

---

**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.

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-230: Cellular Senescence Assay Kit (SA-β-gal Staining)
2. CBA-232: Quantitative Cellular Senescence Assay (SA β-Gal)
3. CBA-240: Cell Viability and Cytotoxicity Assay
4. CBA-251 CytoSelect™ BrdU Cell Proliferation ELISA Kit
5. CBA-253 CytoSelect™ WST-1 Cell Proliferation Assay Reagent

### **Kit Components (shipped on blue ice)**

1. MTT Cell Proliferation Assay Reagent (Part No. 125201): One 10 mL bottle of MTT reagent.
2. Detergent Solution (Part No. 125202): One 100 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.

### **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**

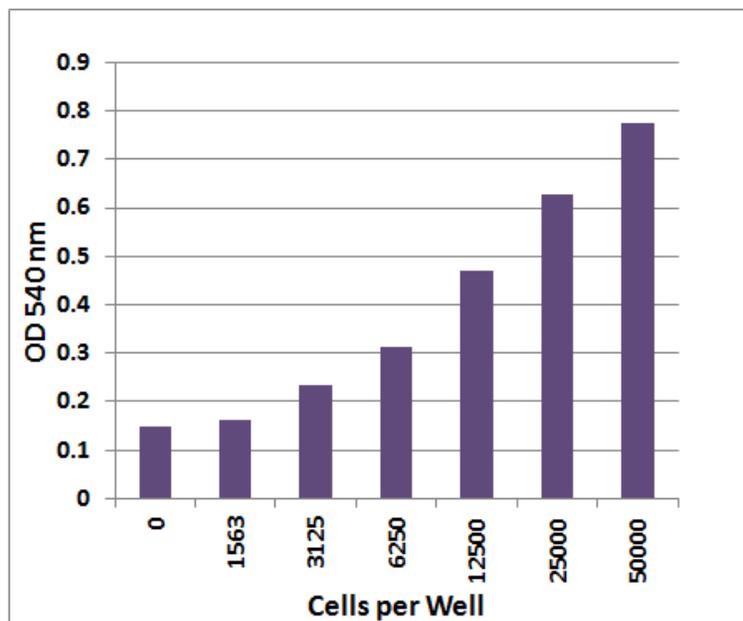
1. Prepare a cell suspension containing 0.1-1.0 x 10<sup>6</sup> cells/ml in medium.
2. Add 100 µL per well to a 96-well cell culture plate or 500 µL per well to a 24-well cell culture plate with or without the compound to be tested. Culture the cells for 24-96 hours at 37°C and 5% CO<sub>2</sub> in 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<sub>2</sub> 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 37°C and 5% CO<sub>2</sub> 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

## **Recent Product Citations**

1. Liu, X. et al. (2023). YIPF5 (p.W218R) mutation induced primary microcephaly in rabbits. *Neurobiol Dis.* **182**:106135. doi: 10.1016/j.nbd.2023.106135.
2. Pardo-Mora, D.P. et al. (2022). Microarray analysis of canine osteosarcoma cells exposed to Colombian propolis. *Phytomedicine Plus.* doi: 10.1016/j.phyplu.2022.100356.
3. Yu, Z. et al. (2021). Receptor interacting protein 3 kinase, not 1 kinase, through MLKL-mediated necroptosis is involved in UVA-induced corneal endothelium cell death. *Cell Death Discov.* **7**(1):366. doi: 10.1038/s41420-021-00757-w.
4. Koga, Y. et al. (2021). DNA-Aptamer Raised against Receptor for Advanced Glycation End Products Improves Survival Rate in Septic Mice. *Oxid Med Cell Longev.* **2021**:9932311. doi: 10.1155/2021/9932311.

