Measuring the ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay on the GloMax® Discover System

Promega Corporation

Materials Required

- ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay (Cat.# E7110 and E7120)
- 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/**

The GloMax® Discover System in conjunction with the ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay provides a convenient and sensitive procedure to measure cell viability and gene expression consecutively within a culture population.

The first part of the ONE-Glo™ + Tox assay is a non-lytic fluorescence assay (CellTiter-Fluor™ Cell Viability Assay) that measures the relative number of live cells present after experimental manipulation. The CellTiter-Fluor™ Assay measures a conserved and constitutive protease activity within live cells and therefore serves as a marker of cell viability. The live-cell-protease activity is restricted to intact viable cells and is measured using a fluorogenic, cell-permeant peptide substrate (glycyl-phenylalanyl-aminofluorocoumarin; GF-AFC) that is cleaved to produce the fluorescent-free AFC fluorophore. Fluorescence can be measured with a microplate reader or CCD imager using an excitation wavelength of 380–400nm and emission wavelength of 505nm.

The second part of the assay uses the ONE-Glo™ Luciferase Assay System to quantify firefly luciferase reporter gene expression from cells made to express this reporter enzyme. Ideally suited for high- and ultrahigh-throughput applications, the ONE-Glo™ Assay contains a new fluoroluciferin substrate, resulting in a reagent that is more stable, more tolerant to sample components, and gives off less odor than standard luciferase assay reagents. Luminescence is measured with a microplate reader or CCD imager.

The ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay is made easy on 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 enable a sensitive and accurate multiplex detection of both cytotoxicity and gene expression on the same plate (Figure 1). This Application Note describes the protocol for measuring fluorescence and luminescence using the GloMax® Discover System with the ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay.

Protocol

For detailed instructions and assay notes for various assay volumes and plate formats, see the *ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay System Technical Manual* #TM356. 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 ionomycin in RPMI 1640 + 10% FBS in the presence of 1nM PMA.
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.
5. Add medium-only and no-ionomycin controls to the plate.
6. Shake the plate on an orbital shaker for 30 seconds, then transfer to a tissue culture incubator at 37°C and 5% CO_2. Incubate for 18 hours.
7. Prepare 5X CellTiter-Fluor™ Reagent by adding 10µl of GF-AFC Substrate to 2ml of Assay Buffer. Vortex to mix.
8. Transfer 20µl of 5X CellTiter-Fluor™ Reagent to each well of the plate.
9. Shake the plate on an orbital shaker for 30 seconds, then transfer the plate back to the tissue culture incubator for 30 minutes.
10. Measure AFC fluorescence (cell viability) with the Discover ONE-Glo™ + Tox protocol.
11. Prepare ONE-Glo™ Reagent by thawing and combining ONE-Glo™ Luciferase Assay Buffer with ONE-Glo™ Luciferase Assay Substrate. Invert to mix.
12. Transfer 100µl of ONE-Glo™ Reagent to each well of the plate.
13. Incubate the plate room temperature for three minutes.
14. Measure luminescence (firefly luciferase activity) on the GloMax® Discover System using the ONE-Glo™ + Tox protocol.

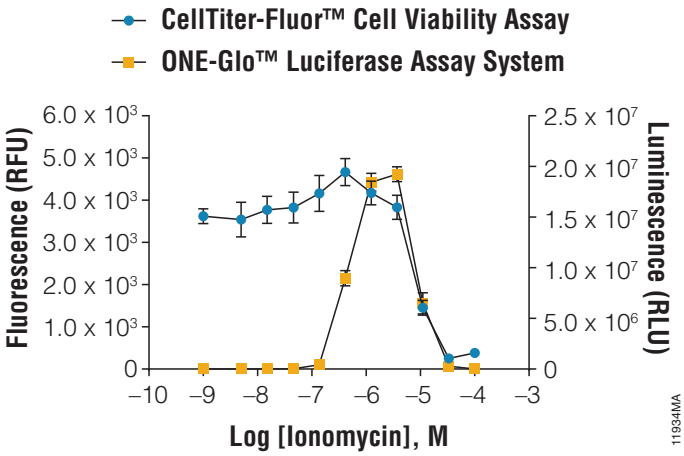

Figure 1. A titration of ionomycin in the presence of PMA. Jurkat cells were prepared in RPMI 1640 + 10% FBS at a density of 2.5 × 10^5 cells/ml and added to a 96-well plate with a serial threefold dilution of ionomycin in RPMI 1640 + 10% FBS in the presence of 1nM PMA. Assays were performed according to Section 4.B of the *ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay Technical Manual*. At specific concentrations, ionomycin and PMA work cooperatively to stimulate NFAT-dependent gene expression (luminescence). At higher concentrations, ionomycin induces cytotoxicity, which results in a decrease in viability (fluorescence). A decrease in reporter expression also is observed due to the increase in cytotoxicity.

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.