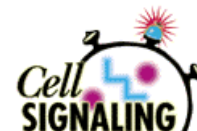


Technically Speaking

# CellTiter 96<sup>®</sup> Non-Radioactive Cell Proliferation Assays and CytoTox 96<sup>®</sup> Non-Radioactive Cytotoxicity Assay



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*Promega currently offers several systems to non-radioactively monitor cell proliferation and cytotoxicity. The CellTiter 96<sup>®</sup> Non-Radioactive Cell Proliferation Assay and CellTiter 96<sup>®</sup> AQ<sub>ueous</sub> Non-Radioactive Cell Proliferation Assay are colorimetric alternatives to [<sup>3</sup>H]thymidine incorporation assays designed to determine cell proliferation and cytotoxicity. A new system, the CellTiter 96<sup>®</sup> AQ<sub>ueous</sub> One Solution Cell Proliferation Assay, was recently introduced as a more convenient alternative to the existing CellTiter 96<sup>®</sup> AQ<sub>ueous</sub> Systems. The CytoTox 96<sup>®</sup> Non-Radioactive Cytotoxicity Assay is a sensitive, colorimetric alternative to <sup>51</sup>Cr release assays for the measurement of cytotoxicity and total cell number.*

## **Q: How does the CellTiter 96<sup>®</sup> Non-Radioactive Cell Proliferation Assay work?**

The CellTiter 96<sup>®</sup> Assay is a non-radioactive, colorimetric assay for measuring the number of viable cells in proliferation, attachment and agent-mediated cytotoxicity assays. Both adherent and suspension cells may be analyzed with this system. The Dye Solution, containing the tetrazolium salt MTT, is added to the cells and is internalized and reduced into an insoluble blue formazan product by cellular metabolism. Only those cells which are living at the time the dye is added will significantly reduce the MTT. The Solubilization Solution is added to lyse the cells and dissolve the formazan dye product. The samples are then read in a 96 well plate reader at 570nm. The intensity of the blue color that appears is directly proportional to the number of viable cells.

## **Q: How does the CellTiter 96<sup>®</sup> AQ<sub>ueous</sub> Assay work and how does this differ from the original CellTiter 96<sup>®</sup> Assay?**

The CellTiter 96<sup>®</sup> AQ<sub>ueous</sub> Assay can be used for the same applications as the CellTiter 96<sup>®</sup> Assay. Both systems measure the conversion of a tetrazolium salt into a colored formazan product by the metabolic activity of living cells. The main difference between the two systems is that the CellTiter 96<sup>®</sup> AQ<sub>ueous</sub> Assay utilizes MTS\* rather than MTT as the tetrazolium reagent. The system also includes PMS, an electron coupling reagent, which facilitates the reduction of MTS. During the assay, MTS is converted into a soluble formazan product, eliminating the need for addition of Solubilization Solution. After incubating the samples for 1-4 hours, they are quantitated using a 96 well plate reader at 490nm. Since the final product is soluble in culture medium, samples may be returned to the incubator for further color development if desired (unlike the CellTiter<sup>®</sup> 96 Assay System). We do not recommend total incubation times beyond 5-6 hours.

*\*The MTS tetrazolium compound is the subject of U.S. Pat. No. 5,185,450 assigned to the University of South Florida, which is licensed exclusively to Promega Corporation.*

## **Q: How does the CellTiter 96<sup>®</sup> AQ<sub>ueous</sub> One Solution Cell Proliferation Assay differ from the CellTiter 96<sup>®</sup> AQ<sub>ueous</sub> Assay?**

The CellTiter 96<sup>®</sup> AQ<sub>ueous</sub> Assay includes the electron carrier phenazine methosulfate (PMS), which mediates the reduction of the tetrazolium salt MTS. The MTS and PMS solutions must be mixed prior to the addition of the dye solution to the cell culture medium. The CellTiter 96<sup>®</sup> AQ<sub>ueous</sub> One Solution Assay contains a single solution of MTS and phenazine ethosulfate (PES). The PES component is an alternative electron carrier which is more stable in solution than PMS. The solution containing MTS and PES is supplied pre-mixed, sterile and ready to add to cell culture medium. The formazan product of the CellTiter 96<sup>®</sup> AQ<sub>ueous</sub> One Solution Assay is also soluble in tissue culture medium. Comparing the relationship of cell number to color formation, the performance characteristics of these two systems are nearly identical.

