Measuring the ONE-Glo™ Luciferase Assay System on the GloMax® Discover System

Promega Corporation

Materials Required

- ONE-Glo™ Luciferase Assay System (Cat.# E6110, E6120 and E6130)
- GloMax® Discover System (Cat.# GM3000)
- white, 96-well tissue culture-treated assay plates (Corning Cat.# 3917)
- RPMI 1640 + 10% FBS
- Jurkat cells stably expressing firefly luciferase under control of NFAT response element
- ionomycin (Sigma Cat.# I0634), 20mM stock in DMSO
- phorbol 12-myristate 13-acetate, PMA (Sigma Cat.# P8139), 20mM stock in DMSO

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 is available at: **www.promega.com/protocols/**

Transcriptional regulation coupled to the expression of a reporter gene 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 applications 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 GloMax® Discover System in combination with the ONE-Glo™ Luciferase Assay System provides a convenient, rapid and sensitive procedure for observing cellular physiology.

Luciferase is a popular choice as a reporter for these applications because functional enzyme is created immediately upon translation and the assay is rapid, reliable and easy to perform. Furthermore, analysis using luciferase as the genetic reporter is well suited to laboratory automation and multi-well plate applications. For these reasons, luciferase is widely used in the biotechnology and pharmaceutical industries.

The ONE-Glo™ Luciferase Assay System is made easy using the GloMax® Discover System, and the protocol comes preloaded on the instrument. The extended dynamic range and limited well-to-well cross talk of the GloMax® Discover System enables a range of signal intensities to be measured on the same plate using the ONE-Glo™ Luciferase Assay System. This Application Note describes the protocol for measuring luminescence using the GloMax® Discover System with the ONE-Glo™ Luciferase Assay System.

Protocol

For detailed instructions and assay notes for various assay volumes and plate formats, see the *ONE-Glo™ Luciferase Assay System Technical Manual* #TM292. A sample procedure follows.

1. Prepare Jurkat cells in RPMI 1640 + 10% FBS at a density of 2.5 × 10^5 cells/ml.
2. Perform a serial threefold dilution of PMA in RPMI 1640 + 10% FBS in the presence of 1µM ionomycin. Include ionomycin- and medium-only wells as controls.
3. Transfer 80µl of cell suspension to a white, 96-well tissue culture-treated assay plate.
4. Transfer 20µl of the PMA titration series to the assay plate in replicates of eight. Include appropriate control wells.
5. Shake the plate on an orbital shaker to mix contents and transfer to a tissue culture incubator at 37°C and 5% CO_2. Incubate for 18 hours.
6. Thaw and combine ONE-Glo™ Luciferase Assay Buffer and ONE-Glo™ Luciferase Assay Substrate. Equilibrate to room temperature.
7. Remove assay plate from the incubator and allow to equilibrate to room temperature for at least 15 minutes.
8. Add 100µl of ONE-Glo™ Luciferase Assay Reagent to each well of the assay plate and then shake on an orbital shaker for 30 seconds.
9. Incubate for three minutes at room temperature, and read luminescence on the GloMax® Discover System using the ONE-Glo™ Luciferase Assay System protocol.

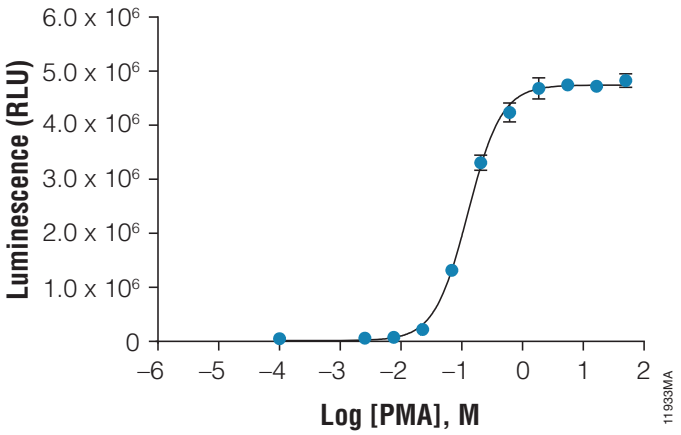

Figure 1. PMA and ionomycin work cooperatively to induce maximal firefly luciferase gene expression through NFAT. A 96-well microplate consisted of Jurkat cells that had been stably transfected with the luciferase reporter gene under control of the NFAT response element and mixed with a 1:3 serial dilution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

GloMax® Discover System

The GloMax® Discover System offers superior sensitivity, dynamic range and limited well-to-well cross talk. The instrument was 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 exporting data to your local network.