Product Manual

CytoSelect™ MTT Cell Proliferation Assay

Catalog Number

CBA-252 960 assays in 96-well plates

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

The measurement and monitoring of cell proliferation is an essential technique in any laboratory focused on cell-based research. This skill allows for the optimization of cell culture conditions as well as the determination of cytokine, growth factor, or hormone activity. More importantly, the cytostatic nature of anticancer compounds in toxicology testing, the efficacy of therapeutic chemicals in drug screening, and cell-mediated cytotoxicity can all be assessed through the quantification and monitoring of cell proliferation.

Cell proliferation characteristics include cellular metabolic activity and cell membrane integrity. One method for measuring metabolic activity is to incubate the cells with a tetrazolium salt such as WST-1, which is cleaved into a colored formazan product by metabolically active cells. Similarly, the green fluorescent dye Calcein AM can measure intracellular esterase activity in proliferating live cells, which is another indicator of cell viability.

Assay Principle

Cell Biolabs' CytoSelect™ MTT Cell Proliferation Assay provides a colorimetric format for measuring and monitoring cell proliferation. The kit contains sufficient reagents for the evaluation of 960 assays in 96-well plates or 192 assays in 24-well plates. Cells can be plated and then treated with compounds or agents that affect proliferation. Cells are then detected with the proliferation reagent, which is converted in live cells from the yellow tetrazole MTT to the purple formazan form by a cellular reductase (Figure 1). An increase in cell proliferation is accompanied by an increased signal, while a decrease in cell proliferation (and signal) can indicate the toxic effects of compounds or suboptimal culture conditions. The assay principles are basic and can be applied to most eukaryotic cell lines, including adherent and non-adherent cells and certain tissues. This cell proliferation reagent can be used to detect proliferation in bacteria, yeast, fungi, protozoa as well as cultured mammalian and piscine cells.

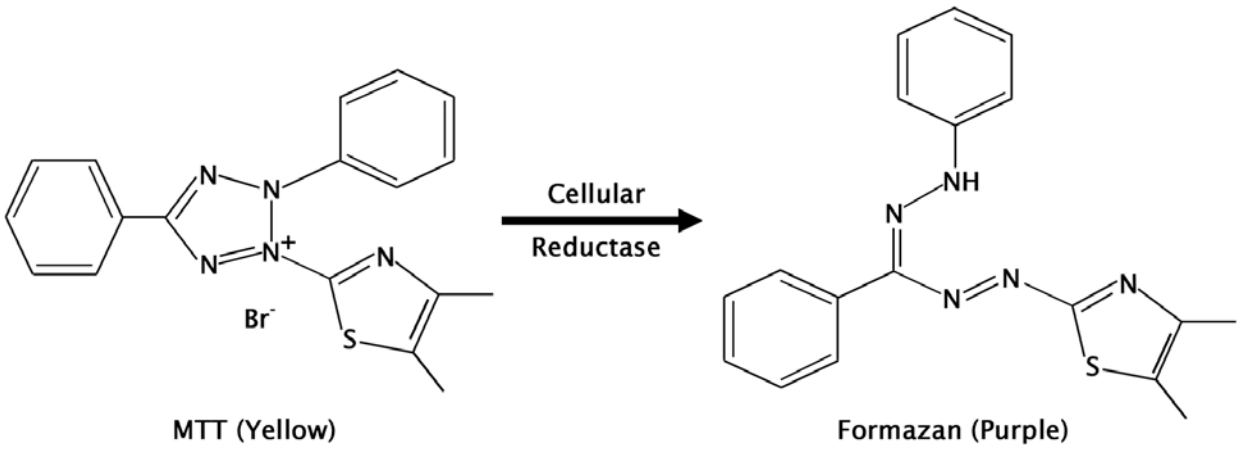

Figure 1. Chemical Structures of Yellow MTT and Purple Formazan Product in Living Cells.

Related Products

1. CBA-080: CytoSelect™ 24-Well Anoikis Assay
2. CBA-081: CytoSelect™ 96-Well Anoikis Assay
3. CBA-230: Cellular Senescence Assay Kit (SA-β-gal Staining)
4. CBA-231: 96-Well Cellular Senescence Assay (SA β-Gal Activity)
5. CBA-232: Quantitative Cellular Senescence Assay (SA β-Gal)
6. CBA-240: Cell Viability and Cytotoxicity Assay
7. CBA-250 CytoSelect™ Fluorometric Cell Proliferation Assay Reagent
8. CBA-251 CytoSelect™ BrdU Cell Proliferation ELISA Kit
9. CBA-253 CytoSelect™ WST-1 Cell Proliferation Assay Reagent

Kit Components

1. MTT Cell Proliferation Assay Reagent (Part No. 125201): One 10 mL bottle of MTT reagent.
2. Detergent Solution (Part No. 125202): One 20 mL bottle of Detergent Solution.

Materials Not Supplied

1. Cells for measuring proliferation
2. Cell culture medium
3. 24-well or 96-well clear cell culture plates.
4. Microtiter plate reader capable measuring absorbance at 540-570 nm.

