Measuring Cell Viability Using the CellTiter-Fluor™ Cell Viability Assay and GloMax® Discover System

Promega Corporation

Materials Required

- CellTiter-Fluor™ Cell Viability Assay (Cat.# G6080, G6081 and G6082)
- GloMax® Discover System (Cat.# GM3000)
- Black 96-well plates

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 *CellTiter-Fluor™ Cell Viability Assay Technical Bulletin* #TB371 are available at: **www.promega.com/protocols/**

Assessing cell viability is a necessary step in the drug development process to monitor cell health during compound screening. The GloMax® Discover System in combination with the CellTiter-Fluor™ Cell Viability Assay provides a convenient, rapid and sensitive procedure to determine the number of viable cells in culture. The presence of viable cells is detected by fluorescence quantitation.

The CellTiter-Fluor™ Cell Viability Assay is a nonlytic, single-reagent-addition fluorescence assay that measures the relative number of live cells in a cell population after experimental manipulation. The CellTiter-Fluor™ Cell Viability Assay measures a conserved and constitutive protease activity within live cells that 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 (glycylphenylalanyl- aminofluorocoumarin; GF-AFC). The substrate enters intact cells, where it is cleaved by the live-cell protease activity to generate a fluorescent signal proportional to the number of viable cells (Figure 1). This live-cell protease becomes inactive upon loss of cell membrane integrity and leakage into the surrounding culture medium.

The CellTiter-Fluor™ Cell Viability Assay also can be used in a single-well, sequential, multiplex format with other downstream chemistries to normalize data by cell number. Data from the assay can serve as an internal control and allow identification of errors resulting from cell clumping or compound cytotoxicity. The CellTiter-Fluor™ Cell Viability Assay is compatible with most Promega luminescence assays or spectrally distinct fluorescence assay methods, such as assays measuring caspase activation, reporter gene expression or orthogonal measures of viability.

Performing the CellTiter-Fluor™ Cell Viability 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 96-well same plate using the CellTiter-Fluor™ Reagent. Using the GloMax® Discover System, data are collected in a standard 96-well plate or 384-well plate format. This Application Note describes the protocol for measuring cell viability in a 96-well plate using the CellTiter-Fluor™ Cell Viability Assay and GloMax® Discover System.

Protocol for Measuring Cell Viability

For detailed instructions and assay notes, see the *CellTiter-Fluor™ Cell Viability Assay Technical Bulletin* #TB371. The following procedure can be used to determine cell viability in 100µl of culture medium in a 96-well plate format.

Note: 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 *CellTiter-Fluor™ Cell Viability Assay Technical Bulletin* (Section 4.A or 4.B).

Reagent Preparation and Storage

1. Completely thaw the CellTiter-Fluor™ Cell Viability Assay components in a 37°C water bath.
2. Transfer the GF-AFC Substrate (10µl for Cat.# G6080 and G6081; 50µl for Cat.# G6082) to the Assay Buffer container (10ml for Cat.# G6080 and G6081; 50ml for Cat.# G6082) to form the 2X CellTiter-Fluor™ Reagent. Mix by vortexing until the substrate is thoroughly dissolved.

Example Viability 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 the appropriate wells so that the final volume in each well is 100µl.
3. Culture cells for the desired test exposure period.
4. Add an equal volume of CellTiter-Fluor™ Reagent (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 for more than 3 hours, and be sure to shield plates from ambient light.
5. Measure resulting fluorescence using the GloMax® Discover System (405nm$_{Ex}$/495–505nm$_{Em}$) as follows:
   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 "CellTiter-Fluor" 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.

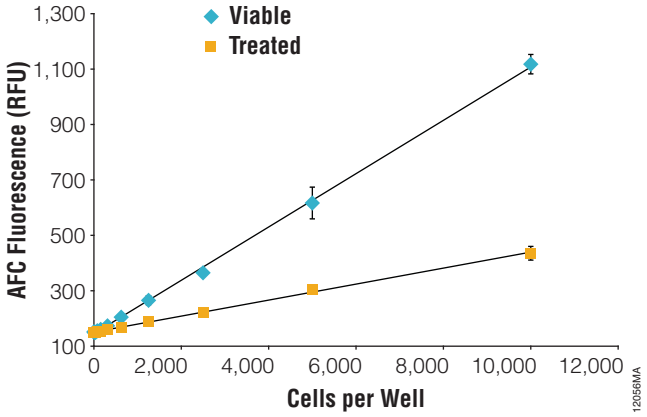

Figure 1. The CellTiter-Fluor™ Cell Viability Assay signal derived from viable cells is proportional to cell number. K562 cells were treated with Lysis Solution (Cat. G1821) (treated) or water (viable) then added to a 96-well plate (0–10,000 cells per well, 100µl). The 2X CellTiter-Fluor™ Reagent was added, and fluorescence was measured. Dead cells (treated) do not contribute appreciable signal in the assay.

Conclusion

The GloMax® Discover shows good assay linearity of fluorescence detection using the CellTiter-Fluor™ Cell Viability 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 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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