

THE CELLTITER-BLUE™ CELL VIABILITY ASSAY: MONITORING CELL VIABILITY USING A FLUORESCENT REDOX INDICATOR DYE

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Here we introduce the CellTiter-Blue™ Cell Viability Assay, a single-addition, homogeneous cell viability assay that is based on the ability of cells to reduce the indicator compound resazurin to resorufin. The assay gives researchers the option to measure cell viability using fluorescence or absorbance, and it also allows users to multiplex some types of assays in a single well of cells.

Introduction

Methods to monitor and track cell viability essentially fall into two categories: those that detect changes in cell membrane integrity and those that function based on the ability of a viable cell to incorporate or metabolize a particular substrate. Researchers continually seek viability assays based on cell metabolism that are single-addition, homogeneous, sensitive and cost-effective. In some cases, a particular means of recording data may be advantageous, so the flexibility to choose absorbance, fluorescence or luminescence as a method of detection is important. The CellTiter-Blue™ Cell Viability Assay is a single-addition, homogeneous assay that measures cell viability using a redox indicator dye resazurin and allows users to measure changes using fluorescence (preferred) or absorbance.

Assay Principle

The CellTiter-Blue™ Assay is based on cellular reduction of resazurin to the fluorescent product resorufin. Viable cells are able to metabolize and reduce the dye; dead cells rapidly lose this capacity once their membrane has been compromised. We formulated and optimized the assay for robust and sensitive detection of viable cells directly in culture medium without multiple additions or washes. Although the actual reduction takes place within the cell, the resorufin diffuses into the surrounding culture medium (1). Upon reduction, resorufin undergoes a dramatic increase in fluorescence, and in most experimental situations, this increase is directly proportional to the number of viable cells. The resorufin excitation and emission peaks in culture medium containing serum are approximately 579nm and 584nm, respectively, allowing fluorescence measurements. In addition, there is an absorbance shift from 605nm to 573nm when resazurin is reduced to resorufin. This absorbance shift allows the assay to be monitored by absorbance at 570nm using 600nm as a reference wavelength. However,

fluorescence is the preferred method for recording data, because it is more sensitive. The CellTiter-Blue™ Assay typically can detect fewer than 150 cells/well using a 384-well format and recording fluorescence.

Simple Protocol

Because the CellTiter-Blue™ Assay is a single-addition, homogeneous assay capable of being used in a variety of multiwell formats, it is convenient for primary or secondary screening assays where cell viability information is sought. The CellTiter-Blue™ Assay protocol is simple and flexible; add the CellTiter-Blue™ Reagent directly to the assay plate containing cells, incubate for 1–4 hours and record fluorescence or absorbance. A recommended ratio of 20µl CellTiter-Blue™ Reagent added to 100µl of cells in culture medium (typical 96-well plate volumes) will be adequate for most situations. For 384-well formats, the addition should be scaled so that 5µl of Reagent is added to 25µl of cells in culture medium. If there is a need to stop the reaction, we recommend adding 3% SDS (sodium dodecyl sulfate; 50µl per 100µl initial culture volume). Plates can be read up to 24 hours after stopping the reaction if they are protected from light and evaporation and stored at room temperature.

Optimizing the Assay

Because every cell line has a different metabolic capacity for reducing resazurin (as well as tetrazolium salts), the fluorescence generated can vary dramatically as illustrated in Figure 1. For optimum performance, the user may empirically determine the length of the incubation period with resazurin and the number of cells, providing a linear response.

One way to optimize initial starting conditions for a cell viability assay or screen is to determine Z'-factor for the assay under different conditions. Although these initial experiments typically involve a large number of data points, they are

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The CellTiter-Blue™ Cell Viability Assay

