

A GloMax[®] 20/20 Luminometer Method for *Renilla-Glo*[™] Luciferase Assay System



INTRODUCTION

The GloMax[®] 20/20 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 specifically maximizes the sensitivity of the assay reagent while providing a luminescent signal half-life greater than 60 minutes. The light signal can be measured between 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, MEM α , 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 *Renilla-Glo*[™] Reagent permits detection of very low levels of luciferase activity. The GloMax[®] 20/20 Luminometer can detect as little as 1×10^{-18} moles luciferase enzyme. Measurements are linear from 1×10^{-18} to 1×10^{-13} moles of luciferase or 5 orders of magnitude (Figure 1). All tests were conducted using the *Renilla-Glo*[™] Luciferase Assay System (Cat.# E2710) and purified recombinant *Renilla* luciferase enzyme.

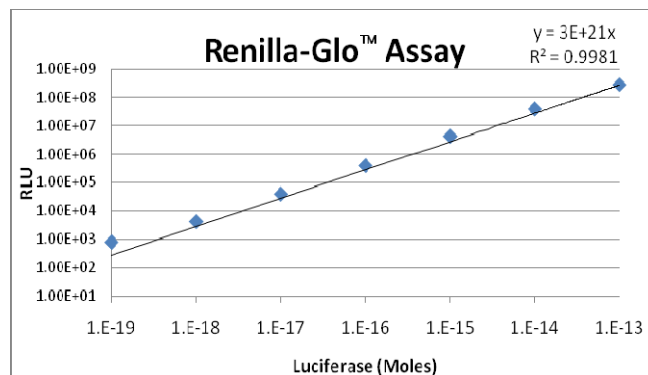


Figure 1. *Renilla-Glo*[™] Assay performed on the GloMax[®] 20/20 Luminometer using the *Renilla-Glo*[™] Luciferase Assay System and recombinant luciferase.

MATERIALS REQUIRED

- GloMax[®] 20/20 Luminometer
- 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, as 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

- Turn ON the GloMax® 20/20. .
- Touch "Run Promega Protocol" from the "Protocols" menu.
- Select "Protocol" and then "Default Protocol" or "Run Promega Protocol" and select any of the single luminescent 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 ten 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.

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