Measuring Luminescence of the *Renilla*-Glo® Luciferase Assay System using the GloMax® Discover System

Promega Corporation

Materials Required

- *Renilla*-Glo® Luciferase Assay System (Cat.# E2710, E2720 and E2750)
- GloMax® Discover System (Cat.# GM3000)
- recombinant *Renilla* enzyme (0.78mg/ml, 36kDa)
- phenol red-free DMEM (Hyclone Cat.# SH30284.01)
- gelatin (Sigma Cat.# G6144)
- white, 96-well flat-bottom assay plate (Corning Cat.# 3912)

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

Protocols: *GloMax® Discover System Technical Manual* #TM397 and Renilla-*Glo® Luciferase Assay System Technical Manual* #TM329 are available at: **www.promega.com/protocols/**

Genetic reporters are used in cell biology to study gene expression and other cellular events coupled to gene expression, such as receptor activity, intracellular signal transduction, mRNA processing, protein folding and protein:protein interactions. *Renilla* luciferase, a monomeric 36kDa protein, catalyzes coelenterate luciferin (coelenterazine) oxidation to produce light. Post-translational modification is not required for its activity, and the enzyme can function as a genetic reporter immediately following translation. The *Renilla*-Glo® Luciferase Assay System was developed for reporter quantitation in mammalian cells and, when used in conjunction with the GloMax® Discover System, provides a rapid, convenient and sensitive method for detecting *Renilla reniformis* luciferase.

The *Renilla*-Glo® Luciferase Assay System includes a single-addition reagent that generates a glow-type signal with *Renilla* luciferase. When reconstituted, the reagent has the capacity to lyse cells, reduce autoluminescence of the coelenterazine substrate and produce a stable signal (i.e., half-life greater than 60 minutes at 22°C). The *Renilla*-Glo® Luciferase Assay System measures *Renilla* luciferase activity as a primary reporter to study transcriptional regulation or as a co-reporter for normalization of experimental variations such as differences in transfection efficiencies.

The *Renilla*-Glo® Luciferase Assay System is made easy on the GloMax® Discover System, and the protocol comes preloaded on the instrument. The extended dynamic range and minimal well-to-well cross talk of the GloMax® Discover System allows you to easily measure signals of varying intensities on the same plate. This Application Note describes the protocol for measuring luminescence using the *Renilla*-Glo® Luciferase Assay System and GloMax® Discover System.

Renilla-Glo® Luciferase Assay System Protocol

For detailed instructions and assay notes, see the Renilla-*Glo® Luciferase Assay System Technical Manual* #TM329. A sample procedure follows.

Assay Protocol

1. Prepare a solution of phenol red-free DMEM + 1mg/ml gelatin by combining 15ml of medium with 15mg of gelatin. Ensure the gelatin is completely dissolved by warming the solution at 37°C for 15–30 minutes before use.
2. Prepare a working stock of 1E–14 moles/µl (=1E–12 moles/100µl reaction) of recombinant *Renilla* enzyme in phenol red-free DMEM + 1mg/ml gelatin.
   - 2a. Perform a 1:100 intermediate dilution of recombinant *Renilla* enzyme by combining 1µl of stock *Renilla* enzyme (0.78mg/ml, 36kDa) with 99µl of phenol red-free DMEM + 1mg/ml gelatin.
   - 2b. Create the final working stock of recombinant *Renilla* enzyme by combining 46µl of the 1:100 intermediate dilution with 954µl of phenol red-free DMEM + 1mg/ml gelatin.
3. Perform a 1:10 serial dilution of the recombinant *Renilla* enzyme working stock in phenol red-free DMEM + 1mg/ml gelatin.
4. Transfer 100µl of each dilution to triplicate wells of an assay plate.
5. Transfer 100µl of phenol red-free DMEM + 1mg/ml gelatin to three wells of the assay plate for background control.
6. Transfer 100µl of *Renilla*-Glo® Reagent to each well of the assay plate, and shake on an orbital shaker for 30 seconds.
7. Incubate for 10 minutes at room temperature, and measure luminescence on the GloMax® Discover System.

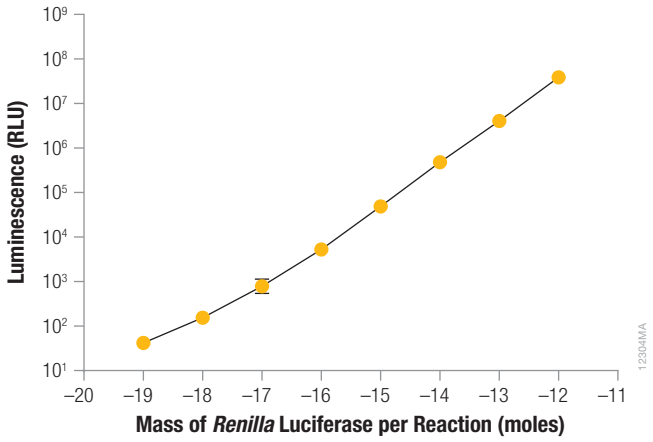

Figure 1. The luminescent signal is correlated with the amount of *Renilla* luciferase enzyme present in the reaction. A working solution of recombinant *Renilla* luciferase was prepared in phenol red-free DMEM + 1mg/ml gelatin. A serial tenfold titration of enzyme was performed in phenol red-free DMEM + 1mg/ml gelatin. Sample and background controls were transferred to the assay plate in triplicate 100µl aliquots. *Renilla*-Glo® Reagent (100µl) was added to each well containing sample or background control. The assay plate was transferred to an orbital shaker for 30 seconds, followed by a 10-minute incubation at room temperature. Luminescence was measured using the GloMax® Discover and a 0.3-second integration per well. Background luminescence was subtracted from wells containing enzyme and results plotted.

Conclusion

This Application Note demonstrates that the GloMax® Discover can measure luminescence using the *Renilla*-Glo® Luciferase Assay System.

The GloMax® Discover System

The GloMax® Discover System offers superior sensitivity and dynamic range and limited well-to-well cross talk. The instrument was developed and optimized with Promega cell and gene reporter assays and may be integrated into low- and medium-throughput automation workflows. The GloMax® Discover System allows flexible use of filters to measure fluorescence intensity, filtered luminescence, BRET, FRET and UV-visible absorbance for 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 exporting data to your local network.

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