

# Measuring the Dual-Glo<sup>®</sup> Luciferase Assay on the GloMax<sup>®</sup> Discover System

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## Materials Required

- Dual-Glo<sup>®</sup> Luciferase Assay System (Cat.# E2920, E2940, E2980)
- GloMax<sup>®</sup> Discover System (Cat.# GM3000)
- QuantiLum<sup>®</sup> Recombinant Luciferase (Cat.# E1701)
- Recombinant *Renilla* Luciferase
- Phenol Red-Free DMEM (HyClone Cat.# SH20384.01)
- Gelatin (Sigma Cat.# G6144-100G)
- White, 96-well assay plate (Corning 3912)

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

**Protocols:** The *GloMax<sup>®</sup> Discover System Technical Manual #TM397* is available at: [www.promega.com/protocols/](http://www.promega.com/protocols/)

In cell biology research and pharmaceutical discovery, it is common to test a variety of experimental conditions or a large number of chemical compounds for their effects on cellular physiology. The ease-of-use and sensitivity of firefly luciferase assays have made it relatively simple to monitor the upregulation of genetic elements; however, it is harder to measure downregulation of genes because of difficulties in discriminating between cell death and cellular downregulation. Normalizing the expression of an experimental reporter to the expression of a control reporter can help differentiate between specific and nonspecific cellular responses. This normalization can also control for transfection efficiencies. Together, the GloMax<sup>®</sup> Discover System and the Dual-Glo<sup>®</sup> Luciferase Assay System provide a convenient, rapid, and sensitive method for measuring changes in cellular physiology.

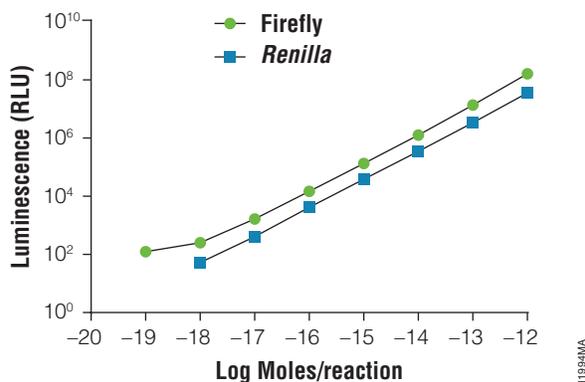
This Application Note describes the protocol for measuring luminescence using the GloMax<sup>®</sup> Discover System with the Dual-Glo<sup>®</sup> Luciferase Assay System.

The Dual-Glo<sup>®</sup> Luciferase Assay System is designed to allow high-throughput analysis of firefly and *Renilla* luciferase expression in mammalian cells grown in 96- or 384-well plates. The Dual-Glo<sup>®</sup> Luciferase Reagent can be added directly to cells in growth medium without washing or preconditioning. The Reagent induces cell lysis and acts as a substrate for firefly luciferase, which has a half-life of approximately two hours. Addition of the Dual-Glo<sup>®</sup> Stop & Glo<sup>®</sup> Reagent quenches the luminescence from the firefly reaction by at least 10,000-fold and provides the substrate for *Renilla* luciferase in a reaction that can also be read within 2 hours (with a similar retention in signal). The Dual-Glo<sup>®</sup> Luciferase Assay System is designed to work in growth media commonly used for mammalian cells with or without added serum.

The Dual-Glo<sup>®</sup> System is easy to use with the GloMax<sup>®</sup> Discover, and the protocol comes pre-loaded on the instrument. The extended dynamic range of the GloMax<sup>®</sup> Discover System allows you to easily measure various sample signal intensities on the same plate over a seven-log range in luciferase concentration using the Dual-Glo<sup>®</sup> System (Figure 1).

## Protocol

1. Combine the QuantiLum<sup>®</sup> Recombinant Luciferase and Recombinant *Renilla* luciferase to a final concentration of  $1.33 \times 10^{-14}$  mols/ $\mu$ l in phenol red free DMEM + 1mg/ml gelatin.
2. Perform a serial tenfold dilution of the recombinant luciferase solution in phenol red free DMEM + 1mg/ml gelatin.
3. Add 75 $\mu$ l of each dilution in the titration series to a white, 96-well assay plate (Corning Cat.# 3912)
4. Warm the Dual-Glo<sup>®</sup> Luciferase Buffer and Dual-Glo<sup>®</sup> Luciferase Substrate (lyophilized) to room temperature and combine.
5. Warm Dual-Glo<sup>®</sup> Stop & Glo<sup>®</sup> Buffer and Dual-Glo<sup>®</sup> Stop & Glo<sup>®</sup> substrate to room temperature and combine.
6. Add 75 $\mu$ l of Dual-Glo<sup>®</sup> Luciferase Reagent to the plate and shake for 30 seconds on an orbital shaker.
7. Incubate the plate at room temperature for 10 minutes and read luminescence (firefly luciferase activity) on the GloMax<sup>®</sup> Discover System using the Dual-Glo<sup>®</sup> protocol.
8. Add 75 $\mu$ l of Dual-Glo<sup>®</sup> Stop & Glo<sup>®</sup> Reagent to the plate and shake for 30 seconds on an orbital shaker.
9. Incubate the assay plate at room temperature for 10 minutes and read luminescence (*Renilla* luciferase activity) on the GloMax<sup>®</sup> Discover System using the Dual-Glo<sup>®</sup> protocol.



**Figure 1. Light output from both firefly and *Renilla* luciferases is directly proportional to the amount of enzyme present in the reaction.** A serial tenfold dilution of the  $1.33 \times 10^{-14}$  mols/ $\mu$ l recombinant enzyme solution (QuantiLum<sup>®</sup> Recombinant Luciferase and Recombinant *Renilla* luciferase) was performed in phenol red free DMEM + 1mg/ml gelatin, and 75 $\mu$ l of each dilution added to the wells of a white 96-well plate. Assays were performed according to the Dual-Glo<sup>®</sup> Luciferase Assay System protocol and luminescence was recorded 10 minutes after each reagent addition on the GloMax<sup>®</sup> Discover System using the Dual-Glo<sup>®</sup> pre-set protocol. Sample signal intensities could be detected on the same plate over a seven-log range in luciferase concentration.

## GloMax<sup>®</sup> Discover System

The GloMax<sup>®</sup> Discover System offers superior sensitivity, dynamic range and limited well-to-well cross-talk. The instrument has been developed and optimized with Promega's industry leading Cell and Gene Reporter assays and may be integrated into low- and medium-throughput automation workflows. The GloMax<sup>®</sup> Discover System also provides flexible use of filters for fluorescence intensity, filtered luminescence, BRET, FRET, and UV-visible absorbance measurements for adaptation into a wide variety of laboratory applications. The instrument is operated by an integrated Tablet PC, which provides quick and easy navigation through the control options. Exporting your results is made seamless with a variety of options, including to your local data network.

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