Measuring Fluorescence Using the CellTiter-Blue® Cell Viability Assay with the GloMax® Discover System

Promega Corporation

Materials Required

- CellTiter-Blue® Cell Viability Assay (Cat.# G8080, G8081 and G8082)
- GloMax® Discover System (Cat.# GM3000)
- 96-well black plate

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocols: *CellTiter-Blue® Cell Viability Assay Technical Bulletin* #TB317 is available at: **www.promega.com/protocols/**

The viability of a population of cells in vitro can be determined using a variety of experimental methods. One parameter used to define cell viability is whether or not metabolic processes remain active. Viable cells must carry out metabolic reactions to generate the energy required to maintain homeostatic processes, including synthesis of critical components and maintenance of membrane potential. When cells lose membrane integrity in vitro, their ability to carry out metabolic processes ceases. The CellTiter-Blue® Cell Viability Assay used in combination with the GloMax® Discover System provides a convenient, rapid, and sensitive procedure for measuring metabolically active cells through fluorescence quantitation.

The CellTiter-Blue® Cell Viability Assay provides a homogeneous, fluorometric method for monitoring cell viability. The assay is based on the metabolic capacity of living cells to convert a redox dye (resazurin) into an end product (resorufin), which is highly fluorescent ($579_{Ex}/584_{Em}$). Nonviable cells rapidly lose metabolic capacity, do not reduce the indicator dye, and thus do not generate a fluorescent signal. The CellTiter Blue® Reagent is a buffered solution containing highly purified resazurin. The conditions for using resazurin reduction as an indicator of cell viability and the other ingredients of the CellTiter-Blue® Reagent have been optimized for use as a cell viability assay.

The CellTiter-Blue® Cell Viability Assay is made easy on the GloMax® Discover System. The extended dynamic range and minimal well-to-well cross talk of the GloMax® Discover System allows the user to easily measure various sample signal intensities on the same plate. Using the GloMax® Discover System, data are collected using a standard 96- or 384-well plate, and fluorescent output is correlated to cells per well (Figure 1). This Application Note describes the protocol for measuring fluorescence using the GloMax® Discover System with the CellTiter-Blue® Cell Viability Assay.

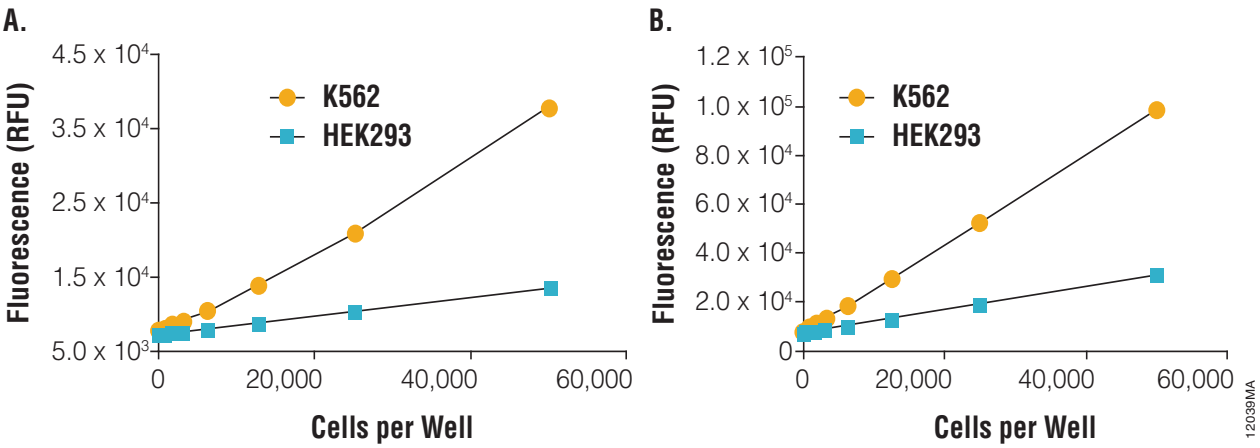

Figure 1. Relative ability of different cell types to reduce resazurin. Serial twofold dilutions of HEK293 or K562 cells were prepared at 100µl/well in a 96-well plate and cultured for 1.5–4 hours at 37°C. CellTiter-Blue® Reagent (20µl/well) was added, and cells were incubated for 1 hour before recording fluorescence (520_{Ex}/580–640_{Em}) using a GloMax® Discover System. Data points represent the mean and standard deviation of triplicate samples. **Panel A.** One and a half-hour incubation. **Panel B.** Four-hour incubation.

Example Cytotoxicity Assay Protocol

For detailed instructions and assay notes, see the *CellTiter-Blue Cell Viability Assay Technical Bulletin* #TB317. The following procedure can be used for caspase-3 and -7 detection in cultured cells in a 96-well plate format but can easily be adapted for measuring caspase activity in purified caspase preparations as well as for 384-well format.

1. Set up 96-well assay plates containing cells in culture medium.
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. Remove assay plates from 37°C incubator and add 20µl/well CellTiter-Blue® Reagent.
5. Shake for 10 seconds.
6. Incubate using standard cell culture conditions for 1–4 hours. **Note:** Extended incubation periods may be used for some applications.
7. Shake plate for 10 seconds, and record fluorescence on the GloMax® Discover ($520nm_{Ex}$/580–$640nm_{Em}$).

GloMax® Discover System

The GloMax® Discover System 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 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. Thus together with the CellTiter-Blue® Assay, the GloMax® Discover offers a complete and easy to use solution to measure cell viability.