utilizing a dual reporter system. One of the reporter genes is correlated with the promoter of interest and is used to assess the effects of specific experimental conditions, while the second reporter is used as a control and serves as a baseline response. The SuperLight™ Dual-Luciferase Reporter Gene Assay allows for the sequential measurement of the activity of two different luciferases, firefly (*FFL*) and *Renilla* (*RL*), in a single sample. The firefly luciferase luminescence is measured first by addition of the *FFL* Reagent. Next, the *RL* Reagent is added to the same well. The *RL* Reagent simultaneously quenches the firefly luciferase luminescence and initiates the *Renilla* luciferase reaction. The light production of both reactions can be conveniently measured on a luminometer.

Reaction schemes:



This bioluminescent dual reporter gene assay is extremely sensitive and is especially suitable for quantifying dual luciferase expression in recombinant cells or in cell free transcription/translation reactions. Assays can be performed in tubes, cuvettes or multi-well plates.

KEY FEATURES

High sensitivity and wide detection range: detection of as little of 2 fg luciferase.

Compatible with routine laboratory and HTS formats: assays can be performed in tubes or microplates, and measured with any luminometer. Can be readily automated on HTS liquid handling systems.

Fast and convenient: three step assay allows detection of dual luciferase levels within 20 minutes.

APPLICATIONS

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.

KIT CONTENTS (100 ASSAYS IN 96-WELL PLATES)

Lysis Buffer: 25 mL **FFL Reagent:** 25 mg
Assay Buffer: 25 mL **RL Reagent:** 400 mg

Custom bulk reagent available upon request.

Storage conditions. The kit is shipped at ambient temperature. Store all Reagents at -20°C. Shelf life: 12 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Reagent Preparation: fresh reconstitution of the *FFL* and *RL* Reagents in Assay Buffer is recommended. Calculate the amount of each reagent needed (100 µL of each per assay for 96 well plates or 25 µL per assay for 384 well plates) and weigh out sufficient amounts of each reagent (2 mg/mL for *FFL* Reagent and 35 mg/mL for *RL* Reagent). Gently shake each reconstituted reagent until they are completely dissolved (~10 min). The reconstituted *FFL* Reagent is stable for up to 4 weeks when stored at -20 °C; however, prolonged storage of reconstituted *RL* Reagent is not recommended and ideally it should be used within 4 hours.

Sample Preparation

1. Remove media from cell cultures and wash cells with PBS.
2. Add enough Lysis Buffer to fully cover the cell monolayer (e.g. 50 µL per well of a 96 well plate) and place culture plates on an orbital shaker. Gently shake at RT for 15 min.
3. Transfer lysates to tubes for storage (lysates can be stored for 8 hours on ice or overnight at -20°C). Alternatively, the dual luciferase assay

can be performed directly in the culture plate if cells were cultured in opaque plates.

Dual Luciferase Assay Procedure

1. If not assaying directly in the culture plate, transfer lysate to an opaque white plate (50 µL for 96 well plates, 12.5 µL for 384 well plates).
2. Add reconstituted *FFL* Reagent to each well. (100 µL for 96 well plates, 25 µL for 384 well plates).
3. Measure the firefly luciferase 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, LJM Analyst, Top Count, MicroBeta Counters, CLIPR and LeadSeeker), integration 1 to 5 sec is appropriate.
4. Add reconstituted *RL* Reagent to each well and gently tap to mix. (100 µL for 96 well plates, 25 µL for 384 well plates).
5. Measure *Renilla* luciferase luminescence on a luminometer.

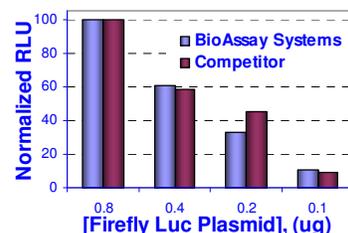
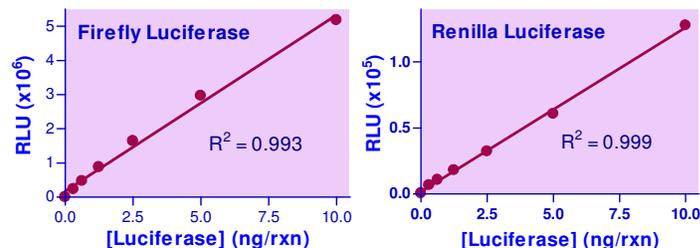
GENERAL CONSIDERATIONS

Incubation time. Both luciferase reactions are fast, so it is recommended that samples are read as soon as possible after addition of each reagent, preferably within 1 min.

Cell number. For assay optimization, it is recommended that the optimal number of cells per well be determined by serial dilution of cells.

DATA ANALYSIS

The light intensity (RLU) is directly proportional to the luciferase concentration. For each individual sample, the RLU of the experimental reporter should be normalized by RLU of the control reporter.



Upper Figures. Luciferase Titrations: Luciferases diluted in cell lysate and assayed in a 96 well plate.

Left Figure. Titration of firefly luciferase plasmid in HEK293 cells with 0.2 ug of *Renilla* luciferase plasmid as the control. 99.97% of firefly luciferase light is quenched during the *Renilla* luciferase assay.

REFERENCES

1. Paguio A, et al. (2010). Improved dual-luciferase reporter assays for nuclear receptors. *Curr Chem Genomics*. 4: 43-9.
2. Rakauskaitė R, et al. (2011). A rapid, inexpensive yeast-based dual-fluorescence assay of programmed-1 ribosomal frameshifting for high-throughput screening. *Nucleic Acids Res*. 39(14): e97.
3. Jia S, et al. (2011). Relative quantification of protein-protein interactions using a dual luciferase reporter pull-down assay system. *PLoS One*. 6(10): e26414.

