

A GloMax[®] Multi Microplate Luminometer Method for Dual-Luciferase[®] Reporter Assay System

INTRODUCTION

The Dual-Luciferase[®] Reporter Assay (DLR) from Promega is ideal as a dual genetic reporting luminescence-based assay system for quantitation of gene expression. Two different luciferase reporter enzymes, the firefly (*Photinus pyralis*) and *Renilla* (sea pansy or *Renilla reniformis*), are expressed simultaneously in each cell creating an efficient means of performing two reporter assays in a single sample.

The GloMax[®] Multi Microplate Luminometer with dual injectors provides a convenient, rapid, and sensitive method for quantifying gene expression when used with the Dual-Luciferase[®] Reporter (DLR) Assay kit. The superior sensitivity and wide dynamic range of the GloMax[®] Multi Microplate Luminometer make it highly suited for the Dual-Luciferase[®] Reporter (DLR) Assay application. The GloMax[®] Multi Microplate Luminometer has a pre-programmed protocol for quick setup of the Dual-Luciferase[®] Reporter Assay. Results are reported in RLU for each of the luciferase readings and in Ratio (firefly RLU/*Renilla* RLU) for quick analyses. The GloMax[®] Multi Microplate Luminometer detects less than 1×10^{-19} moles of firefly luciferase enzyme using Luciferase Assay Reagent II (LAR II) and 1×10^{-18} moles of *Renilla* enzyme using Stop & Glo[®] Reagent. Measurements are linear for more than eight and six orders of magnitude for firefly and *Renilla* substrates, respectively. All tests were conducted using purified recombinant firefly luciferase enzyme (Ca.# E1701) and purified *Renilla* recombinant enzyme (Chemicon Catalog 4400).the GloMax[®] 96 Microplate Luminometer.

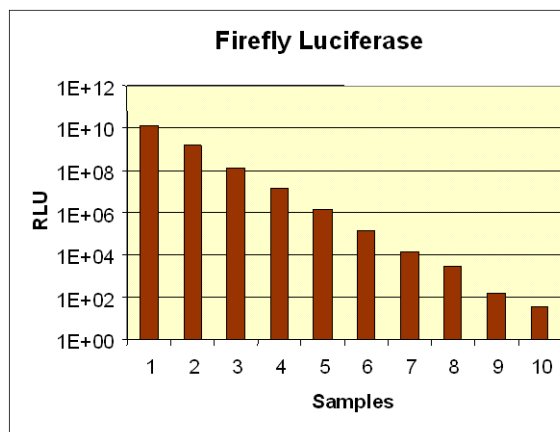


Figure 1. Firefly luciferase, 1×10^{-11} to 1×10^{-20} moles

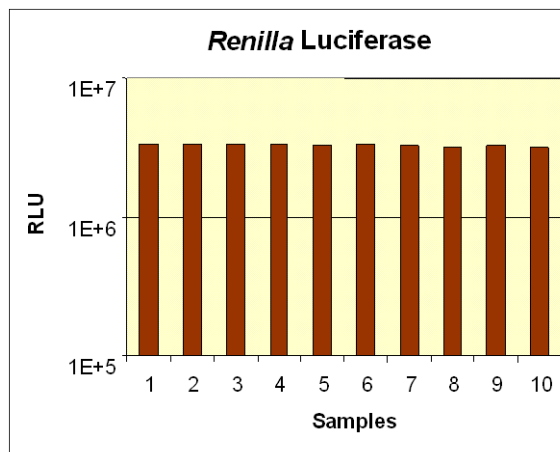


Figure 2. *Renilla* luciferase, 1×10^{-14} moles.

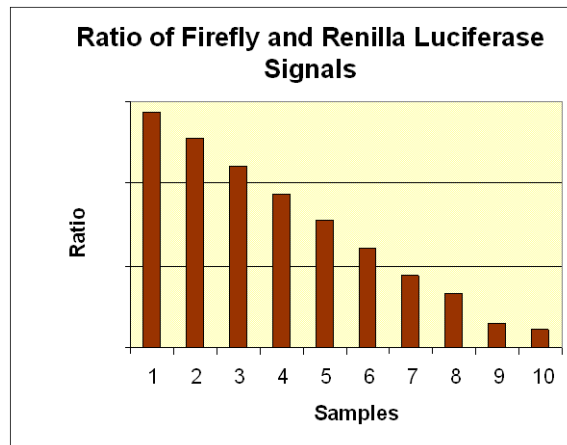


Figure 3. Ratio of firefly and *Renilla* luciferase signals. DLR assay was performed on the GloMax[®] Multi Microplate Luminometer using the Dual-Luciferase[®] Reporter Assay system with recombinant firefly luciferase and *Renilla* luciferase.

