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

Promega Corporation

Materials Required

- CellTiter-Glo® Luminescent Cell Viability Assay (Cat.# G7570, G7571, G7572 and G7573)
- GloMax® Discover System (Cat.# GM3000)
- Opaque-walled multiwell 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-Glo® Luminescent Cell Viability Assay Technical Bulletin* #TB288 are available at:
www.promega.com/protocols/

The GloMax® Discover System in combination with the CellTiter-Glo® Luminescent Cell Viability Assay provides a convenient, rapid and sensitive procedure to determine the number of viable cells in culture by quantifying ATP, an indicator of metabolically active cells.

The homogeneous CellTiter-Glo® Luminescent Cell Viability Assay relies on the properties of a thermostable luciferase (Ultra-Glo™ Recombinant Luciferase), which generates a stable "glow-type" luminescent signal and improves performance across a wide range of assay conditions with a half-life of greater than five hours. This extended half-life provides flexibility for continuous or batch-mode processing of multiple plates. The homogeneous "add-mix-measure" format results in cell lysis and generation of a luminescent signal proportional to the amount of ATP, which is directly proportional to cell number. The CellTiter-Glo® Assay is designed for use with multiwell-plate formats, making it ideal for automated high-throughput screening and cell proliferation and cytotoxicity assays.

Performing the CellTiter-Glo® Assay is easy with the GloMax® Discover System, and the protocol comes preloaded on the instrument. The extended dynamic range of the GloMax® Discover System allows the user to easily measure signals of varying intensities on the same plate using the CellTiter-Glo® Assay. The GloMax® Discover System can detect luminescence from 50 mammalian cells, with a signal more than three times background and a linear range of 50 to 50,000 cells per well (Figure 1). This Application Note describes the protocol for measuring luminescence of the CellTiter-Glo® Luminescent Cell Viability Assay using the GloMax® Discover System.

Cell Viability Assay Protocol

For detailed instructions and assay notes for various assay volumes and plate formats, see the *CellTiter-Glo® Luminescent Cell Viability Assay Technical Bulletin* #TB288. A sample procedure is provided below.

1. Thaw the CellTiter-Glo® Buffer, and equilibrate the buffer and lyophilized CellTiter-Glo® Substrate to room temperature.
2. Transfer the appropriate volume of CellTiter-Glo® Buffer to the amber bottle containing CellTiter-Glo® Substrate to reconstitute the lyophilized substrate and form the CellTiter-Glo® Reagent.
3. Prepare opaque-walled multiwell plates with mammalian cells in 100µl of culture medium per well. Prepare control wells containing medium without cells to measure background luminescence.
4. Add test compound to experimental wells, and incubate according to your culture protocol.
5. Equilibrate the plate contents at room temperature for approximately 30 minutes.
6. Add 100µl of CellTiter-Glo® Reagent to each well. Mix contents for 2 minutes on an orbital shaker to induce cell lysis.
7. Incubate the plate at room temperature for 10 minutes to stabilize luminescent signal.
8. Measure luminescence using the GloMax® Discover System 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-Glo" from the list of protocols.
   f. To assign the 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 completed select "Export". The data will be exported as a CSV file and an Excel® file.

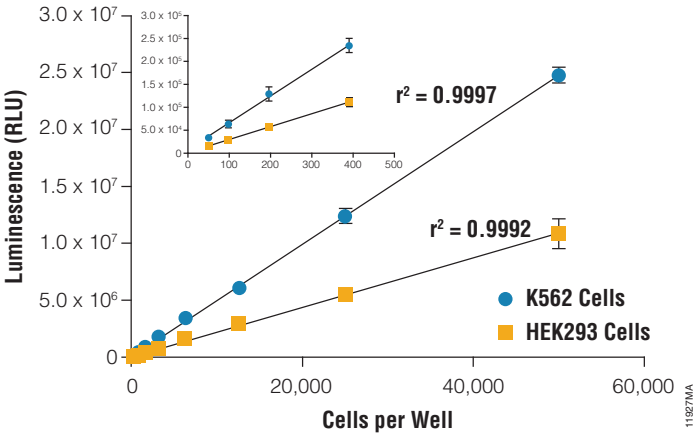

Figure 1. Cell number correlates with luminescent output. Serial twofold dilutions of K562 and HEK293 cells were prepared in a 96-well plate in RPMI or DMEM medium with 10% fetal bovine serum. CellTiter-Glo® assays were performed as described in Section 3.B of the *CellTiter-Glo® Luminescent Cell Viability Assay Technical Bulletin*. Luminescence was recorded 10 minutes after reagent addition using the GloMax® Discover System. Values represent the mean of six replicates for each cell number. Error bars are ± 1 standard deviation. There is a linear relationship (r^2>0.99) between the luminescent signal and cell number from 50 to 50,000 cells per well.

GloMax® Discover System

The GloMax® Discover System and CellTiter-Glo® Luminescent Cell Viability Assay offer superior sensitivity and dynamic range (Figure 1) as well as limited well-to-well cross talk (data not shown). 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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