5. Uranowska, K. et al. (2021). A chondroitin sulfate proteoglycan 4-specific monoclonal antibody inhibits melanoma cell invasion in a spheroid model. *Int J Oncol.* **59**(3):70. doi: 10.3892/ijo.2021.5250.
6. Yoshizawa, R. et al. (2021). p52Shc regulates the sustainability of ERK activation in a RAF-independent manner. *Mol Biol Cell.* doi: 10.1091/mbc.E21-01-0007.
7. Nagasu, S. et al. (2019). Y-box-binding protein 1 inhibits apoptosis and upregulates EGFR in colon cancer. *Oncol Rep.* **41**(5):2889-2896. doi: 10.3892/or.2019.7038.
8. Pardo-Mora, D.P. et al. (2021). Apoptosis-related gene expression induced by Colombian propolis samples in canine osteosarcoma cell line. *Vet World.* **14**(4): 964-971. doi: 10.14202/vetworld.2021.964-971.
9. Uranowska, K. et al. (2021). Expression of chondroitin sulfate proteoglycan 4 (CSPG4) in melanoma cells is downregulated upon inhibition of BRAF. *Oncol Rep.* **45**(4):14. doi: 10.3892/or.2021.7965.
10. Housein, Z. et al. (2021). In vitro anticancer activity of hydrogen sulfide and nitric oxide alongside nickel nanoparticle and novel mutations in their genes in CRC patients. *Sci Rep.* **11**(1):2536. doi: 10.1038/s41598-021-82244-x.
11. Yoshiya, S. et al. (2020). Impact of Capicua on Pancreatic Cancer Progression. *Ann Surg Oncol.* doi: 10.1245/s10434-020-09339-z.
12. Zhou, K.I. et al. (2019). Regulation of Co-transcriptional Pre-mRNA Splicing by m6A through the Low-Complexity Protein hnRNPG. *Mol Cell.* pii: S1097-2765(19)30535-0. doi: 10.1016/j.molcel.2019.07.005.
13. Souza, A.D.G. et al. (2018). Extracellular vesicles as drivers of epithelial-mesenchymal transition and carcinogenic characteristics in normal prostate cells. *Mol Carcinog.* **57**(4): 503–511. doi: 10.1002/mc.22775.
14. Dahiya, N.R. et al. (2018). A natural molecule, urolithin A, downregulates androgen receptor activation and suppresses growth of prostate cancer. *Mol Carcinog.* **57**(10):1332-1341. doi: 10.1002/mc.22848.
15. Zhang, Q.A. et al. (2018). miR-34 increases in vitro PANC-1 cell sensitivity to gemcitabine via targeting Slug/PUMA. *Cancer Biomark.* **21**(4):755-762. doi: 10.3233/CBM-170289.
16. Kolluru V, et al. (2017). Induction of Plac8 promotes pro-survival function of autophagy in cadmium-induced prostate carcinogenesis. *Cancer Lett.* **408**:121-129. doi: 10.1016/j.canlet.2017.08.023
17. Kim, M. et al. (2017). Novel mouse models of hepatic artery infusion. *J. Surg. Res.* doi: 10.1016/j.jss.2017.05.083.
18. Tsubouchi, H. et al. (2017). Ghrelin does not influence cancer progression in a lung adenocarcinoma cell line. *Endocr. J.* **64**(Suppl):S41-S46.
19. Kim, M. et al. (2016). Preclinical validation of a single-treatment infusion modality that can eradicate extremity melanomas. *Cancer Res.* **76**:6620-6630.
20. Wu, H. et al. (2015). MicroRNA-21 is a potential link between non-alcoholic fatty liver disease and hepatocellular carcinoma via modulation of the HBP1-p53-Srebp1c pathway. *Gut.* doi:10.1136/gutjnl-2014-308430.
21. Zhang, L. et al. (2014). miR-125b can enhance skin tumor initiation and promote malignant progression by repressing differentiation and prolonging cell survival. *Genes Dev.* **28**:2532-2546.
22. Ren, Z. et al. (2014). Anti-tumor effect of a novel soluble recombinant human endostatin: administered as a single agent or in combination with chemotherapy agents in mouse tumor models. *PLoS One.* **9**:e107823.

## **Warranty**

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

## **Contact Information**

Cell Biolabs, Inc.  
5628 Copley Drive  
San Diego, CA 92111  
Worldwide: +1 858-271-6500  
USA Toll-Free: 1-888-CBL-0505  
E-mail: [tech@cellbiolabs.com](mailto:tech@cellbiolabs.com)  
[www.cellbiolabs.com](http://www.cellbiolabs.com)

©2014-2023: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.