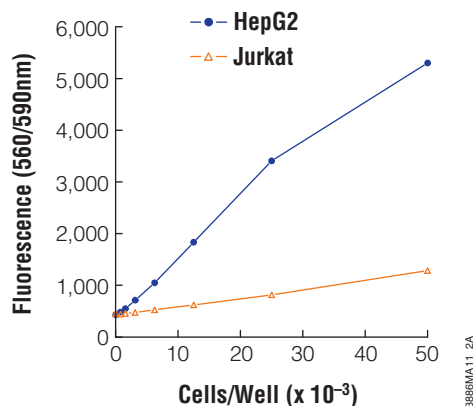


Figure 1. Relative ability of different cell types to reduce resazurin. Serial twofold dilutions of Jurkat or HepG2 cells were prepared at 100µl/well in a 96-well plate and cultured for 1.5 hours at 37°C. CellTiter-Blue™ Reagent (20µl/well) was added, and cells were incubated 1 hour before recording fluorescence 560(20)_{Ex}/ 590(10)_{Em} using a Labsystems Fluoroskan Ascent plate reader.

invaluable for optimizing the starting cell number, the means of dispensing cells into the well, the incubation kinetics with the detection method, and the instrumentation.

We determined a Z'-factor using the CellTiter-Blue™ Assay with L929 cells in a 384-well plate format. The Z'-factor value calculated is affected by the signal dynamic range and variability associated with background and sample. As shown in Table 1, this value increased with the number of cells added to each well, as well as with the length of time the cells were incubated with the CellTiter-Blue™ Reagent. Actual data points used for determining a Z'-factor value with 500 cells are shown in Figure 2. Here, the separation band is large, and the Z'-factor is 0.80, yielding an excellent screening assay (2).

Table 1. Effect of Cell Number and Incubation Time on Z'-Factor Values.

Cells/Well	Mean Fluorescence After 2-Hour 37°C Incubation (n = 96)	Standard Deviation	Z'-Factor Calculation
0	431.97	5.02	
500	710.02	18.43	0.75
2,000	1575.97	67.46	0.81
2,500	1828.19	76.96	0.82

Cells/Well	Mean Fluorescence After 4-Hour 37°C Incubation (n = 96)	Standard Deviation	Z'-Factor Calculation
0	455.28	5.76	
500	1074.58	34.67	0.80
2,000	3056.88	125.37	0.85
2,500	3602.1	132.98	0.87

Four cell suspensions containing varying amounts of L929 cells were prepared. Ninety-six replicates of each cell concentration were dispensed into 384-well plates at 25µl/well. The plate was equilibrated at 37°C for 1 hour before 5µl CellTiter-Blue™ Reagent was added to each well. Fluorescence 560(20)_{nmEx}/ 590(10)_{nmEm} was recorded after 2 and 4 hours of incubation at 37°C using a Labsystems Fluoroskan Ascent plate reader.

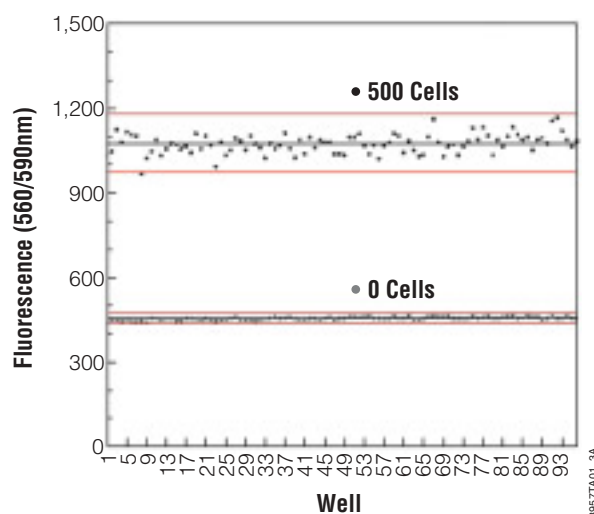


Figure 2. Data points used to obtain a Z'-factor value for 500 cells at 4 hours. Resulting fluorescence after 4-hour incubation at 37°C with CellTiter-Blue™ Reagent of all 96 data points for 0 and 500 cells/well. Black line across represents the mean for that series; red lines above and below represent 3 standard deviations from the mean. A Z'-factor value of 0.80 was achieved with these conditions.

