Promega

Application Note

A GloMax® 96 Microplate Luminometer Method for Dual-Glo® Luciferase

1. INTRODUCTION

The GloMax® 96 Microplate Luminometer in combination with the Dual-Glo® Luciferase Assay System provides a convenient, rapid, sensitive procedure for quantifying gene expression. Transcriptional regulation, coupled to the expression of a luciferase reporter gene, is regularly used to study a wide range of biological events in cultured cells. Luciferase is an ideal reporter because of the absence of endogenous luciferase activity in mammalian cells and the functional enzyme is created immediately upon translation[1,2].

The Dual-Glo® Assay System contains two different luciferase reporter enzymes that are expressed simultaneously in each cell. Typically, the experimental reporter is correlated with the effect of specific experimental conditions, while the activity of the co-transfected "control" reporter gene provides an internal control, which serves as the baseline response. Normalizing the experimental reporter gene to the activity of an internal control minimizes the variability caused by differences in cell viability and transfection efficiency. Thus, dual-reporter assays allow for more reliable interpretation of the experimental data by reducing extraneous influences. The experimental and control luciferase enzymes used in the Dual-Glo® Luciferase Assay have distinct evolutionary origins. The firefly luciferase and the *Renilla* (sea pansy) luciferase can discriminate between their respective bioluminescent substrates and do not cross-activate.

The firefly and *Renilla* substrates maximize the sensitivity of the assay reagent and still provide a long-lasting luminescent signal. This system is widely used in the pharmaceutical and biotechnology industries because of the superior light generation and high signal to noise ratio. The Dual-Glo® Reagents are compatible with commonly used culture media for mammalian cells including RPMI 1640, MEMα, DMEM, and Ham's F12.

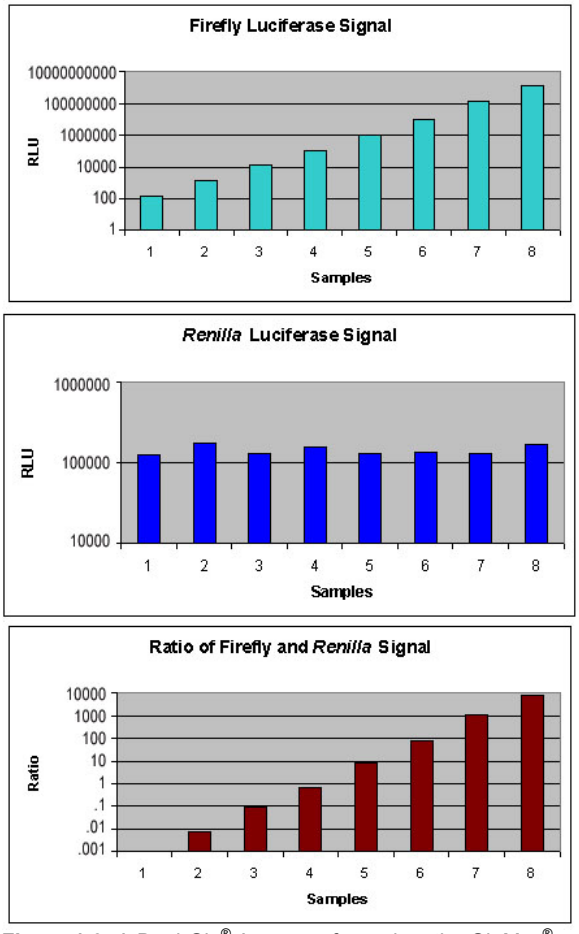

Figure 1-3: A Dual-Glo® Assay performed on the GloMax® 96 Microplate Luminometer using the Dual-Glo® System with recombinant firefly ($1x10^{-18}$ to $1x10^{-11}$ moles) and *Renilla* luciferase ($1x10^{-14}$ moles).

The highly sensitive GloMax® 96 Microplate Luminometer is particularly suited for the Dual-Glo® Luciferase Assay. The GloMax® 96 can detect as little as $1×10^{-18}$ moles firefly luciferase enzyme using the Dual-Glo® Reagent and $1×10^{-17}$ moles *Renilla* enzyme using Stop & Glo® Reagent. Measurements are linear for more than 7 and 5 orders of magnitude for firefly and *Renilla* substrates, respectively. All tests were conducted using purified recombinant firefly luciferase enzyme (Cat.# E1701) and purified *Renilla* recombinant enzyme (Chemicon Cat.# 4400).

