Measuring Cytotoxicity Using the CytoTox-Glo™ Cytotoxicity Assay and GloMax® Discover System

Promega Corporation

Materials Required

- CytoTox-Glo™ Cytotoxicity Assay (Cat.# G9290, G9291 and G9292)
- GloMax® Discover System (Cat.# GM3000)
- 96-well, white-walled tissue culture plates (clear or solid bottom)
- Positive control cytotoxic compound

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 *CytoTox-Glo™ Cytotoxicity Assay Technical Bulletin* #TB359 are available at: **www.promega.com/protocols/**

Measuring cell death within a cell population is important during drug development to understand potential cytotoxicity effects of drug compounds. The GloMax® Discover System in combination with the CytoTox-Glo™ Cytotoxicity Assay provides a convenient, rapid and sensitive procedure to determine the number of dead cells in cell populations. A distinct protease activity associated with cytotoxicity produces a luminescent signal that can be used to quantify dead cells.

The homogeneous CytoTox-Glo™ Cytotoxicity Assay is a single-reagent-addition, luminescent assay that measures the number of dead cells in cell populations. The assay uses a luminogenic peptide substrate (alanyl-alanylphenylalanyl-aminoluciferin; AAF-Glo™ Substrate) to measure "dead-cell protease activity", which is released from cells that have lost membrane integrity. The AAF-Glo™ Substrate cannot cross the intact membrane of live cells, does not generate any appreciable signal from the live-cell population and therefore selectively detects dead cells.

The CytoTox-Glo™ Assay relies on the properties of a proprietary thermostable luciferase (Ultra-Glo™ Recombinant Luciferase), which uses aminoluciferin as a substrate to generate a stable "glow-type" luminescent signal and is formulated to improve performance across a wide range of assay conditions. With the addition of the Lysis Reagent (provided), the CytoTox-Glo™ Cytotoxicity Assay delivers a luminescent signal indicative of the total number of cells in each assay well (Figure 1). Viability can be calculated by subtracting the luminescent signal resulting from experimental cell death from total luminescence values.

Performing the CytoTox-Glo™ Cytotoxicity Assay is easy with the GloMax® Discover System. The extended dynamic range of the GloMax® Discover System allows you to easily measure signals of varying intensities on the same plate using the CytoTox-Glo™ Reagent. This Application Note describes the protocol for measuring cytotoxicity using the CytoTox-Glo™ Cytotoxicity Assay and GloMax® Discover System.

CytoTox-Glo™ Cytotoxicity Assay Protocol

For detailed instructions and assay notes, see the *CytoTox-Glo™ Cytotoxicity Assay Technical Bulletin* #TB359. The following protocol can be used to determine cytotoxicity in 100µl of culture in a 96-well plate format.

Note: If you have not performed this assay with your cell line, we strongly recommend that you determine the assay sensitivity for your cells using one of the two methods described in the *CytoTox-Glo™ Cytotoxicity Assay Technical Bulletin* (Section 4.A or 4.B).

Reagent Preparation and Storage

1. Thaw the CytoTox-Glo™ Cytotoxicity Assay components in a 37°C water bath. Mix the components to ensure homogeneity.
2. Prepare the CytoTox-Glo™ Cytotoxicity Assay Reagent by transferring the contents of one bottle of Assay Buffer to the AAF-Glo™ Substrate bottle.
3. Prepare the Lysis Reagent by transferring Digitonin (33µl for Cat.# G9290 and G9291; 162µl for Cat.# G9292) to the Assay Buffer (5ml for Cat.# G9290 and G9291; 25ml for Cat.# G9292). Mix well to ensure homogeneity.

Example Cytotoxicity Assay Protocol and Viability (by Lysis) Assay Protocol

1. Set up 96-well assay plates containing cells in culture medium at the desired density.
2. Add test compounds and vehicle controls to appropriate wells so that the final volume is 100µl in each well.
3. Culture cells for the desired test exposure period.

   Note: All enzymatic markers for cytotoxicity have finite activity half-lives. Although the protease marker(s) measured in this assay demonstrate an improved stability profile compared to other enzymatic markers under most circumstances, we recommend exposing the cells to the test compound for 24 hours or less to ensure that cytotoxicity is not underestimated.
4. Add 50µl of CytoTox-Glo™ Cytotoxicity Assay Reagent to all wells. Mix briefly by orbital shaking, and incubate for 15 minutes at room temperature.
5. Measure luminescence using the GloMax® Discover System.
   a. Select the door icon at the top right on the home page.
   b. Once the door opens, place the plate in the holder with well A1 of the plate at the front left corner.
   c. Select the door icon to close the door.
   d. Select “Protocols” from the home page, then select “Preset” on the left menu.
   e. Select “CytoTox-Glo” from the list of protocols.
   f. To assign assay wells, select the plate icon next to the lock icon. Highlight the assay wells (green wells will be assayed; white wells will not). Select “OK”.
   g. Select “Start” to begin the protocol.
   h. Once measurements are completed select “Export”. The data will be exported as a CSV file and an Excel® file.
6. Add 50µl of Lysis Reagent to all wells. Mix, and incubate at room temperature for 15 minutes.
7. Measure luminescence using the GloMax® Discover System.
8. Calculate viable cell luminescence by subtracting the luminescent signal resulting from experimental cell death (Step 5) from total luminescence death (Step 7).

Total luminescence	–	Experimental dead cell luminescence	=	Viable cell luminescence

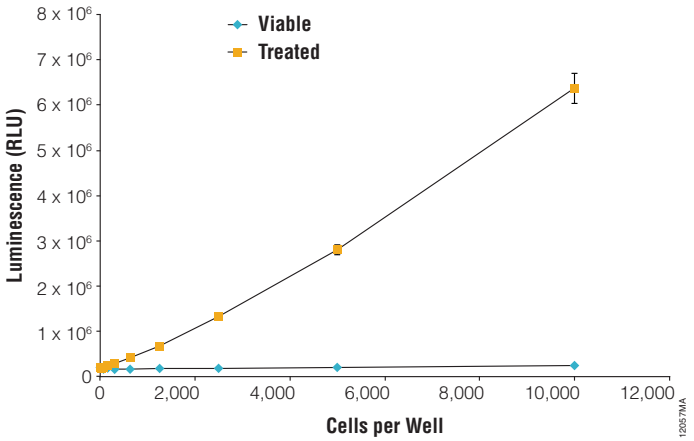

Figure 1. The CytoTox-Glo™ Cytotoxicity Assay is extremely sensitive, and the signal derived from lysed cells is proportional to cell number, demonstrating selective detection of dead cells. K562 cells were treated with Lysis Solution (Cat.# G1821) (treated) or water (viable). Cells were serially diluted in a 96-well plate, and 50µl of CytoTox-Glo™ Cytotoxicity Assay Reagent was added to each well. Luminescence was measured using the GloMax® Discover System. Each point represents the average of three replicates; error bars represent the standard deviation.

Conclusion

The GloMax® Discover shows good assay linearity of luminescence detection using the CytoTox-Glo™ Cytotoxicity Assay over the range of cell numbers shown in Figure 1.

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 applications. The instrument is operated by an integrated Tablet PC, which provides quick and easy navigation through the control options. Exporting your results is seamless with a variety of options, including exporting data to your local network.

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