

A GloMax[®]-Multi Jr Method for Steady-Glo[®] Luciferase Assay System

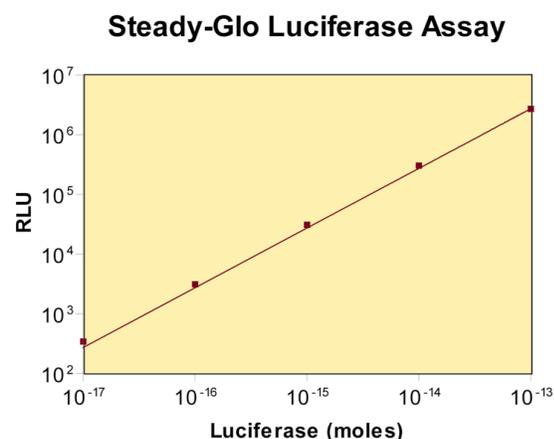


INTRODUCTION

The GloMax[®]-Multi Jr in combination with the Steady-Glo[®] Luciferase Assay System provides a convenient procedure for quantifying gene expression under the luciferase reporter system. Transcriptional regulation, coupled to the expression of a luciferase reporter gene, commonly is 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.

Using the GloMax[®]-Multi Jr Luminescence Module, measurements are linear from 1×10^{-18} to 1×10^{-11} moles of luciferase or orders of magnitude (Figure 1). All tests were conducted using Steady-Glo[®] Luciferase Assay System (Cat.# E2520) and purified recombinant firefly luciferase enzyme (QuantiLum[®] Recombinant



Luciferase; Cat.# E1701).

Figure 1. Steady-Glo[®] Luciferase Assay was performed on the GloMax[®]-Multi Jr using the Steady-Glo[®] Luciferase Assay System and QuantiLum[®] Recombinant Luciferase.

MATERIALS REQUIRED

- GloMax[®]-Multi Jr with Luminescence Module
- 1.5 mL microcentrifuge tubes
- Steady-Glo[®] Luciferase Assay System (Cat.# E2510, E2520, E2550)
- p200 pipette and pipette tips

3. 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 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.

2. Instrument Setup

- Power OFF the GloMax[®]-Multi Jr. Install the Luminescence Module according to the Technical Manual.
- Power ON the GloMax[®]-Multi Jr. A 5-minute countdown immediately after power up allows a warm-up period for the GloMax[®]-Multi Jr.
- After warm-up, the GloMax[®]-Multi Jr is ready to measure your samples. The default protocol with 1-second integration is suitable for the Steady-Glo[®] Assay.

3.3 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 5 minutes to allow for sufficient cell lysis. Then transfer the sample to a 1.5 mL centrifuge tube for analysis.
- Insert the tube into the GloMax[®]-Multi Jr, and touch “Measure Luminescence” to begin measurement.
- Record the results in Relative Light Units (RLU). The GloMax[®]-Multi Jr displays the most recent 20 results on the touch screen.

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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.

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