Product Manual

CytoSelect™ Cell Proliferation Assay Reagent (Fluorometric)

Catalog Number

CBA-250	10 mL (960 assays in 96-well plate format)

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

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 MTT, 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™ Cell Proliferation Assay Reagent (Fluorometric) provides a fluorometric format for measuring and monitoring cell proliferation. The kit contains sufficient reagents for the evaluation of 960 assays in ten 96-well plates or 192 assays in eight 24-well plates. Cells can be plated and then treated with compounds or agents that affect proliferation. Cells are then incubated with the proliferation reagent. Upon entering metabolically active live cells, the non-fluorescent proliferation reagent is converted into a bright red fluorescent form. An increase in cell proliferation is accompanied by increased fluorescent 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.

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-253: CytoSelect™ Cell Proliferation Assay Reagent (Colorimetric)

Materials Not Supplied

1. Cells for measuring proliferation
2. Cell culture medium
3. 24-well or 96-well clear or black-walled fluorescence microtiter cell culture plates.

4. Fluorescence plate reader capable measuring fluorescence at 560 nm excitation wavelength and 590 nm emission wavelength.

Storage

Store at 4ºC and protect from light.

Assay Protocol

1. Prepare a cell suspension containing 0.1-1.0 x 10^6 cells/ml in medium.
2. Add 100 µL of cell suspension 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 one tenth volume of the CytoSelect™ Cell Proliferation Assay Reagent per well (for example add 10 µL of CytoSelect™ Cell Proliferation Assay Reagent per 100 µL of cell culture).
4. Incubate plate at 37°C and 5% CO_2 for 4-8 hours.
5. Read the fluorescence with a fluorescence plate reader at 560 nm excitation wavelength and 590-600 nm emission wavelength.

Example of Results

The following figure demonstrates typical results with the CytoSelect™ Cell Proliferation Assay (Fluorometric). Fluorescence measurement was performed on SpectraMax Gemini XS Fluorometer (Molecular Devices) with a 560/590 nm filter set and 590 nm cutoff. One should use the data below for reference only. This data should not be used to interpret actual results.

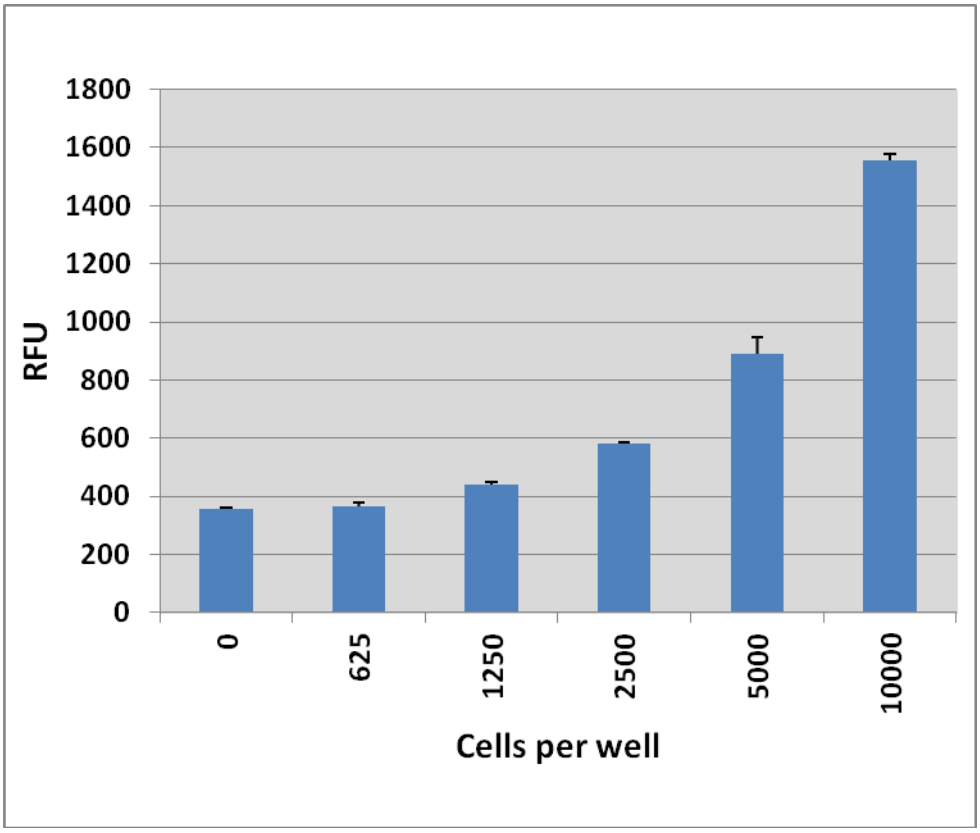

Figure 1. Human HEK 293 Cell Density. HEK 293 cells were seeded at various densities in triplicate as indicated above and allowed to culture for 24 hours. Cells were then treated with the CytoSelect™ Cell Proliferation Assay Reagent for 6 hours at 37ºC and 5% CO2.

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. Poole CA, Brookes NH, Clover GM. (1993) *J Cell Sci* **106**, 685.
4. Wang XM, Terasaki PI, Rankin GW Jr, Chia D, Zhong HP, Hardy S. (1993) *Hum Immunol* **37**, 264.
5. Weil M, Jacobson MD, Coles HS, Davies TJ, Gardner RL, Raff KD, Raff MC. (1996) *J Cell Biol* **133**, 1053.
6. Zurgil N, Shafran Y, Fixler D, Deutsch M. (2002) *Biochem Biophys Res Commun* **290**, 1573.

Warranty

In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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