Measuring the Dual-Luciferase® Reporter Assay on the GloMax® Discover System

Promega Corporation

Materials Required

- Dual-Luciferase® Reporter Assay System (Cat.# E1910, E1960)
- GloMax® Discover System (Cat.# GM3000)
- QuantiLum® Recombinant Luciferase (Cat.# E1701, E1702)
- Recombinant *Renilla* Luciferase
- Gelatin (Sigma Cat.# G6144-100G)
- Nuclease-Free Water (Cat.# P1195)
- 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® Discover System Technical Manual* #TM397 is available at: **www.promega.com/protocols/**

Genetic reporter systems are widely used to study eukaryotic gene expression and cellular physiology. Applications include the study of receptor activity, transcription factors, intracellular signaling, mRNA processing and protein folding. Dual reporters are commonly used to improve experimental accuracy by normalizing the activity of the experimental reporter to the activity of a control receptor,which minimizes experimental variability caused by differences in cell viability or transfection efficiency.

The Dual-Luciferase® Reporter (DLR™) Assay System provides an efficient way to perform dual-reporter assays. In the DLR™ Assay, the activities of firefly (*Photinus pyralis*) and *Renilla* (*Renilla reniformis*) luciferases are measured sequentially from a single sample. The firefly luciferase reporter is measured first by adding Luciferase Assay Reagent II (LAR II) to generate a stabilized luminescent signal. After quantifying firefly luminescence, the reaction is quenched and the *Renilla* luciferase reaction is initiated by adding Stop & Glo® Reagent. The Stop & Glo® Reagent produces a stabilized signal from *Renilla* luciferase, which decays slowly over the course of the measurement. In the DLR™ Assay System, both reporters yield linear assays with sub-attomole sensitivities and no endogenous activity of either reporter in the experimental host cells. Furthermore, the integrated format of the DLR™ Assay provides rapid quantitation of both reporters either in transfected cells or in cell-free transcription/translation reactions.

The Dual-Luciferase® Reporter Assay System is easy to perform on the GloMax® Discover System, as the protocol comes pre-loaded on the instrument. The extended dynamic range and minimal well-to-well cross-talk of the GloMax® Discover System enable accurate detection of multiple sample intensities. Measuring the Dual-Luciferase® Reporter Assay System on the GloMax® Discover System yields linear and sensitive results of less than 1×10^{-21} moles of luciferase under optimal conditions (Figure 1).

This Application Note describes the protocol for measuring luminescence using the GloMax® Discover System with the Dual-Luciferase® Reporter Assay System.

Protocol

1. Dilute stocks of QuantiLum® Recombinant Luciferase and Recombinant *Renilla* luciferase and combine in 1X Passive Lysis Buffer + 1mg/ml gelatin.
2. Perform a serial 1:10 dilution of the luciferase solution in 1X Passive Lysis Buffer + 1mg/ml gelatin.
3. Transfer 20µl of each dilution to eight rows of a white, 96-well assay plate.
4. Transfer 20µl of 1X Passive Lysis Buffer + 1 mg/ml gelatin into additional wells of the same assay plate for use as a background control.
5. Prime 0.5ml of Luciferase Assay Reagent II and Stop & Glo® Reagent in lines 1 and 2, respectively, of the GloMax® Discover injectors.
6. Run the Dual-Luciferase® Reporter Assay protocol injecting 100µl of reagent per well with a 2-second wait and 10-second integration.

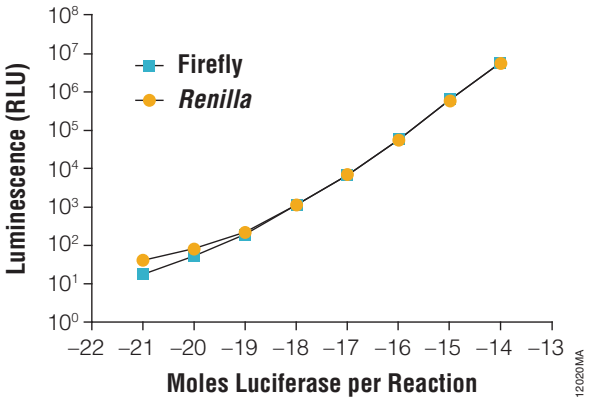

Figure 1. Dynamic range of the Dual-Luciferase® Reporter Assay System analyzed on the GloMax® Discover System. A 1:10 dilution series of QuantiLum® Recombinant Luciferase and Recombinant *Renilla* luciferase ranging from 1×10^{-14} moles to 1×10^{-21} moles was created. A total volume of 20µl of each dilution of the series was loaded on a white, 96-well, flat-bottom assay plate. Data is represented as signal–background. Data points and standard deviations were calculated from triplicate samples. Light output from firefly and *Renilla* luciferase activities is directly proportional to the amount of enzyme present in the reaction.

GloMax® Discover System

The GloMax® 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® 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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