Promega

Application Note

A GloMax® 96 Microplate Luminometer Method for the Bright-Glo™ Assay

1. INTRODUCTION

The GloMax® 96 Microplate Luminometer in combination with the Bright-Glo™ Assay kit provides a convenient, rapid, and 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 Bright-Glo™ Luciferase Assay System specifically maximizes the sensitivity of luciferase. This system is widely used in life science research because of the superior light generation and high signal to noise ratio. The Bright-Glo™ Reagent is compatible with commonly used culture media for mammalian cells (RPMI 1640, MEMα, DMEM and Ham's F12) and tolerates phenol red and organic solvents.

The GloMax® 96 Microplate Luminometer uses a unique system for light detection to maximize the sensitivity and range of the Bright-Glo™ Luciferase Assay. The GloMax® 96 can detect as little as 1×10^{-19} moles luciferase enzyme using the Bright-Glo™ Reagent. Measurements were linear from 1×10^{-19} moles to 3×10^{-11} moles luciferase or more than 8 orders of magnitude (Figure 1). All tests were conducted using Bright-Glo™ Luciferase Assay kit (Cat.# E2620) and purified recombinant firefly luciferase enzyme (Cat.# E1701).

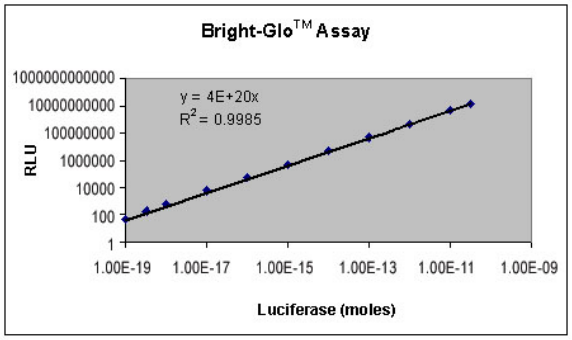

Figure 1. Bright-Glo™ Assay performed on the GloMax®96 Microplate Luminometer using Bright-Glo™ Reagent and recombinant luciferase.

2. MATERIALS REQUIRED

- GloMax® 96 Microplate Luminometer
- 96-well plates, white (E&K Scientific EK-25075)
- Bright-Glo™ Luciferase Assay kit (Cat.# E2610, E2620, E2650)
- p200 pipette and pipette tips

3. PROTOCOL

3.1 Reagent Preparation

Bright-Glo™ Substrate: Use as supplied. Store at -20°C, where it is stable for up to 6 months. The substrate may also be stored at 4°C for up to one month.

Bright-Glo™ Buffer: Use as supplied. Store below 25°C.

Bright-Glo™ Reagent: Transfer the contents of one bottle of Bright-Glo™ Buffer to one bottle of Bright-Glo™ Substrate. Mix by inversion until the substrate is thoroughly dissolved. Use reconstituted reagent on the same day it is prepared or aliquot into working volume and store at -20°C for up to two weeks.

Note: The temperature of the Bright-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 reagent performance. Mix well after thawing. The simplest method for thawing is placing 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 GloMax® 96" dialog box.

3.2.3 Select "BrightGlo" from the list of Promega protocols.

3.2.4 Click on "Options" from the "Main Dialog Box" to select the wells to be read, modify the number of runs, or select a delay time between runs from the "Plate Setup and Options" screen. You can also modify the integration time and delay before measurement 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.

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 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

A. If the cells are lysed, add a volume of the Bright-Glo™ Reagent equal to that of the culture medium or cell lysate in each well. For 96-well plates, typically 100 µL of reagent is added to cells grown in 100 µL of medium. Proceed immediately to 3.3.3.

B. If the cells are not lysed, add a volume of the Bright-Glo™ Reagent equal to that of the culture medium in each well. For 96-well plates, typically 100 µL of reagent is added to cells grown in 100 µL of medium. Wait at least two minutes for complete cell lysis before proceeding to 3.3.3.

3.3.3 Insert sample 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.

Note: Opening another Excel Spreadsheet while the GloMax® 96 reads your sample plate is strongly discouraged.

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

3.3.5 Remove your plate after measurements.

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 Bright-Glo™ 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