

Total Cell Quantitation Using the CytoTox 96 (TM) Non-Radioactive Cytotoxicity Assay

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Abstract

Through simple modifications to the protocol, the CytoTox 96(TM) Assay can be used to measure total cell number, without the use of radioisotopes.

Introduction

As more and more laboratories face dramatic increases in the costs associated with the use and disposal of radioisotopes, immediate needs arise to develop alternative non-radioactive methodologies that are sensitive, easy to use and readily adaptable to current protocols. The CytoTox 96(TM) Assay offers solutions for these problems. The CytoTox 96 reagents measure lactate dehydrogenase (LDH) activity released from cells by the generation of a red colored product that is measured in an endpoint fashion using an ordinary ELISA plate reader. Although the CytoTox 96 Assay was designed primarily to measure cell-mediated cytotoxicity, this article describes how the CytoTox 96 Assay also can be adapted easily to quantitate total cell number.

Historical radioactive cell quantitation methods

Cell quantitation has been performed most commonly with the use of tritiated thymidine [³H] incorporation or prelabeling cells with radioactive sodium chromate (⁵¹Cr). Thymidine use requires a 1-4 hour "pulse," wash and harvest of cells followed by scintillation counting. Quantitation with chromium requires pre-labeling of cells with high levels of ⁵¹Cr, washing and eventual counting in a gamma counter. Both methods are time and labor intensive. In addition, they require disposal of liquid and solid radioactive waste.

Total cell quantitation with the CytoTox 96 assay

The CytoTox 96 Assay indirectly measures lactate dehydrogenase activity, which is present in the cytoplasm of intact cells. Cell quantitation therefore can occur only if the cells are lysed to release the LDH present in the cell. Certain detergents (SDS and Cetrimide) have been shown to inhibit the assay's conversion of LDH activity to the final red product. However, the Lysis Solution included with the CytoTox 96 Assay can be used for cell lysis and does not interfere with assay substrate activity when used as recommended.

Cell samples of interest are lysed by adding 15µl of Lysis 10X Solution (9% (v/v) Triton X-100 in water) per 100µl of culture medium, followed by incubation at 37°C for 45-60 minutes. Sample

supernatants (50µl) are then transferred to a fresh 96 well enzymatic assay plate. Reconstituted Substrate Mix (50µl) is added to each supernatant sample, and the enzymatic conversion of LDH activity is allowed to proceed for 30-50 minutes at room temperature, protected from light. The enzymatic assay is then stopped by adding 50µl/well of the Stop Solution. The plate can be read at 490nm using an ordinary ELISA plate reader. The number of cells present will be directly proportional to the absorbance values which represent LDH activity. Resulting data can be plotted with absorbance 490nm values along the "y-axis" and cell number along the "x-axis." [Figure 1](#) summarizes these steps.

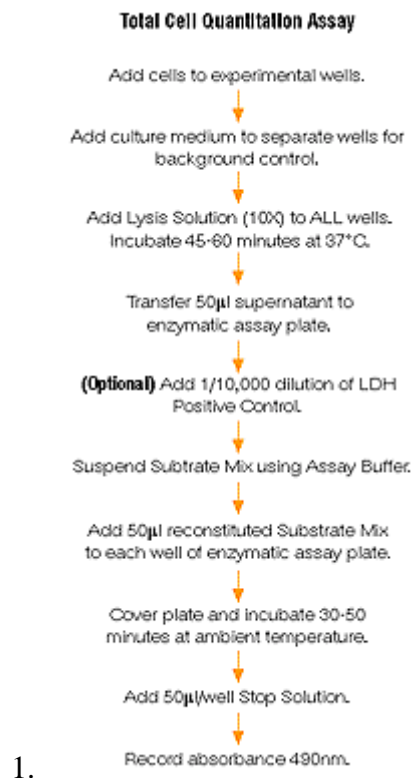


Figure 1. Modified CytoTox 96 Assay procedure for total cell number.

Optimizing background

Two factors will contribute to "background" absorbance (wells without cells present), phenol red in culture medium and active LDH found in varying amounts in serum supplements. Among the sera that we have tested, the rank from lowest to highest LDH activity is: human AB serum, horse serum, fetal bovine serum and calf serum. For maximum sensitivity, we recommend reducing the concentration of serum as much as possible (keeping in mind why and how much serum is necessary for the actual experiment) or replacing serum with 1% bovine serum albumin.

Results

The CytoTox 96 Assay can be used with the slightly modified protocol described above for non-radioactive cell quantitation. Using three different cell types, the resulting absorbance values were shown to be directly proportional ($r^2 > 0.99$) to the number of cells ([Figure 2](#)). Maximum sensitivity can be achieved (<1500 cells/well for all three types) by substituting 1% BSA for whole serum.

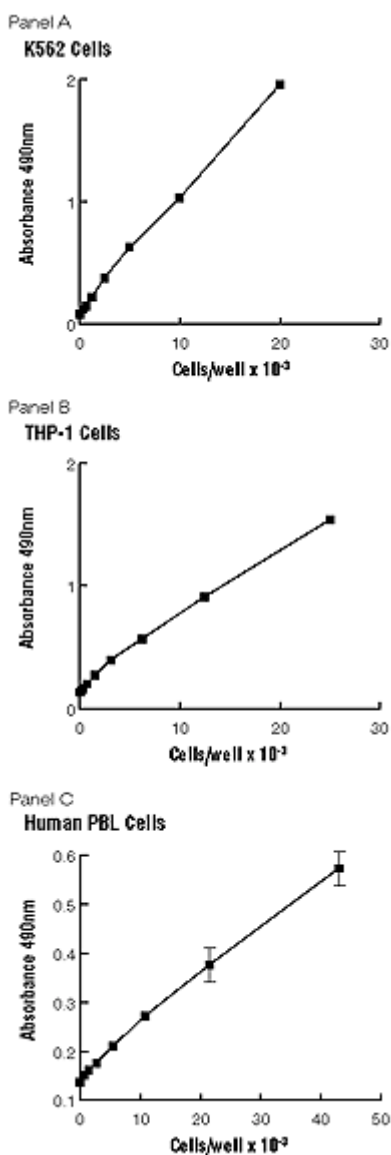


Figure 2. Effect of cell number on absorbance. Various concentrations of K562 cells, THP-1 cells and freshly isolated non-adherent human PBL in culture medium containing phenol red, 15mM HEPES and 1% bovine serum albumin were added to wells of a 96 well plate. Lysis 10X Solution was added to each well and plates incubated at 37°C in a humidified 5% CO₂ incubator for 45-60 minutes. Enzymatic determination of LDH activity was determined as described. Each point represents the mean +/- S.D. of 3 replicates.

Summary

The CytoTox 96 Assay provides a non-radioactive approach to studying cell-mediated cytotoxicity. With some simple modifications to the standard protocol, the CytoTox 96 Assay also can be used to easily quantitate cell numbers without the use of radioactive labels.

Ordering Information

Product	Size	Cat.#
CytoTox 96(TM) Non-Radioactive Cytotoxicity Assay	10 96 well plates	G1780

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