A GloMax®-Multi Jr Method for Dual-Glo® Luciferase Assay System

INTRODUCTION

The GloMax® Multi Jr., in combination with the Dual-Glo® Luciferase Assay System, enables fast, simple quantitation of firefly and *Renilla* luciferase activities in a single sample. Transcription 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 benefit of using a dual-reporter system is that normalizing the expression of an experimental reporter to the expression of the control reporter can help differentiate between specific and nonspecific cellular responses. Firefly and *Renilla* luciferases are widely used as co-reporters for these normalized studies because the assays used to measure their activity are quick, easy and sensitive. Neither enzyme requires post-translational processing, so both firefly and *Renilla* enzymes are functional immediately upon translation.

Firefly and *Renilla* luciferases are evolutionarily distinct enzymes with different substrate specificities. The Dual-Glo® Assay System is a two-substrate reagent system optimized for analysis of mammalian cells expressing firefly and *Renilla* luciferase. The Dual-Glo® Luciferase Reagent is added directly to cells in growth media, whereupon it induces cell lysis and provides the substrate for firefly luciferase. A stable luminescent signal is produced that can be read over a period of two hours with little loss of intensity. Addition of the Dual-Glo® Stop & Glo® Reagent quenches luminescence from the firefly reaction and provides the substrate for the *Renilla* luciferase reaction. The Dual-Glo® Stop & Glo® reagent produces a stable luminescent signal that can be read over a two-hour period with little loss of intensity. The Dual-Glo® Assay System is optimized for use with the following types of media containing 0-10% serum: RPMI 1640, DMEM, MEMα and F12.

Using the Dual-Glo® Assay System with the Luminescence Module on a GloMax® Multi Jr., measurement of firefly and *Renilla* luciferases is linear from 1 x 10^{-18} to 1 x 10^{-13} moles (Figure 1).

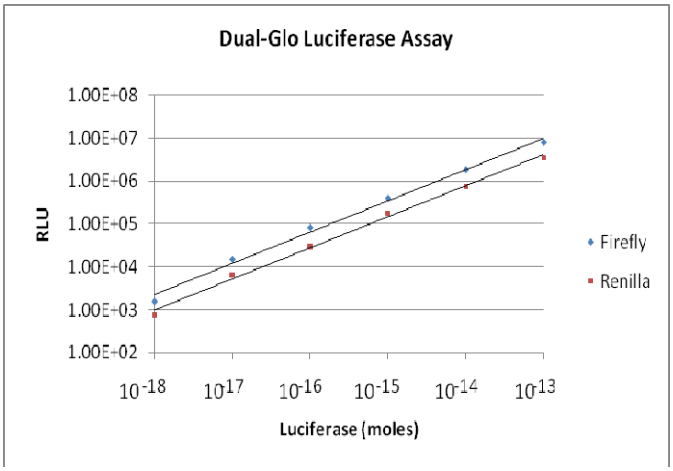

Figure 1. Comparison of the linear range of firefly and* Renilla *luciferases. *The Dual-Glo® Assay was performed with a mixture of purified firefly (QuantiLum® Cat.# E1701) and* Renilla *luciferases prepared in DMEM containing 1 mg/ml BSA. The GloMax® Multi Jr luminescence module was used to measure luminescence. The assay was linear from 10^{-13}–10^{-18} moles for both firefly and* Renilla *luciferases.*

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MATERIALS REQUIRED

- GloMax®-Multi Jr
- 1.5 ml microcentrifuge tubes
- Dual-Glo® Assay System (Cat.# E2920, E2940, E2980)
- 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

Dual-Glo® Luciferase Substrate: Store the lyophilized substrate at -20ºC for period indicated by the expiration date. The substrate may also be stored at 4ºC up to one month

Dual-Glo® Luciferase Buffer: Store the buffer below 25ºC for period indicated by the expiration date. Buffer storage at room temperature is recommended to prevent the need for temperature equilibration when the reagents are reconstituted.