Multiplexing with Other Assays

Researchers often desire multiple endpoints from a single experimental sample. For example, the ability to obtain information on the number of viable cells as well as caspase-3/7 activity in a single sample may help elucidate details on the mechanism of cell death. Short exposure to the CellTiter-Blue™ Reagent is relatively nondestructive to cells, allowing users to multiplex two assays in a single well of cells. Figure 3 shows cell viability data obtained with the CellTiter-Blue™ Reagent and apoptosis data obtained using the Apo-ONE® Homogeneous Caspase-3/7 Assay^(a) from the same experimental samples. Additional information could have been obtained about membrane integrity using the CytoTox-ONE™ Membrane Integrity Assay^(b), which measures LDH activity, if a portion of the culture supernatants had been

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The CellTiter-Blue™ Cell Viability Assay

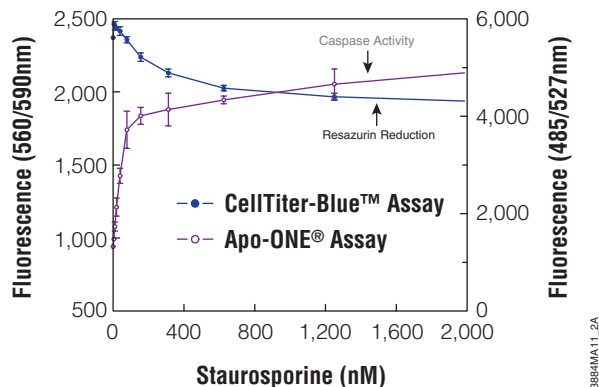


Figure 3. Multiplexing two assays in the same well. Jurkat cells (100µl/well) were treated with various concentrations of staurosporine for 5 hours. CellTiter-Blue™ Reagent (20µl/well) was added to each well immediately after drug addition and incubated simultaneously for 5 hours prior to recording fluorescence (560_{Ex}/590_{Em}). Then caspase activity was measured in the same well by adding 120µl of the Apo-ONE® Homogeneous Caspase 3/7 Assay Reagent. Cells were incubated for an additional hour at ambient temperature prior to recording fluorescence (485_{Ex}/527_{Em}).

Short exposure to the CellTiter-Blue™ Reagent is relatively nondestructive to cells, and two assays can be multiplexed in the same well of cells.

transferred to a new assay plate before adding the CellTiter-Blue™ Reagent to the wells.

The CellTiter-Blue™ Cell Viability Assay is an excellent choice for monitoring cell viability in high-throughput and multiwell formats. The assay is a single-addition, homogeneous assay that can easily be scaled for use in a 96- or 384-well plate, and data can be recorded using a fluorescence reader (preferred method) or an absorbance spectrophotometer. The CellTiter-Blue™ Reagent is extremely stable if protected from extended exposure to light, with no loss of activity after 2 months of storage at 22°C, and no loss in activity when frozen and thawed 10 times. The same assay plate can be read multiple times if increased dynamic range is needed, and the Reagent spectral properties allow multiplexing with the Apo-ONE® Homogeneous Caspase-3/7 Assay. ■

References

1. Voytik-Harbin, S.L. *et al.* (1997) *In Vitro Cell Dev. Biol. Animal* **34**, 239–46.
2. Zhang, H-U. *et al.* (1999) *J. Biomol. Screening* **4**, 67–73.

Protocol

CellTiter-Blue™ Cell Viability Assay Technical Bulletin #TB317
www.promega.com/tbs/tb317/tb317.html

Ordering Information

Product	Size	Cat#
CellTiter-Blue™ Cell Viability Assay	20ml	G8080
	100ml	G8081
	10 × 100ml	G8082
Apo-ONE® Homogeneous Caspase-3/7 Assay ^(a)	1ml	G7792
	10ml	G7790
	100ml	G7791

^(a)This product is covered by U.S. Pat. Nos. 4,557,862 and 4,640,893 and is sold for research use only. All other uses, including but not limited to use as a clinical diagnostic or therapeutic, require a separate license. Please contact Promega Corporation for details relating to obtaining a license for such other use.

^(b)Patent Pending.

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Symposium on Biological Imaging

The Second UW-Madison/Promega Symposium on Biological Imaging, “New Dimensions in In Vivo Imaging,” will be held on May 30, 2003, at Promega Corporation’s BioPharmaceutical Technology Center in Madison, WI. For further information about the symposium please contact Wendy Gelking at: wgelking@promega.com