Promega

Application Note

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

1. INTRODUCTION

The GloMax® 96 Microplate Luminometer in combination with the Steady-Glo® Assay 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 Steady-Glo® Luciferase Assay System has been developed specifically to maximize the sensitivity of the assay reagent while providing a luminescent signal half-life of approximately five hours. The light signal can be measured between 5 minutes and several hours after adding assay reagents. The extended period of luminescence makes Steady-Glo® Luciferase Assay System especially appealing to laboratory automation and high-throughput applications. The Steady-Glo® Reagent is widely used in the pharmaceutical and biotechnology industries. The Steady-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 superior sensitivity of the GloMax® 96 Microplate Luminometer combined with the effectiveness of Steady-Glo® Reagent permits detection of very low levels of luciferase activity. The GloMax® 96 Microplate Luminometer can detect as little as 1×10^{-18} moles luciferase enzyme (Note: Assay limited sensitivity). Measurements are linear from 1×10^{-18} to 1×10^{-11} moles luciferase or 7 orders of magnitude (Figure 1). All tests were conducted using Steady-Glo® Luciferase Assay Kit (Cat.# E2520) and purified recombinant firefly luciferase enzyme (Cat.# E1701).

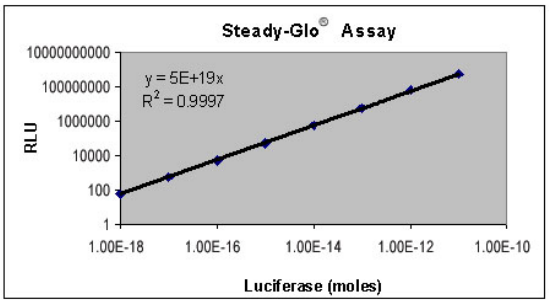

Figure 1. Steady-Glo® Assay performed on the GloMax® 96 Microplate Luminometer using the Steady-Glo® Reagent and recombinant luciferase.

2. MATERIALS REQUIRED

- GloMax® 96 Microplate Luminometer
- 96-well plates, white (E&K Scientific EK-25075)
- Steady-Glo® Luciferase Assay kit (Cat.# E2510, E2520, E2550)
- p200 pipette and pipette tips

3. PROTOCOL

3.1 Reagent Preparation

Steady-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.

Steady-Glo®Buffer: Use as supplied. Store below 25°C.

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

Note: The temperature of the Steady-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 Veritas" dialog box.

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

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

3.2.5 Click on "Options" from the "Main Dialog Box" and select the wells to be read, modify the number of runs, or set a delay time between each run. You can also modify the integration time in the "Other Options" tab. Once you have made your choices, click the "Apply Changes" button to return to 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 Add a volume of the Steady-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.

3.3.3 Wait a minimum of five minutes to allow for sufficient cell lysis, then proceed to measure with the GloMax® 96 Microplate Luminometer.

3.3.4 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.

Note: Opening another Excel spreadsheet while the GloMax® 96 reads your sample plate is not recommended.

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

3.3.6 Please make sure to remove your plate after measurement.

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 Steady-Glo® Substrate contains dithiothreitol (DTT) and is 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