A GloMax®-Multi Jr Method for *Renilla*-Glo™ Luciferase Assay System

INTRODUCTION

The GloMax® Multi Jr. Luminometer in combination with the *Renilla*-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. *Renilla* Luciferase (36kDa) is about half the size of firefly luciferase (61kDa), does not require ATP, and the functional enzyme is created immediately upon translation.

The *Renilla*-Glo™ Luciferase Assay System is a single-addition reagent developed specifically to maximize the sensitivity of the assay reagent while providing a luminescent signal half-life greater than 60 minutes. The light signal can be measured 10–80 minutes after adding assay reagents. The Renilla-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® Multi Jr. Luminometer combined with the effectiveness of the *Renilla*-Glo™ Reagent permits detection of very low levels of luciferase activity. The GloMax® Multi Jr. can detect as little as 1 x 10^{-18} moles luciferase enzyme. Measurements are linear from 1 x 10^{-18} to 1 x 10^{-14} moles of luciferase or 4 orders of magnitude (Figure 1, 2). All tests were conducted using the *Renilla*-Glo™ Luciferase Assay System (Cat.# E2710) and purified recombinant *Renilla* luciferase enzyme.

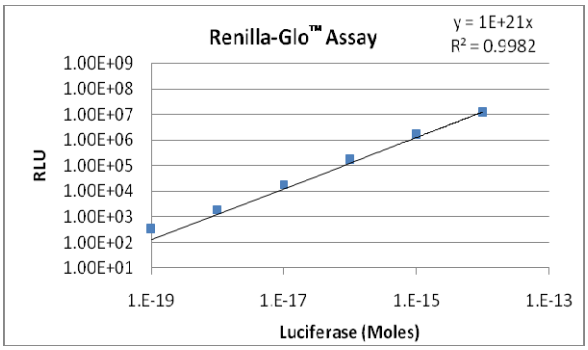

Figure 1. *Renilla-Glo™ Assay performed on the GloMax® Jr Luminometer using the Renilla-Glo™ Luciferase Assay System and recombinant luciferase. Luminescence was measured after 10 minutes.*

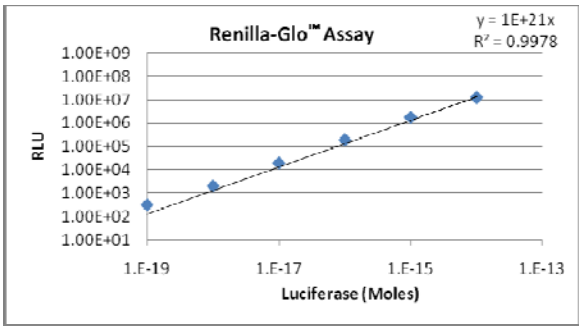

Figure 2. *Renilla-Glo™ Assay performed on the GloMax® Jr Luminometer using the Renilla-Glo™ Luciferase Assay System and recombinant luciferase. Luminescence was measured after after 20 minutes.*

MATERIALS REQUIRED

- GloMax®-Multi Jr
- 1.5 ml microcentrifuge tubes
- *Renilla*-Glo™ Luciferase Assay System (Cat.# E2710, E2720, E2750)
- p200 pipette and pipette tips

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

EXPERIMENTAL PROTOCOL

1. Reagent Preparation

***Renilla*-Glo™ Substrate:** Use as supplied.

***Renilla*-Glo™ Buffer:** Use as supplied.

***Renilla*-Glo™ Reagent:** Add one volume of 100X *Renilla*-Glo™ Luciferase Assay Substrate to 100 volumes of *Renilla*-Glo™ Luciferase Assay Buffer to generate an amount of *Renilla*-Glo™ Luciferase Assay Reagent sufficient to perform the desired experiment. Once reconstituted, the reagent will lose 10% activity in ~2 hours and 50% activity in ~12 hours at room temperature. The stability of the reconstituted reagent is greater at 4°C (10% loss in ~10 hours), but we recommend preparing the reagent immediately before use and not storing reconstituted reagent at any temperature.

Note: The temperature of the *Renilla*-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 to place 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. The instrument will indicate the 60-second countdown required for the instrument to warm up.
- After the 60-second warm up period, the instrument indicates it is ready to measure luminescence. Select “Protocol” and then “Default Protocol” or “Run Promega Protocol” and select any of the single luminescence measurement protocols with a 1 sec integration time.
- The option “Lid start is ON” can be activated by selecting “Settings” and “Lid Start”.

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 *Renilla*-Glo™ Reagent equal to that of the culture medium.
- Wait a minimum of 10 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® Jr using the microcentrifuge tube holder, and touch “Measure Luminescence” to begin measurement.

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