technically speaking

CellTiter-Blue™ Cell Viability Assay

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Abstract

To assist researchers in measuring cell viability in vitro, Promega Corporation has developed the CellTiter-Blue™ Cell Viability Assay. This article addresses questions about the general assay protocol, assay characteristics and test compound influences.

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Q What is the CellTiter-Blue™ Cell Viability Assay?

The CellTiter-Blue™ Cell Viability Assay is a homogeneous, fluorometric method for estimating the number of viable cells present in a cell population. This system contains the CellTiter-Blue™ Reagent, a buffered solution of highly purified resazurin. Resazurin is a redox indicator dye that can be added directly to cells in culture. Cells convert the dark blue oxidized form of the dye (resazurin) into a red reduced form (resorufin). This system is specific for viable cells since nonviable cells rapidly lose metabolic capacity, do not reduce resazurin and thus do not generate fluorescent signal (1). Results can be recorded with a fluorometer or spectrophotometer.

Q Can the CellTiter-Blue™ Cell Viability Assay be multiplexed with other assays?

Because CellTiter-Blue™ Reagent is relatively non-destructive to cells during short-term exposures, it is possible to use the same cells to do more than one type of assay. One example is the measurement of cell viability using the CellTiter-Blue™ Reagent and the measurement of apoptosis using the Apo-ONE® Homogeneous Caspase-3/7 Assay[a] (2). We recommend performing preliminary studies to determine if the assays of interest are compatible with the assay conditions of the CellTiter-Blue™ Cell Viability Assay.

Q What is the protocol for the CellTiter-Blue™ Cell Viability Assay?

Cells are grown in 96-well or 384-well tissue culture plates in culture medium. The test compounds are added to the cells to bring the final volume per well to 100µl for 96-well plates or 25µl for 384-well plates. Plates are incubated at 37°C for the desired time period. Once the plates are removed from the incubator, 20µl of CellTiter-Blue™ Reagent is added per 100µl of medium. Cells are incubated for 1 to 4 hours, and fluorescence is recorded at $560_{Em}/590_{Ex}$.

The recommended ratio of CellTiter-Blue™ Reagent to medium volume results in a high fluorescent signal and a low fluorescent background. Cell washing, removal of medium and multiple pipetting steps are not required, making this homogeneous assay suitable for manual as well as high-throughput, automated screening assays for cell viability and cytotoxicity.

Q What is the optimal incubation time?

The ability of different cell types to reduce resazurin to resorufin varies depending on their metabolic capacity. For most applications a 1- to 4-hour incubation is adequate. For screening assays, the optimal number of cells/well and length of the incubation period should be empirically determined (1,2).

The CellTiter-Blue™ Reagent is designed for use as an endpoint assay rather than a kinetic method for monitoring cell growth. The reagent should be added near the end of the period of exposure to the test compound (3). Longer incubation periods increase assay sensitivity (1). Assays using low cell numbers will produce little fluorescent signal during a 2-hour incubation period with resazurin; however, extending the incubation period to several hours may improve the signal-to-background ratio and the lower limit of detection.

What equipment is needed to perform the assay?

Fluorescence is recorded using a fluorometer with a $560(20)_{Ex}/590(10)_{Em}$ filter set. Options for filter sets include 530–570nm filters for excitation and 580–620nm filters for emission. Care should be taken that excitation and emission band pass do not overlap. The absorbance maximum of resazurin is 605nm, and the absorbance maximum of resorufin is 573nm. Alternatively, visible light absorbance can be recorded using a spectrophotometer. However, measuring fluorescence is the method of choice for gathering data because of the higher sensitivity (1).

How can I stop the reaction?

The CellTiter-Blue™ Assay reaction can be stopped and the fluorescence stabilized by the addition of 50µl of 3% SDS per 100µl of culture volume. The plate can then be stored at ambient temperature for up to 24 hours before recording data, provided that the contents are protected from light and covered to prevent evaporation.

What is the correlation between IC_{50} values obtained using CellTiter-Blue™ Assay and Promega's other cell proliferation and cytotoxicity assays?

Data are available that show a correlation between the CellTiter-Blue™ Assay and the CellTiter 96® AQ_{ueous} One Solution Assay[(b)], which uses the reduction of the tetrazolium salt MTS[(b)] to a colored formazan product to measure cell viability. There is also a correlation between the CellTiter-Blue™ Assay and CellTiter-Glo® Luminescent Cell Viability Assay[(c)], which measures ATP levels (1). For example, when HepG2 cells are exposed to increasing concentrations of tamoxifen, the IC_{50} values determined using these three assays are similar (1). Similarly, there are data showing that the IC_{50} values determined using the CellTiter-Blue™ Assay correlate with the IC_{50} values determined with the CytoTox-ONE™ Homogeneous Membrane Integrity Assay[(c)], which measures lactate dehydrogenase (LDH) release, a marker of membrane integrity and an indicator of cytotoxicity. There is an inverse correlation between resazurin reduction and release of LDH (1).

How is resazurin reduced in a cytotoxicity assay versus a cell viability assay?

Resazurin is a component of both the CytoTox-ONE™ Homogeneous Membrane Integrity Assay and the CellTiter-Blue™ Cell Viability Assay but in different formulations. The CytoTox-ONE™ Homogeneous Membrane Integrity Assay measures the release into the culture medium of lactate dehydrogenase (LDH) from cells with damaged membranes. LDH release results in the conversion of resazurin into resorufin using a coupled enzymatic assay (4).

The reaction conditions recommended in the CytoTox-ONE™ Homogeneous Membrane Integrity Assay protocol drive reaction rates by supplying excess lactate and NAD+ as substrates for LDH. The 10-minute incubation at ambient temperature results in a negligible amount of resazurin reduction by the viable cell population and only a very slight increase in fluorescence in the wells containing viable cell controls (4). The CellTiter-Blue™ Reagent does not contain the coupling reagents needed to drive resazurin reduction by LDH and thus LDH released from dead and damaged cells does not interfere with this assay.

How can I control for the potential effects of cell culture components or test compounds on the CellTiter-Blue™ Viability Assay?

Each experiment should include the proper controls to determine if any components of the cell culture system have an effect on the assay. Suggested controls include: vehicle control to measure any effect of the solvent, a no-cell negative control to determine background, a positive control for cytotoxicity and a test-compound only control to determine the extent to which the test compound can reduce the resazurin or interfere with fluorescence monitoring.

References

1. *CellTiter-Blue™ Cell Viability Assay Technical Bulletin* #TB317, Promega Corporation.
2. Moravec, R. and Riss, T. (2002) *Cell Notes* **5**, 12–14
3. Riss, T. and Moravec, R. (2003) *Promega Notes* **83**, 10–13.
4. *CytoTox-ONE™ Homogeneous Membrane Integrity Assay Technical Bulletin* #TB306, Promega Corporation.

[(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)] The MTS tetrazolium compound is the subject of U.S. Pat. No. 5,185,450 assigned to the University of South Florida and is licensed exclusively to Promega Corporation.

[(c)] Patent Pending.