APPLICATION NOTE

Measure dual-luciferase reporter gene activity with the SpectraMax Mini Multi-Mode Microplate Reader

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Introduction

Firefly luciferase is a widely used reporter to study gene regulation and function. It is a very sensitive reporter due to the lack of any endogenous luciferase activity in mammalian cells or tissue. Firefly luciferase is a 62-kDa protein that is active as a monomer and does not require subsequent processing for its activity. It catalyzes ATP-dependent oxidation of D-luciferin with the resulting emission of light (Figure 1A). Luciferase from the sea pansy *Renilla reniformis* is often used in multiplexed luciferase assays as a second reporter for normalizing transfection efficiency and for studying gene regulation and function. *Renilla* luciferase catalyzes coelenterazine oxidation by oxygen to produce light (Figure 1B).

A homogeneous reagent set (Dual-Glo® Luciferase Assay System) enables the measurement of both firefly and *Renilla* luciferase activity in a single sample, with firefly acting as the experimental reporter and *Renilla* the control. Here the assay was set up with transfected cell samples to assess assay performance and sensitivity. Both luciferase reactions are easily measured in Molecular Devices SpectraMax® Mini Multi-Mode Microplate Reader, with ratiometric data analysis performed using SoftMax Pro® Software.

Benefits

- Detect firefly and *Renilla* luciferase expression in 20 or fewer cells per well
- Improve accuracy of results by normalizing reporter expression to a control
- See your results faster with SoftMax Pro Software's data analysis and graphing

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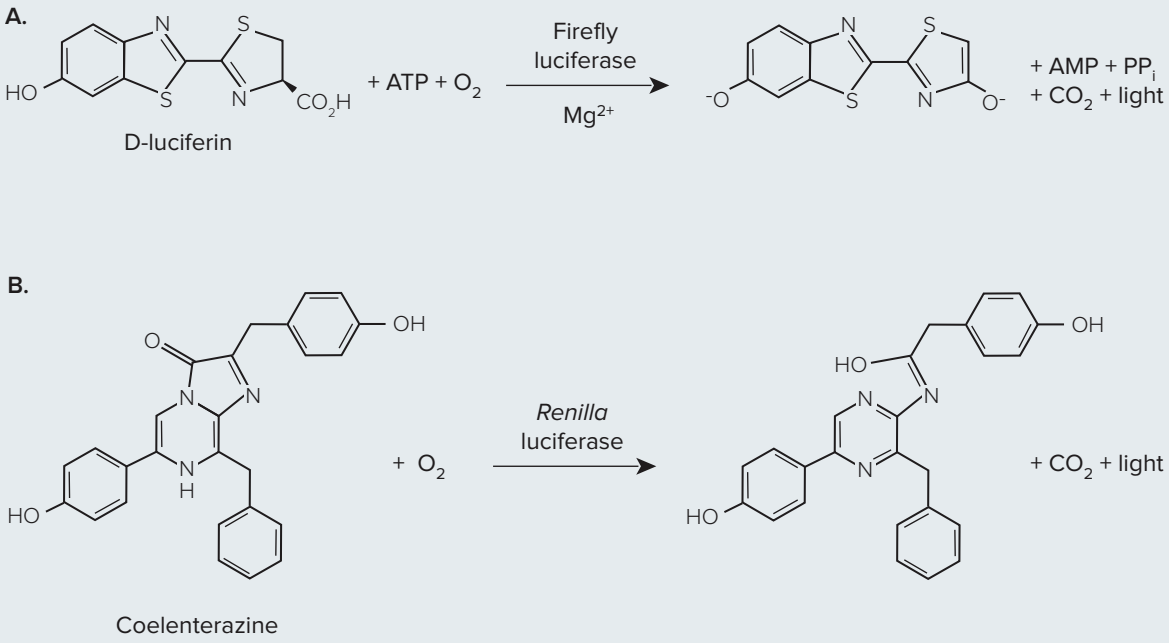

Figure 1. *Bioluminescent reactions catalyzed by (A) firefly luciferase and (B) Renilla luciferase.*

Materials

- Dual-Glo® Luciferase Assay System (Promega cat. #E2920)
- HeLa cells (ATCC cat. #CCL-2)
- pGL4.13[*luc2*/SV40] firefly luciferase expression vector (Promega cat. #E668A)
- pGL4.75[*hRluc*/CMV] *Renilla* luciferase expression vector (Promega cat. #E693A)
- ViaFect™ Transfection Reagent (Promega cat. #E4981)
- Opti-MEM Reduced Serum Medium (ThermoFisher Scientific cat. #31985062)
- Passive Lysis Buffer (Promega cat. #1941)
- Solid white 96-well microplate (Greiner cat. #655075)
- SpectraMax Mini Multi-Mode Microplate Reader (Molecular Devices)
- Luminescence Filter Cube (Molecular Devices P/N 5089334)

Cell-based dual-luciferase assay

Cell transfection

HeLa cells were seeded at $2x10^5$ cells per well in 6-well tissue-culture treated plates and incubated at 37°C/5% CO_2 for 24 hours prior to transfection. The pGL4.13[*luc2*/SV40] firefly luciferase expression vector was diluted in Opti-MEM medium to 1 µg/µL, and the pGL4.75[*hRluc*/CMV] *Renilla* luciferase expression vector was diluted to 100 ng/µL. Three tubes were set up as follows and mixed gently: 400 µL of Opti-MEM medium + 2 µL (2 µg) of pGL4.13[*luc2*/SV40] firefly luciferase expression vector + 2 µL (2 ng) of pGL4.75[*hRluc*/CMV] *Renilla* luciferase expression vector. To each tube, 6 µL of ViaFect reagent was added, and the contents were gently mixed by tapping. Tubes were incubated for 10 minutes at room temperature to allow complexes to develop. 200 µL of transfection complex was added dropwise to each well of the 6-well plate, with gentle swirling to mix.