Storage

The MTT Cell Proliferation Assay Reagent is a clear yellow ready-to-use solution, and it should be stored at -20°C protected from light. Store the Detergent Solution at room temperature. If precipitate or turbidity is observed in the Detergent Solution, warm the solution to 37°C for 10–20 minutes and agitate to dissolve the precipitate prior to use.

Assay Protocol

1. Prepare a cell suspension containing 0.1-1.0 x 10^6 cells/ml in medium.
2. Add 100 µL per well to a 96-well cell culture plate or 500 µL per well to a 24-well cell culture plate with or without the compound to be tested. Culture the cells for 24-96 hours at 37°C and 5% CO_2 in a humidified incubator.
3. Add 10 µL of the CytoSelect™ MTT Cell Proliferation Assay Reagent to each well if using a 96-well plate, or 50 µL to each well of a 24-well plate.
4. Incubate plate at 37°C and 5% CO_2 for 3-4 hours until purple precipitate is visible (cellular precipitate can be more precisely visualized under a light microscope)

5. Add 100 µL of Detergent Solution per well of a 96-well plate, or 500 µL per well of a 24-well plate.
6. Incubate at room temperature for 2 hours to overnight protected from light.

   Note: Longer incubations with Detergent Solution in the wells may result in precipitate or turbidity that can increase background. If precipitate is observed, warm the plate at 37°C for 10-20 minutes and agitate to dissolve the precipitate.
7. Read absorbance using 540-570 nm as the primary wavelength.

Example of Results

The following figure demonstrates typical results with the CytoSelect™ MTT Cell Proliferation Assay. One should use the data below for reference only. This data should not be used to interpret actual results.

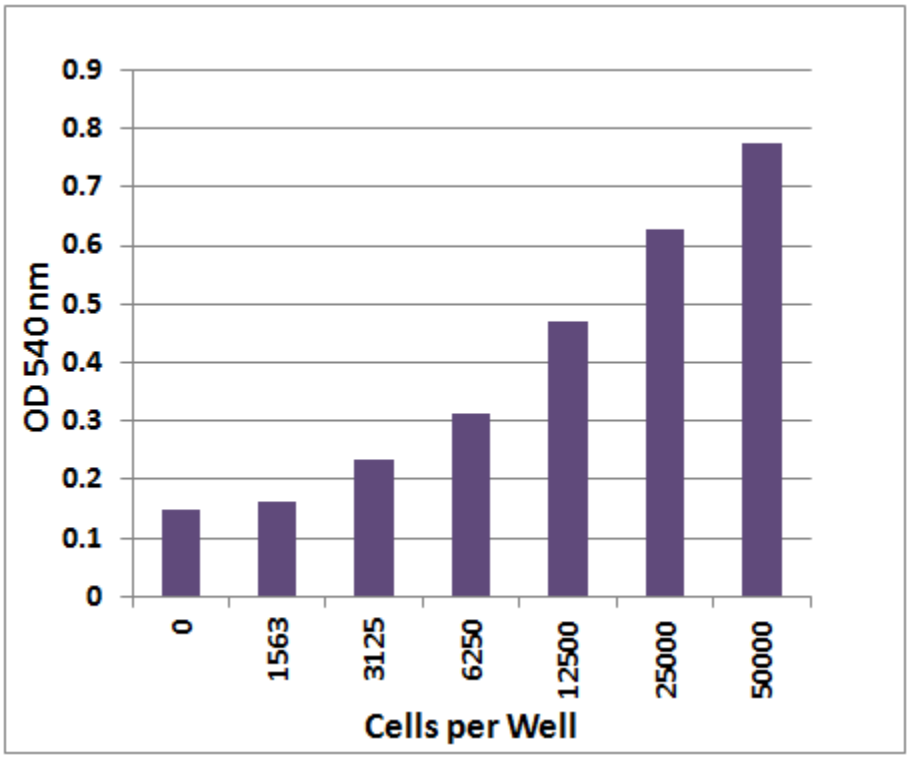

Figure 2. Human HEK 293 Cell Density. HEK 293 cells were seeded at various densities as indicated above and allowed to grow for 24 hours. After adding CytoSelect MTT Cell Proliferation Assay Reagent, cells were then incubated for 3 hours at 37ºC and 5% CO_2 and solubilized with Detergent Solution for 3 hours.

References

1. Jacobsen MD, Weil M, Raff MC. (1996) *J Cell Biol* **133**, 1041.
2. Papadopoulos NG, Dedoussis GV, Spanakos G, Gritzapis AD, Baxevanis CN, Papamichail M. (1994) *J Immunol Methods* **177**, 101.
3. Yamaori S, Ishii H, Chiba K, Yamamoto I, Watanabe K (2013) *Toxicology* **314**, 251
4. Wang Y, Qu L, Gong L, Sun L, Gong R, Si J (2013) *Cancer Biother Radiopharm*. **28**, 623

Warranty

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