

Technically Speaking

CellTiter 96™ and CellTiter 96™ AQueous Non-Radioactive Cell Proliferation Assays

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Promega has recently introduced a number of non-radioactive alternatives to standard research techniques. The CellTiter 96™ Non-Radioactive Cell Proliferation Assay, and more recently, the CellTiter 96™ AQueous Non-Radioactive Cell Proliferation Assay are colorimetric alternatives to [3H] thymidine incorporation assays to determine cell proliferation. In addition to the CellTiter Systems, we also have introduced the CytoTox 96™ Non-Radioactive Cytotoxicity Assay as a sensitive alternative to [51Cr] release assays to study cell-mediated cytotoxicity. The following is a list of the most commonly asked questions on each of the three systems and provides some comparative information to ensure that you can find the product which best meets your needs.

Q. How does the CellTiter 96 Assay work?

The CellTiter 96 Assay is a colorimetric assay system which measures the reduction of a tetrazolium component (MTT) into an insoluble formazan product by the mitochondria of viable cells. After incubation of the cells with the Dye Solution for approximately 1-4 hours, a Solubilization Solution is added to lyse the cells and solubilize the colored product. These samples can be read using an ELISA plate reader at a wavelength of 570nm. The amount of color produced is directly proportional to the number of viable cells.

Q. How does the CellTiter 96 AQ Assay work?

CellTiter 96 AQ Assay, like the CellTiter 96 System, measures the conversion of a tetrazolium compound into a formazan product by the mitochondria of living cells. The main difference between the two systems is that the CellTiter 96 AQ System utilizes MTS rather than MTT as the tetrazolium reagent. During the assay, MTS is converted into a **soluble** formazan product, eliminating the addition of a solubilization solution. Samples are read after a 1-4 hour incubation using an ELISA plate reader at 490nm. Because the final product is soluble, samples may be returned to the incubator for further color development if desired.

Q. Can CellTiter 96 or CellTiter 96 AQ Assays directly replace [3H]thymidine incorporation assays?

Yes, the addition of either dye solution can be substituted at the time point in the assay when radioactive thymidine is added.

Q. How do the CellTiter96 and the CellTiter 96 AQ Assay results compare to [3H]thymidine incorporation assays?

Since no cell harvesting or medium changes are necessary with either CellTiter 96 Assay, the standard deviation values are less than [3H]thymidine incorporation assays. The CellTiter 96 and CellTiter 96AQ Assays require less cell manipulations, reducing the possibility of error. Direct comparisons between [3H]thymidine incorporation and the CellTiter 96 Assay have demonstrated less than a 5% difference for determination of growth factor content of several samples (1).

Q. How many cells are required to obtain an efficient reading with the CellTiter Assay?

For most tumor cells, hybridomas and fibroblast cell lines, we recommend 5,000 cells per well to initiate proliferation assays, although as few as 1,000 cells per well have been used successfully. The known exception to this is blood lymphocytes (2) which require approximately ten-fold more cells (25,000-250,000 cells/well) to obtain a sufficient absorbance reading.

Q. Are there cell types which will not work with the CellTiter Assays?

Cells with functional mitochondria are needed to convert the tetrazolium dye into their respective reduced forms. Most eukaryotic cells in culture, including mammalian (suspension and adhesion cell types), plant and yeast cell types, reduce the dye sufficiently to perform accurate assays.

Q. Can the CellTiter Systems be used for cytotoxicity studies?

Yes, CellTiter 96, CellTiter 96 AQ and CytoTox 96 Systems can be used to study cell death mediated by a cytotoxic agent. For **cell-mediated** cytotoxicity assays, we recommend the CytoTox 96 Assay System.

Q. How does the CytoTox 96 Non-Radioactive Assay work?

The CytoTox 96 Assay is a colorimetric assay system which measures lactate dehydrogenase (LDH), a stable cytosolic enzyme that is released upon cell lysis in much the same way as [51Cr] 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 (3). The amount of color formed is proportional to the number of lysed cells. Visible wavelength absorbance (490nm) data are collected using an ordinary 96 well plate reader.

Q. How do the CytoTox 96 results compare to [51Cr] release assays?

Variations on this technology have been reported for measuring natural cytotoxicity and have been demonstrated to be identical within experimental error to values determined in parallel [51Cr] release assays (4,5). Studies at Promega have shown good correlation between the two assays.

Q. Will the number of target cells vary for different cell types?

Yes, because various target cell types contain different amounts of LDH, we recommend performing a preliminary experiment using the cell type of interest to determine the optimum number of target cells to use with the CytoTox 96 Assay to ensure an adequate signal to noise ratio.

Q. When should I use the CytoTox 96 Assay instead of the CellTiter 96 Assays?

CellTiter 96 indirectly measures cell proliferation, while CytoTox 96 indirectly measures cell death. The CytoTox 96 Assay was developed primarily as a replacement for the [51Cr] release assay to study **cell-mediated** cytotoxicity because the CellTiter 96 Assay Systems cannot differentiate between a mixture of viable cells. However, both systems can be used to study cell death when only one cell type is present.

Q. Can I alter the CytoTox 96 Assay protocol to measure cell death with only one cell type present if I wish to measure LDH?

Yes. For example, a typical drug chemosensitivity experiment might be set up using a constant number of cells which have various concentrations of the test component added. A background control consisting of medium alone would be used as the "blank." A relative amount of LDH release (absorbance 490nm, Y-axis) versus concentration of the cytotoxic agent (X-axis, log scale) could be plotted to determine IC50 value.

Q. What factors affect background readings?

There are a number of factors which affect the background absorbance for the CytoTox 96 Assay, including the spontaneous release of LDH from effector cells, target cells, LDH present in serum used in the medium, and phenol red. The controls correct for all of these factors. The quantity of LDH in animal sera varies. Generally, calf serum contains the highest LDH levels, followed by fetal bovine serum, horse serum and finally human AB serum. Eliminating phenol red and reducing serum concentrations in media help to reduce background absorbance.

References

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