Dual-Glo® Stop & Glo® Substrate: Store the substrate at -20ºC for period indicated by the expiration date. The substrate may also be stored at 4ºC up to one month.

Dual-Glo® Stop & Glo® Buffer: Store the buffer below 25ºC for period indicated by the expiration date. Buffer storage at room temperature is recommended to prevent the need for temperature equilibration when the reagents are reconstituted. The Dual-Glo® Stop & Glo® Buffer can precipitate upon freezing. Precipitate can be resolubilized by heating to 37°C for up to 2 hours with vigorous shaking.

Dual-Glo® Luciferase Reagent: Transfer the contents of the Dual-Glo® Luciferase Assay Buffer to one bottle of the Dual-Glo® Luciferase substrate. Mix by inversion until the substrate is dissolved. Use as directed. Any remaining Dual-Glo® Luciferase reagent can be stored in working aliquots at -70ºC for up to one month.

Dual-Glo® Stop & Glo® Reagent: The Dual-Glo™ Stop & Glo® Reagent should be prepared fresh as needed. Calculate the amount of Dual-Glo™ Stop & Glo® needed for the desired experiment. Dilute the Dual-Glo® Stop & Glo® Substrate 1:100 in the appropriate volume of Dual-Glo® Stop & Glo® Buffer.

Note: The optimum temperature for the activity of both luciferases is approximately room temperature, so it is important that the reagents be equilibrated to room temperature before use

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 (required for the instrument to warm up) will begin.
- The instrument will indicate it is ready to measure luminescence. If reading one or two samples, the preprogrammed Dual-Glo® method can be used. When analyzing multiple samples, it is faster and more convenient to read samples in batches using the default protocol or any of the single luminescent measurement protocols with a 1-sec integration time (i.e., Steady-Glo®, Bright-Glo®, CellTiter-Glo®).

3. Sample Analysis

- Remove cell cultures from the incubator. For maximum reproducibility, equilibrate the cultures to room temperature before luminescence measurement.
- Add a volume of Dual-Glo® Luciferase Reagent equal to the culture media volume to each well and mix. For 96-well plates, typically 75µl of reagent is added to cells grown in 75µl media.
- Leave the reagent on the cells for at least 10 minutes to allow sufficient time for lysis. Transfer each sample to a 1.5 ml microcentrifuge tube. Optimal results will be generated if luminescence is measured within 2 hours of the addition of the Dual-Glo® Luciferase Reagent.
- Insert each microfuge tube into the GloMax® Multi Jr. and touch “Measure Luminescence” to measure firefly luminescence.
- Record the Relative Light Units (RLU). The GloMax® Multi Jr. will display the most recent 20 results on the touchscreen.

  Note: The GloMax® Multi Jr. does not store more than 20 measurements at one time. Measurements are not stored between power cycles.

- To measure *Renilla* luciferase, add a volume of Dual-Glo® Stop & Glo® Reagent equal to the original culture media volume to each sample and mix. For 96-well plates, this volume is typically 75µl.
- Wait at least 10 minutes then measure luminescence. Optimal results will be generated if the luminescence is measured within 2 hours of the addition of the Dual-Glo® Luciferase Reagent.

  Note: Dual-Glo® Stop & Glo® Reagent should be added to the samples within 4 hours of the addition of the Dual-Glo® Luciferase Reagent.
- Record the Relative Light Units (RLU). The GloMax® Multi Jr. will display the most recent 20 results on the touchscreen.

  Note: The GloMax® Multi Jr. does not store more than 20 measurements at one time. Measurements are not stored between power cycles.
- Calculate the ratio of luminescence from the experimental reporter to luminescence from the control reporter. Normalize this ratio to the ratio of a control, or series of controls. Relative response ratios can be calculated from the normalized ratios. See the *Dual-Glo® Luciferase Assay System Technical Manual* (TM058) for more information and sample calculations.

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