Measuring Luminescence of the Bright-Glo™ Luciferase Assay System using the GloMax® Discover System

Promega Corporation

Materials Required

- Bright-Glo™ Luciferase Assay System (Cat.# E2610, E2620 and E2650)
- GloMax® Discover System (Cat.# GM3000)
- white, 96-well tissue culture-treated assay plates (Corning Cat.# 3917)
- RPMI 1640 medium + 10% fetal bovine serum (FBS)
- Jurkat cells stably expressing firefly luciferase under control of the NFAT response element
- ionomycin (Sigma Cat.# I0634), prepared as a 10mM stock in DMSO
- phorbol 12-myristate 13-acetate (PMA, (Sigma Cat.# P8139), prepared as a 20mM stock in DMSO
- DMSO (Sigma Cat.# D2650)

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 *Bright-Glo™ Luciferase Assay System Technical Manual* #TM052 are available at: **www.promega.com/protocols/**

Transcriptional regulation, coupled to reporter gene expression, is routinely used to study a wide range of physiological events. A common example is analysis of receptor function by quantifying the action of specific receptor response elements on gene expression. Other examples include the study of signal transduction, transcription factors, protein:protein interactions, and viral infection and propagation. Events downstream of transcription, such as mRNA processing and protein folding, also can be analyzed. The Bright-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 firefly luciferase.

Quantitation of luciferase expression in mammalian cells requires highly sensitive reagents that are adaptable to continuous-process robotic systems. Firefly luciferase is a 61kDa monomer that catalyzes the mono-oxygenation of beetle luciferin. The gene encoding firefly luciferase (*luc*) is incorporated into a number of reporter vectors and is a popular choice as a reporter gene because functional enzyme is created immediately upon translation, and the assay is rapid, reliable and easy to perform. The enzyme uses ATP as a cofactor, although most of the energy for photon production comes from molecular oxygen. The Bright-Glo™ Luciferase Assay System is designed specifically to meet the needs of continuous-process systems by providing robust, homogeneous assay chemistry that achieves high assay sensitivity and approximately 30-minute signal half-life without prior sample processing. These attributes also benefit scientists analyzing fewer samples who still require high sensitivity and ease of use. The provided Bright-Glo™ Luciferase Assay Buffer and Substrate are combined to form Bright-Glo™ Reagent, which when added to the well, lyses cells and initiates firefly luciferase activity.

The Bright-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 Bright-Glo™ Luciferase Assay System and GloMax® Discover System.

Bright-Glo™ Luciferase Assay System Protocol

For detailed instructions and assay notes, see the *Bright-Glo™ Luciferase Assay System Technical Manual* #TM052. A sample procedure follows.

1. Prepare Jurkat cells in RPMI 1640 medium + 10% FBS at 2.5 × 10^5 cells/ml.
2. Prepare 2ml of RPMI 1640 medium + 10% FBS + 1µM ionomycin by combining 11.994ml of RPMI 1640 medium + 10% FBS with 6µl of 10mM ionomycin. This solution will also be used as a no-PMA control.
3. Prepare a 5X (250nM) working solution of PMA.
   3a. Create a 20µM intermediate dilution of 20mM PMA by combining 1µl of 20mM PMA with 999µl of DMSO.
   3b. Combine 25µl of 20µM PMA with 1.975ml of RPMI 1640 medium + 10% FBS + 5µM ionomycin to create a 5X working solution of PMA.
4. Perform a 10-point 1:3 serial dilution of PMA in RPMI 1640 medium + 10% FBS + 5µM ionomycin by combining 400µl of diluent with 200µl of the 250nM working stock of PMA. Mix well. Repeat the titration series an additional nine times to create the 10-point titration series.
5. Add 80µl of cell suspension to each well in columns 1–11 of a white, 96-well tissue culture-treated assay plate.
6. Add 20µl of the PMA titration series to wells in columns 1–10 of the assay plate in replicates of eight. Add 20µl of RPMI 1640 medium + 10% FBS + 5µM ionomycin to column 11 (no-PMA control) in replicates of eight. Add 100µl of RPMI 1640 medium + 10% FBS (medium-only control) to column 12 in replicates of eight.
7. Shake the assay plate on an orbital shaker to mix contents, and transfer plate to a tissue culture incubator at 37°C and 5% CO_2. Incubate for 18 hours.
8. Thaw and combine Bright-Glo™ Luciferase Assay Buffer and Bright-Glo™ Luciferase Assay Substrate to make Bright-Glo™ Luciferase Assay Reagent. Equilibrate to room temperature.
9. Remove assay plate from the incubator and allow to equilibrate to room temperature for at least 15 minutes.
10. Add 100µl of Bright-Glo™ Luciferase Assay Reagent to each well of the assay plate, and shake on an orbital shaker for 30 seconds.
11. Incubate for 2 minutes at room temperature, and measure luminescence on the GloMax® Discover System using the Bright-Glo™ Luciferase Assay System protocol.

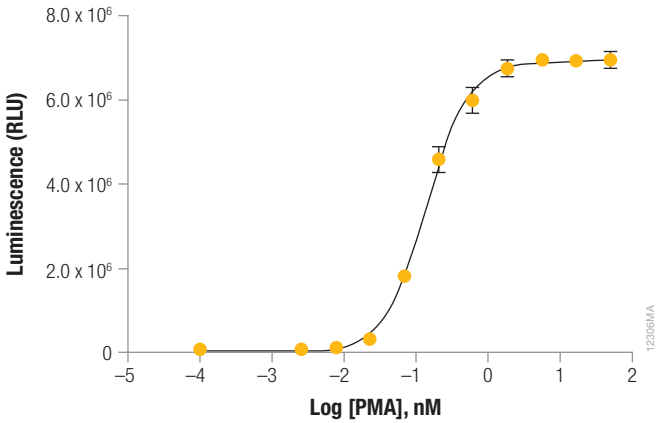

Figure 1. PMA and ionomycin work cooperatively to induce maximal firefly luciferase gene expression through NFAT. A 96-well plate consisted of Jurkat cells stably transfected with the luciferase reporter gene under control of the NFAT response element and mixed with a 1:3 serial dilution of PMA in RPMI 1640 medium + 10% FBS + 1µM ionomycin. The luciferase assay was conducted as described in Section 3.C of the *Bright-Glo™ Luciferase Assay System Technical Manual* #TM052, and luminescence was measured using the GloMax® Discover System and the Bright-Glo™ Luciferase Assay System protocol. Firefly luciferase expression is directly proportional to PMA concentration.

Conclusion

This Application Note demonstrates that the GloMax® Discover can measure luminescence using the Bright-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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