## **Q: How can background readings with the CellTiter 96<sup>®</sup> and CellTiter 96<sup>®</sup> AQ<sub>ueous</sub> Assays be reduced?**

Background absorbance readings using the MTT-based CellTiter 96<sup>®</sup> Assay are usually less than 0.05 absorbance units in assay wells containing no viable cells and no background correction is usually necessary. Background readings from untreated cells using the CellTiter 96<sup>®</sup> AQ<sub>ueous</sub> Systems are typically 0.1-0.3 absorbance units. These values can be subtracted from the absorbance values of treated cells to obtain a corrected absorbance. To reduce background absorbance, avoid including high concentrations of reducing agents such as DTT, ascorbic acid, coenzyme A, glutathione, NADH or NADPH in the cell culture medium. These agents can reduce the tetrazolium salts and increase background absorbance. High pH can also cause increased background absorbance. Medium containing

HEPES buffer in addition to carbonate will help maintain a constant pH. If carbonate-buffered medium is used, the tissue culture incubator door should be kept closed as much as possible to keep the CO<sub>2</sub> levels constant and the pH of the medium between 7.0-7.4.

**Q: Have these assays been used with cell types other than mammalian cultured cells?**

The MTT and MTS-based systems have been used with yeast (1), bacterial (2), protozoan (3) and plant cells. Primary cell cultures have also been used with these types of assays (4).

**Q: Will the agent used in proliferation or cytotoxicity screening affect the reduction of MTT or MTS?**

With all screening assays, we recommend a control experiment containing medium with the agent being tested, MTT or MTS and no cells. This will determine if the agent alone will reduce the tetrazolium salt, and provides a background absorbance value which can be subtracted from all wells.

**Q: How does the CytoTox 96<sup>®</sup> Non-Radioactive Cytotoxicity Assay work?**

The CytoTox 96<sup>®</sup> Assay is a colorimetric cytotoxicity assay which serves as a substitute for <sup>51</sup>Cr release assays. The assay can be used to monitor cell-mediated or agent-mediated cytotoxicity. The assay measures lactate dehydrogenase (LDH), a stable cytosolic enzyme that is released upon cell lysis, in much the same way as <sup>51</sup>Cr is released in radioactive assays. Released LDH in culture supernatants is measured in a 30 minute coupled enzymatic assay which results in the conversion of a tetrazolium salt (INT) into a red formazan product. The amount of color formed is proportional to the number of lysed cells. Visible wavelength absorbance data at 490nm are collected using a standard 96 well plate reader.

**Q: Should the CellTiter 96<sup>®</sup> Cell Proliferation or CytoTox 96<sup>®</sup> Cytotoxicity Assays be used when performing biocompatibility testing with a single cell type?**

Either the CellTiter 96<sup>®</sup> Cell Proliferation or CytoTox 96<sup>®</sup> Cytotoxicity Assay may be used to measure biocompatibility or cytotoxicity. There are several considerations for each choice. The CellTiter 96<sup>®</sup> Assays require a minimum of 1,000 cells/well for accurate determinations. The CytoTox 96<sup>®</sup> Assays can detect as few as 150 cells/well under serum-free conditions. However, the CytoTox 96<sup>®</sup> Assay requires additional control experiments which are not necessary when using the CellTiter 96<sup>®</sup> Assays. See references 5 and 6 for a detailed discussion of biocompatibility testing and total cell number quantitation using the CytoTox 96<sup>®</sup> Assay.

**Q: How can background readings be reduced when using the CytoTox 96<sup>®</sup> Assay?**

Background absorbance is caused mainly by the spontaneous release of LDH from the target or effector cells. To minimize this spontaneous release, the health of the cells must be carefully maintained. Culture cells prior to the assay at low cell density (<1.5 x 10<sup>6</sup> cells/ml) in the optimal culture medium containing serum and/or cytokine supplements. When handling cells, avoid excessive centrifugation forces (>250 x g), rapid temperature changes during washes and vigorous pipetting. After isolating effector cells from their host, a 1-2 hour rest period in complete medium in a cell culture incubator will reduce cell stress and spontaneous LDH release. Target cell-only and effector cell-only control wells will account for spontaneous release of LDH from each cell type.

**Q: How can culture medium background levels be reduced when using the CytoTox 96<sup>®</sup> Assay?**

The most common source of medium background is the LDH found in serum supplements. Serum concentrations of 10% are generally too high for this assay. Most cell types remain healthy in medium containing 3-5% serum. The amount of serum in the medium can be optimized to find the level which promotes cell health but keeps LDH background levels low. Different types of sera typically contain different levels of LDH. Common types of sera listed in order of increasing LDH levels include: human AB serum, horse serum, fetal bovine serum and calf serum. Phenol red is a minor contributor to culture medium background and background readings can be subtracted from the experimental values. However, phenol red-free media may be used. A cell culture medium-only control will account for LDH or reductases in the medium and serum.

## References

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