

Measuring the Luciferase Assay System on the GloMax[®] Discover

Promega Corporation



Materials Required

- Luciferase Assay System (Cat.# E1500)
- GloMax[®] Discover System (Cat.# GM3000)
- QuantiLum[®] Recombinant Luciferase (Cat.# E1701, E1702)
- Gelatin (Sigma Cat.# G6144)
- 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/

The GloMax[®] Discover System used in conjunction with the Luciferase Assay System provides a convenient and sensitive way to quantify genetic reporter activity. Genetic reporters are used commonly 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. Firefly luciferase is widely used as a reporter because it is rapid, sensitive and does not require post-translational processing.

The Luciferase Assay System is substantially improved in both sensitivity and simplicity over conventional assay methods. Light is produced by converting the chemical energy of luciferin oxidation through an electron transition, forming oxyluciferin. In the conventional assay for luciferase, a flash of light is generated that decays rapidly after the enzyme and substrates are combined. The Luciferase Assay System incorporates coenzyme A (CoA) for improved kinetics, allowing greater enzymatic turnover and resulting in increased light intensity that is nearly constant for at least one minute.

The Luciferase Assay System is made easy on the GloMax[®] Discover System as the protocol comes preloaded 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 Luciferase 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). Generally, 100-fold greater sensitivity can be achieved over chloramphenicol acetyltransferase (CAT) assays.

This Application Note describes the protocol for measuring luminescence using the GloMax[®] Discover with the Luciferase Assay System.

Protocol

1. Dilute QuantiLum® Recombinant Luciferase in 1X Passive Lysis Buffer + 1mg/ml gelatin (1X PLB).
2. Perform a serial 1:10 dilution of enzyme in 1X PLB + 1mg/ml gelatin.
3. Dispense 20µl of the dilution series to the wells of a white, 96-well assay plate.
4. Dispense 20µl of 1X PLB + 1mg/ml gelatin into additional wells of the same assay plate for use as a background control.
5. Prime 0.5ml of Luciferase Assay System reagent to line 1 of the GloMax® Discover injector.
6. Run the Luciferase Assay System protocol on the GloMax® Discover (injecting 100µl of reagent per well with a 2-second wait and a 10-second integration).

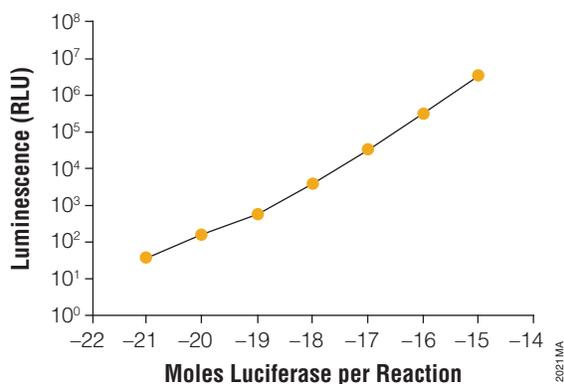


Figure 1. Dynamic range of the Luciferase Assay System

analyzed on the GloMax® Discover System. A 1:10 dilution series of QuantiLum® Recombinant Luciferase (Cat.# E1701) ranging from 1×10^{-15} 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 Luciferase activity 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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