Promega

Application Note

A GloMax® 96 Microplate Luminometer Method for

the Dual-Luciferase® Reporter (DLR) Assay

1. INTRODUCTION

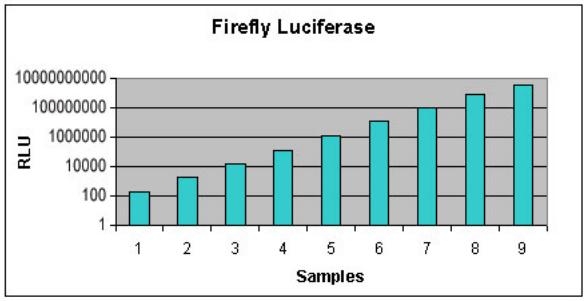
The GloMax® 96 Microplate Luminometer in combination with the Dual-Luciferase® Reporter (DLR) Assay provides a convenient, rapid, sensitive procedure for quantifying gene expression. Transcriptional regulation, coupled to the expression of a luciferase reporter gene, is regularly used to study a wide 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].

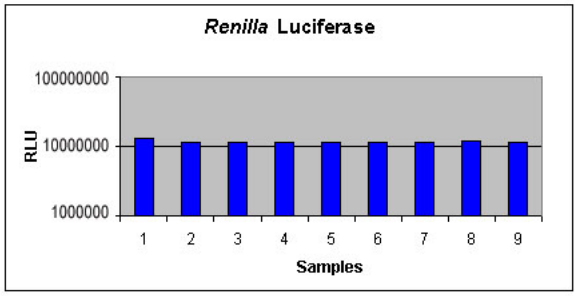
The Dual-Luciferase® Reporter (DLR) Assay System contains two different luciferase reporter enzymes that are expressed simultaneously in each cell. Typically, the experimental reporter is correlated with the effect of specific experimental conditions, while the activity of the co-transfected "control" 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 more reliable interpretation of the experimental data by reducing extraneous influences. The experimental and control luciferase enzymes used in the Dual-Luciferase® Reporter (DLR) 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.

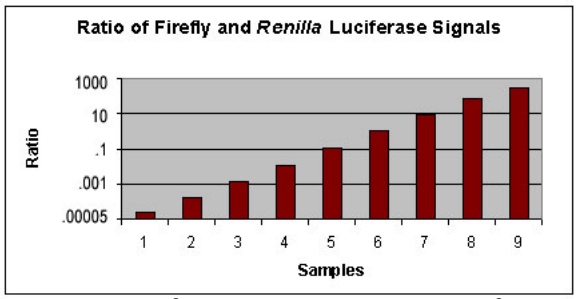
The firefly and *Renilla* substrates have been developed specifically to maximize the sensitivity of the assay reagent. This system is widely used in life science research because of superior light generation and high signal-to-noise ratio. The Dual-Luciferase® Reporter (DLR) reagents are compatible with commonly used culture media for mammalian cells including RPMI 1640, MEMα, DMEM, and Ham's F12. These reagents can also be used with the Passive Lysis Buffer that comes with the kit and is available separately (Cat.# E1910).

The sensitivity and wide dynamic range offered by the GloMax® 96 Microplate Luminometer make it highly suited for the Dual-Luciferase® Reporter (DLR) Assay application. The GloMax® 96 software facilitates a quick set-up for the Dual-Luciferase® Reporter Assay with a pre-installed template. The internal automatic injectors are easy to use and completely accessible.

The GloMax® 96 Microplate Luminometer can detect as little as 1×10^{-19} moles firefly luciferase enzyme using Luciferase Assay Reagent II (LAR II) and 1×10^{-18} moles *Renilla* enzyme using Stop & Glo® Reagent. Measurements are linear for more than 8 and 6 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).







Figures 1–3. DLR® assay performed on the GloMax® 96 Microplate Luminometer using the Dual-Luciferase® Reporter Assay System with recombinant firefly luciferase (1×10^{-19} moles to 1×10^{-11} moles) and *Renilla* luciferase (1×10^{-14} moles).

2. MATERIALS REQUIRED

- GloMax® 96 Microplate Luminometer
- 96-well plates, white (E&K Scientific EK-25075)
- Dual-Luciferase Reporter® Assay kit (Cat.# E1980)
- p200 pipette and pipette tip

3. EXPERIMENT PROTOCOL

3.1 Reagent Preparation

Luciferase Assay Buffer II and Luciferase Assay Substrate: Use as supplied. Store at -20°C, where it is stable for up to 6 months. The Luciferase Assay Substrate may also be stored at 4°C for up to one month.

Transfer the contents of one bottle of Luciferase Assay Buffer II into one vial of Luciferase Assay Substrate. Mix by inversion until the substrate is thoroughly dissolved. Use reconstituted Luciferase Assay II Reagent (LAR II) on the same day it is prepared, or aliquot into working volume and store at -20°C for 1 month or 70°C for up to one year.

Stop & Glo® Substrate and Stop & Glo® Buffer: Use as supplied. Store below -20°C.

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

To make Stop & Glo® Reagent, dilute the 50X Stop & Glo® Substrate to 1X concentration using Stop & Glo® Buffer in a glass or siliconized polypropylene tube. Mix by inversion. Use reconstituted Stop & Glo® Substrate on the same day it is prepared or store at –20°C for up to two weeks.

Passive Lysis Buffer: To make 1X Passive Lysis Buffer, dilute the 5X Passive Lysis Buffer in DI water. Store below 25°C.

Note: The temperature of the Luciferase Assay Buffer II and Stop & Glo® Buffer should be held constant at room temperature while quantifying luminescence, as luciferase activity is temperature-dependent. Reagent stored frozen after reconstitution must be thawed below 25°C to ensure reagent 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 "Run Promega Protocol" from the "Welcome to Veritas" dialog box.

3.2.3 Browse the files in the "DLR" folder and choose the appropriate protocol. For example, if you are using DLR with one injector select "DLR with one injection."

3.2.4 Click on "Options" to select the wells to be read. The default settings on the

Promega protocols pre-installed are optimized. However, you may wish to modify the integration time, delay before measurement and injection volume in the "Other Options" tab. Once you have made your choices, click the "Apply Changes" button to accept changes or the "Save Protocol As" button to save the protocol. You can return to this screen by clicking "Options" from the "Main Dialog Box".

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

3.3 Sample Analysis

3.3.1 Prepare the 96-well plate containing lysed cell cultures.

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

3.3.2 Prepare the injectors. Place the intake tubing for injector 1 into the bottle of LAR II. Place the intake tubing for injector 2 into the bottle of Stop & Glo® Reagent. Prime both injectors using the "Prime" tab located on the "Main Dialog Box".

Note: Do not switch injectors. Residual Stop & Glo® Reagent will quench the firefly luciferase reporter activity. It is recommended to dedicate a single injector for Stop & Glo® Reagent and another injector for LAR II.

3.3.3 Insert the plate into the GloMax® 96 Microplate Luminometer and click "Start" to begin assay. RLU values measured by the GloMax® 96 Microplate Luminometer will appear in the Excel spreadsheet after all the selected wells in each row have been read. If you encounter an error message, refer to the troubleshooting guide for more information.

3.3.4 Once the measurements are complete you can access Excel to analyze your data.

3.3.5 Remove your plate after the measurements are complete. You can choose the "Reverse Purge" tab to return unused reagent to the bottle. Be sure to flush injectors thoroughly after use.

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.

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CAUTION: The lyophilized Luciferase® Assay 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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