2. MATERIALS REQUIRED

- GloMax® 96 ™ Microplate Luminometer
- 96-well plates, white (E&K Scientific EK-25075)
- Dual-Glo® Luciferase Assay System (Cat.# E2920, E2940, E2980)
- p200 pipette and pipette tips

3. PROTOCOL

3.1 Reagent Preparation

Dual-Glo® Luciferase Assay Buffer: Use as supplied. Store below 25°C.

Dual-Glo® Luciferase Substrate: Use as supplied. Store at -20°C.

Stop & Glo® Buffer: Use as supplied. Store below 25°C.

Stop & Glo® Substrate: Use as supplied. Store at -20°C.

Transfer the contents of one bottle of Dual-Glo® Luciferase Buffer to one bottle of Dual-Glo® Luciferase Substrate to create the Dual-Glo® Luciferase Reagent. Mix by inversion until the substrate is thoroughly dissolved. Use reconstituted Dual-Glo® Luciferase Reagent on the same day it is prepared or aliquot into working volume and store at -70°C for up to one month.

Calculate the amount of Dual-Glo® Stop & Glo® Reagent needed to perform desired experiments. Dilute the Dual-Glo® Stop & Glo® Substrate 1:100 into an appropriate volume of Dual-Glo® Stop & Glo® Buffer in a new container. Prepare reconstituted Dual-Glo® Stop & Glo® Reagent on the day it is to be used.

Note: The temperature of the Dual-Glo® Luciferase Buffer and Dual-Glo® Stop & Glo® Reagent should be held constant at room temperature while quantifying luminescence since luciferase activity is temperature dependent. Reagent stored frozen after reconstitution must be thawed below 25°C to ensure performance. Mix well after thawing. The simplest method for thawing is to place the reagent in a water bath at room temperature.

3.2 Instrument Setup

3.2.1 Double-click on the GloMax® 96 icon to start the software.

3.2.2 Click on the "Create New Protocol" button. The wizard will help you create a new protocol with zero injectors.

3.2.3 Enter your information into the "Experiment", "Operator", "Plate No.", and "Notes" fields in the "Main Dialog Box".

3.3 Sample Analysis

3.3.1 Remove the 96-well plate containing cell cultures from the incubator.

Note: For maximum reproducibility, equilibrate cell cultures to room temperature before adding reagent.

3.3.2 To measure the firefly luciferase activity, add a volume of Dual-Glo® Luciferase Reagent equal to the culture medium volume to each well and mix. For 96-well plates, typically 75 µL of reagent is added to cells grown in 75 µL of medium.

3.3.3 Wait for a minimum of 10 minutes, then place the sample plate in the GloMax® 96 ™ Microplate Luminometer. Click "Start" to begin measurement. Optimal results will be generated if the sample plate is read within 2 hours after the addition of Dual-Glo® Luciferase Reagent.

3.3.4 To measure the *Renilla* luciferase activity, add a volume of Dual-Glo® Stop & Glo® Reagent equal to the original culture medium volume to each well and mix. Again, this volume is typically 75 µL for 96-well plates.

3.3.5 Wait for a minimum of 10 minutes, then place the sample plate in the GloMax® 96 Microplate Luminometer. Click "Start" to begin measurement. Optimal results will be generated if the sample plate is read within 2 hours after the addition of Stop & Glo® Reagent.

3.3.6 Once the measurements are complete, remove your sample plate.

4. REFERENCES

1. Ow, D.W. *et al.* (1986) Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants. *Science* **234**, 856–9.

2. De Wet, J.R. *et al.* (1987) Firefly luciferase gene: structure and expression in mammalian cells, *Mol. Cell. Biol.* **7**, 725–37.

CAUTION: The lyophilized Dual-Glo® Luciferase Substrate contains dithiothreitol (DTT) and is therefore classified as hazardous. The reconstituted reagent is not known to present any hazards as the concentration of DTT is less than 1%. However, we recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents. Promega assumes no liability for damage resulting from handling or contact with these products.

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