Cells were incubated at 37°C/5% CO_2 for 48 hours after transfection and then harvested for assays.

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Preparation of cell samples

Cellular samples suitable for testing instrument performance were prepared as follows. Transfected cells in the 6-well plate were trypsinized, counted, and divided into aliquots containing about 60,000 cells each. Cells were then spun down at 1500 rpm for 5 minutes and washed once with PBS. PBS was removed, and cell pellets were stored at -80°C until the time of assay.

To make cell lysates representing known numbers of transfected cells, an aliquot of frozen cells was thawed to room temperature and lysed by adding 150 µL of Passive Lysis Buffer and pipetting up and down gently, followed by a 15-minute incubation at room temperature to ensure complete lysis. Lysates were diluted with 450 µL of cell culture medium to arrive at a sample containing about 10^5 cells/mL. From this lysate, a series of 1:2 dilutions were made in medium.

Assay setup

75 µL of each dilution of cell lysate, representing from about 15 to 7500 cells per well, was pipetted into triplicate wells of a 96-well white plate. A set of replicates containing medium or buffer only was included as a blank.

75 µL of Dual-Glo Luciferase Assay Reagent was added to each assay well; the plate was incubated at room temperature for 10 minutes, and then firefly luminescence was measured on the SpectraMax Mini reader using a 0.5-second integration time. 75 µL of Dual-Glo Stop & Glo Reagent was then added to assay wells, the plate was incubated at room temperature for 10 minutes, and *Renilla* luminescence was measured on the SpectraMax Mini reader using a 0.5-second integration time.

SoftMax® Pro software was used to analyze and graph the data. The luminescence measured (relative light units, RLU) for firefly luciferase in each sample was divided by its corresponding *Renilla* measurement to calculate the normalized signal.

Representative data

Figure 2 shows representative plots for firefly and *Renilla* luciferase detected in transfected HeLa cells. These results indicate that both luciferases can be detected in fewer than 20 cells per well using the Dual-Glo assay. Normalization of firefly to *Renilla* luciferase activity (Figure 3) helps to correct for experimental variability such as cell number and well-to-well reporter gene transfection efficiency. The SpectraMax Mini reader offers luminescence sensitivity enabling a variety of experimental workflows for easy detection of luciferase assay readouts, with easy data analysis using SoftMax Pro Software.

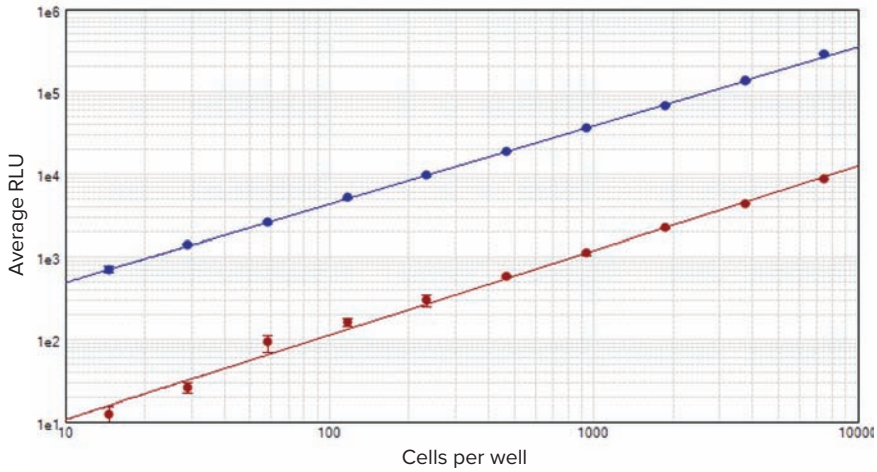

Figure 2. *Cell-based assay standard curve. Firefly (blue plot) and Renilla (red plot) curves were plotted using a log-log curve fit in SoftMax Pro Software ($r^2 > 0.99$ for each). N = 3 replicates at each cell number.*

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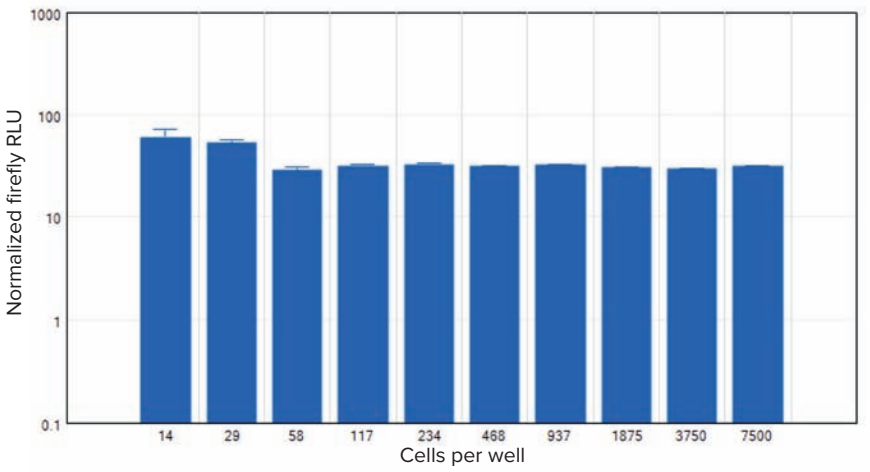

Figure 3. *Normalized firefly luciferase RLU vs. cell concentration. SoftMax Pro Software can automatically calculate and graph luminescence values for firefly luciferase normalized to Renilla luciferase values. N = 3 replicates at each cell number.*

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