MATERIALS REQUIRED

- GloMax[®] Multi Microplate Multimode Reader
- GloMax[®] Multi Microplate Luminescence Module
- Promega Dual-Luciferase Reporter[®] Assay Kit (Cat.# E1980)
- 96-well, white microplates (E&K Scientific, EK-25075)
- P10, P200 pipette and pipette tips

EXPERIMENTAL PROTOCOL

Reagent Preparation Recommendation

1. **Luciferase Assay Buffer II and Luciferase Assay Substrate:** Use as supplied. Store at -20°C, where it is stable for up to six 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 Reagent II (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.

2. **Stop & Glo[®] Substrate and Stop & Glo[®] Buffer:** Use as supplied. Store below -20°C.
3. **Stop & Glo[®] Buffer Substrate Solvent:** Use as supplied. Store below 25°C.
4. **Stop & Glo[®] Reagent:** To make the 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.
5. **Passive Lysis Buffer:** To make 1x Passive Lysis Buffer, dilute the 5x Passive Lysis Buffer in DI water with 0.1% Gelatin. Store below 25°C.

Note: Since luciferase activity is temperature dependent, the temperature of the Luciferase Assay Buffer II and Stop & Glo[®] Buffer should be held constant at room temperature while quantifying luminescence. 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 placing the reagent in a water bath at room temperature.

Instrument Setup

1. From the Home screen, touch Select Protocol and follow the protocol wizard to select the preset Dual-Luciferase 2 injectors protocol. Enter the following: Luminescence; at the Preset tab, select Dual-Luciferase 2 Injectors; Finish.
2. The Instrument Control screen shows all the reading parameters: integration, each injector and injection volume, and delay time. All the wells in the plate are selected to be read. If desired, change the delay and integration time settings and save the changed protocol under a different protocol name in the User protocol folder.
3. Select the wells on the Plate Map according to the samples loaded into the plate.
4. Refer to the on-screen Help topics, Quick Start Guide, or Operating Manual for detailed instructions.

Sample Analysis

5. Prepare the 96-well plate containing lysed cell cultures accordingly.
Note: For maximum reproducibility, equilibrate cell cultures to room temperature before adding reagent.
6. 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.
7. Prime both injectors using the Setup icon on the Read Screen in Instrument Control. Place the Waste Collection Tray in the instrument. Follow the step-by-step injector set-up wizard to prime.
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.
8. Open the instrument door by using the Door icon on the touch screen. Place the plate with A1 well at the top right corner of the microplate sample tray. Close the door by using the Door icon.
9. Touch the Start icon on the touch screen to begin reading.
10. RLU values measured by the GloMax[®] Multi Microplate Luminometer will appear on the Results screen of the touch screen display immediately after each well is measured. Both readings for each well are displayed side-by-side. Toggle the Ratio icon to alternately view the RLU values and the ratio of the two (Injector 1 value/Injector 2 value).
11. Once the measurements are complete, data can be transferred to an external computer for further data analysis in Excel by using the provided USB flash drive.
12. Remove the plate after measurement completion.
13. Use the Reverse Purge function in the Set-Up wizard to return unused reagent to the bottle.

14. Rinse the injectors using the Flush function in the Set-Up wizard thoroughly after use.
15. Refer to the Technical Manual for detailed instructions of the use of the injectors.

RESULTS**Sensitivity:**

- $< 1 \times 10^{-19}$ moles of firefly luciferase using Luciferase Assay Reagent II (LAR II)
- $< 1 \times 10^{-18}$ moles of *Renilla* luciferase using Stop & Glo[®] Reagent

Dynamic Range:

- $>$ eight orders of magnitude for firefly luciferase
- $>$ six orders of magnitude for *Renilla* luciferase

CONCLUSION

The GloMax[®] Multi Microplate Luminometer offers superior sensitivity and dynamic range for luminescence detection, such as the luminescence based dual-reporter assays. The GloMax[®] Multi Microplate Luminometer achieves superior performance with a combination of unique detection and optical designs, premium components such as the photomultiplier tube (PMT), low-noise circuitry, and proprietary dual-masking system.

The modular approach of the GloMax[®] Multi Microplate Luminometer allows for instrument capability expansion as needs in the lab change. Fluorescence and/or absorbance detection modules as well as other accessories can be added after the initial purchase.

Superior performance, ease of use, and utmost flexibility of the GloMax[®] Multi Microplate make it an ideal microplate reader for today's life science laboratory.

The GloMax[®] Multi Microplate has passed the DLReady[™] validation criteria.

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.

CONTACT INFORMATION

Toll-Free: (800) 356-9526

Fax: (800) 356-1970

www.promega.com

Email: custserv@promega.com

Mailing Address:

Promega Corporation
2800 Woods Hollow Rd.
Madison, WI 53711 USA