

A Modulus™ Single Tube Reader Method for Promega Steady-Glo®

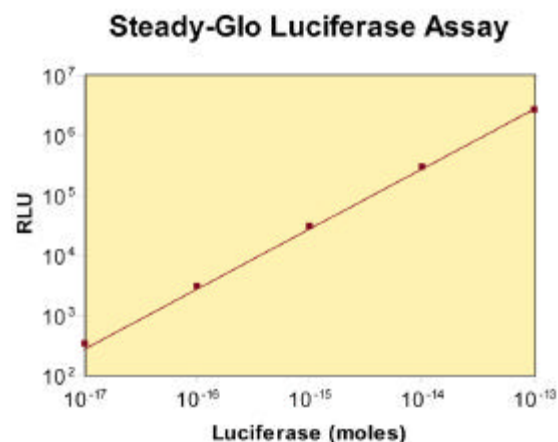


1. INTRODUCTION

The Modulus™ by Turner BioSystems in combination with Promega's Steady-Glo® Assay kit provides a convenient procedure for quantifying gene expression under the luciferase reporter system. 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.

The luminescent signal half-life of the Steady-Glo Reagent is approximately five hours. The light signal can be measured between 5 minutes and several hours after adding the reagent. The Steady-Glo Reagent is compatible with commonly used culture media for mammalian cells (RPMI 1640, MEM α , DMEM and Ham's F12) and tolerates phenol red and organic solvents.

In combination with the Luminescence Module and Modulus, measurements are linear from 1×10^{-18} to 1×10^{-11} moles luciferase or orders of magnitude (Figure 1). All tests were conducted using Steady-Glo Luciferase Assay Kit (Promega Catalog # E2520) and purified recombinant firefly luciferase enzyme (Promega



Catalog # E1701).

Figure 1. Steady-Glo Assay was performed on the Modulus using Promega's Steady-Glo Reagent kit and recombinant luciferase.

2. MATERIALS REQUIRED

- Modulus with Luminescence Module (P/N 9200-001 or 9200-002)
- 1.5 mL microfuge tubes
- Steady-Glo Luciferase Assay kit (Promega Catalog #'s E2510, E2520, E2550)
- p200 pipette and pipette tips

3. EXPERIMENT 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 Power OFF the Modulus. Install the Luminescence Module according to the *Operating Manual*.

3.2.1 Power ON the Modulus. A 5-minute countdown immediately after power up allows a warm-up period for the Modulus.

3.2.2 After the warm-up, the Modulus is ready to measure your samples. The default protocol with 1-second integration is suitable for the Steady-Glo Assay.

3.3 Sample Analysis

3.3.1 Remove the 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.

3.3.3 Wait a minimum of 5 minutes to allow for sufficient cell lysis. Then transfer the sample to a 1.5 mL centrifuge tube for analysis.

3.3.4 Insert the tube into the Modulus and touch "Measure Luminescence" to commence measurement.

3.3.5 Record the results in Relative Light Units (RLU). The Modulus displays the most recent 20 results on the touchscreen.

4. ABOUT PROMEGA CORPORATION

Steady-Glo is a trademark of Promega Corporation and is registered with the U.S. Patent and Trademark Office. Orders for Promega's products may be placed by:

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5. ABOUT TURNER BIOSYSTEMS

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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 and Turner BioSystems assume no liability for damage resulting from handling or contact with these products.