Measuring the Output of the CytoTox-Fluor™ Cytotoxicity Assay on the GloMax® Discover System

Promega Corporation

Materials Required

- CytoTox-Fluor™ Cytotoxicity Assay (Cat.# G9260, G9261 and G9262)
- GloMax® Discover System (Cat.# GM3000)
- 96-well, opaque-walled tissue culture plates (clear- or solid-bottom)
- positive control cytotoxicity compound or lytic detergent (e.g., digitonin, Calbiochem Cat.# 300410 at 20mg/ml 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/

CytoTox-Fluor™ Cytotoxicity Assay Technical Manual #TB350 is available at:
www.promega.com/protocols/

The GloMax® Discover System in combination with the CytoTox-Fluor™ Cytotoxicity Assay provides a convenient, rapid and sensitive procedure for determining the number of dead cells in cell populations. Fluorescence correlates with the presence of a distinct protease activity associated with cytotoxicity and loss of membrane integrity.

The CytoTox-Fluor™ Cytotoxicity Assay is a single-reagent-addition, homogeneous, fluorescent assay that measures the relative number of dead cells in cell populations. The assay uses a fluorogenic peptide substrate (bis-alanyl-alanyl-phenylalanyl-rhodamine 110; bis-AAF-R110) to measure a "dead-cell protease activity" that has been released from cells that have lost membrane integrity. The bis-AAF-R110 Substrate cannot cross the intact membrane of live cells and therefore gives no signal from live cells. The CytoTox-Fluor™ Assay is designed to accommodate downstream multiplexing with any Promega luminescent assay or spectrally distinct fluorescent assay methods, such as assays measuring caspase activation, reporter expression or orthogonal measures of viability.

The CytoTox-Fluor™ Cytotoxicity Assay is made easy on the GloMax® Discover System. The extended dynamic range of the GloMax® Discover System allows the user to easily measure various sample signal intensities on the same plate using the CytoTox-Fluor™ Reagent. GloMax® Discover System data are collected using a standard 96-well plate, and the amount of fluorescence produced is proportional to the number of lysed cells (Figure 1). This Application Note describes the protocol for measuring fluorescence using the GloMax® Discover System with the CytoTox-Fluor™ Cytotoxicity Assay.

Protocol

For detailed instructions and assay notes, follow the procedure as indicated in the *CytoTox-Fluor™ Cytotoxicity Assay Technical Bulletin*, #TB350. The following procedure can be used for 100µl of culture in a 96-well plate format, but the assay can be scaled to accommodate multiplexing as well as variable plate formats. If you have not performed this assay on your cell line previously, we recommend determining assay sensitivity using your cells and one of the two methods described in the Technical Bulletin (Section 4.A or 4.B).

Reagent Preparation and Storage

1. Thaw the CytoTox-Fluor™ Cytotoxicity Assay components in a 37°C water bath.
2. Transfer the bis-AAF-R110 Substrate into the Assay Buffer container for a 2X reagent. Mix by vortexing the contents until the substrate is thoroughly dissolved to create the reagent.

Example Cytotoxicity Assay Protocol

1. Set up 96-well assay plates containing cells in culture medium at desired density.
2. Add test compounds and vehicle controls to appropriate wells so the final volume is 100µl in each well.
3. Culture cells for the desired test exposure period.
4. Add CytoTox-Fluor™ Cytotoxicity Assay Reagent in an equal volume (100µl per well) to all wells, mix briefly by orbital shaking, then incubate for at least 30 minutes at 37°C.

   Note: Longer incubations may improve assay sensitivity and dynamic range. However, **do not** incubate more than 3 hours.
5. Measure resulting fluorescence on the GloMax® Discover System (475nm$_{Ex}$/500–550nm$_{Em}$).

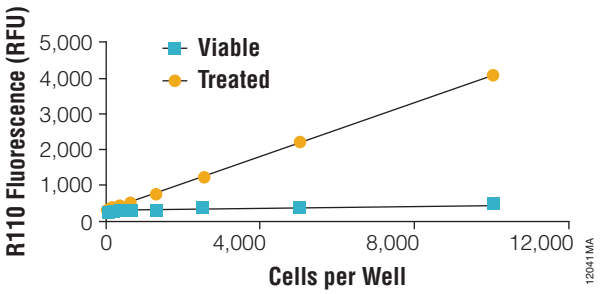

Figure 1. The CytoTox-Fluor™ Cytotoxicity Assay signals derived from untreated cells (Viable) or treated cells (Lysed) are proportional to dead-cell number. K562 cells were treated with CellTox™ Green Cytotoxicity Assay Lysis Solution (Lysed) or water (Viable). Cells were serially diluted in a 96-well plate and 50µl of CytoTox-Fluor™ Cytotoxicity Assay Reagent was added to each well. Fluorescence was read on the GloMax® Discover System. Points represent the average of three replicates with standard deviation.

GloMax® Discover System

The GloMax® Discover System, developed and optimized with Promega cell and gene reporter assays, 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 to your local network. Thus, together with the CytoTox-Fluor™ Cytotoxicity assay, the GloMax® Discover offers a complete and easy-to-use solution to measure cytotoxicity.

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