Promega

Application Note

A GloMax® 20/20 Luminometer Method for Steady-Glo® Luciferase Assay System

1. INTRODUCTION

The GloMax® 20/20 Luminometer in combination with the Steady-Glo® Luciferase Assay System 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 5 hours. The light signal can be measured between 5 minutes and several hours after adding assay reagents. The Steady-Glo® Reagent is used widely in the pharmaceutical and biotechnology industries. It 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® 20/20 Luminometer combined with the effectiveness of the Steady-Glo® Reagent permits detection of very low levels of luciferase activity. The GloMax® 20/20 Luminometer can detect as little as 1 x 10^{-19} moles luciferase enzyme. Measurements are linear from 1 x 10^{-19} to 1 x 10^{-11} moles of luciferase or 8 orders of magnitude (Figure 1). All tests were conducted using the Steady-Glo® Luciferase Assay System (Cat.# E2520) and purified recombinant firefly luciferase enzyme (Cat.# E1701).

MATERIALS REQUIRED

- GloMax® 20/20 Luminometer
- 1.5 mL microcentrifuge tubes
- Steady-Glo® Luciferase Assay System (Cat.# E2510, E2520, E2550)
- p200 pipette and pipette tips

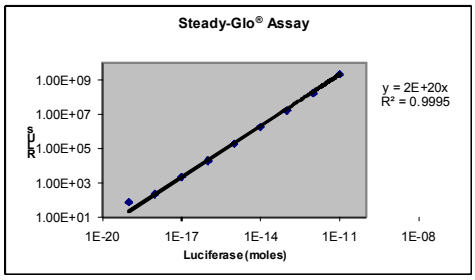

Figure 1. *Steady-Glo® Assay was performed on the GloMax® 20/20 Luminometer using the Steady-Glo® Luciferase Assay System and recombinant luciferase.*

EXPERIMENT PROTOCOL

1. Reagent Preparation

Steady-Glo® Substrate: Use as supplied. Store at –20°C, where it is stable for up to 6 months. The substrate also may be stored at 4°C for up to 1 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 2 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.

2. Instrument Setup

- Turn ON the GloMax® 20/20. A five-minute warm-up period is recommended but not necessary.
- Touch "Run Promega Protocol" from the "Protocols" menu.
- Select "Steady-Glo" from the list of Promega protocols. The "Parameters" screen appears next with pre-programmed settings that are optimized for the Steady-Glo® Assay.
- Touch "Advanced Options" to access "Replicates" and "Automatic Blank Subtraction" features (optional).
- Touch "OK" to go to the "Home" screen.

3. Sample Analysis

- Remove the cell cultures from the incubator. **Note:** For maximum reproducibility, equilibrate cell cultures to room temperature before adding reagent.
- Add a volume of the Steady-Glo® Reagent equal to that of the culture medium.
- Wait a minimum of five minutes to allow for sufficient cell lysis, then transfer the sample to a 1.5 mL microcentrifuge tube for analysis.
- Insert the tube into the GloMax® 20/20 using the microcentrifuge tube holder, and touch "Measure Luminescence" to begin measurement.

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 Steady-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.