Product Manual

CytoSelect™ Cell Viability and Cytotoxicity Assay Kit

Catalog Number

CBA-240	96 assays (96-well plate)

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 viability 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 viability and growth.

Cell viability 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. The green fluorescent viability dye Calcein AM can measure intracellular esterase activity, which is another indicator of cell viability. Live cells can convert the nonfluorescent, cell-permeable polyanionic calcein acetoxymethyl (Calcein AM) dye to the highly fluorescent calcein. The cleaved calcein remains in the cells. Ethidium homodimer (EthD-1) is an excellent marker for measuring dead cells. EthD-1 is a red fluorescent dye that can only penetrate damaged cell membranes. EthD-1 will fluoresce with a 40-fold enhancement upon binding ssDNA, dsDNA, RNA, oligonucleotides, and triplex DNA. Background fluorescence levels are very low because the dyes are virtually non-fluorescent before interacting with cells. This method of detection is more efficient, safer, less expensive, and a more sensitive method for determining cell viability or cytotoxicity compared to traditional viability assays such as ^{51}Cr release or trypan blue exclusion.

Cell Biolabs' CytoSelect™ Cell Viability and Cytotoxicity Assay Kit provides a colorimetric and fluorometric format for measuring and monitoring cell viability. The kit contains MTT reagent, Calcein AM, and Ethidium Homodimer. Detergent and Lysis Buffer are provided for extracting the MTT reagent or the Calcein AM/EthD-1 from cell samples. Saponin, a cell death initiator, is also included as a control. The kit is suitable for use with light and fluorescence microscopes, colorimetric and fluorometric multiwell plate scanners, flow cytometers, and other colorimetric or fluorometric detection systems. The kit contains sufficient reagents for the evaluation of 96 assays in a 96-well plate, or 24 assays in a 24-well plate. Cells can then be treated with compounds or agents that affect viability. Live cells are detected with the MTT reagent as well as the Calcein AM. Dead cells are detected with the EthD-1 reagent. Finally, cell viability/cytotoxicity is determined using the colorimetric and fluorometric detection reagents. An increase in cell viability is accompanied by cell growth, while a decrease in cell viability 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. The kit does not react with bacteria or yeast.

Related Products

1. CBA-230: Cellular Senescence Assay Kit (SA-β-gal Staining)
2. CBA-231: 96-Well Cellular Senescence Assay (SA β-Gal Activity)
3. CBA-232: Quantitative Cellular Senescence Assay (SA β-Gal)
4. AKR-100: β Galactosidase Staining Kit

Kit Components

1. MTT Colorimetric Reagent (Part No. 113502): One vial – 1 mL
2. Detergent Solution (Part No. 113501): One bottle – 10 mL
3. Calcein AM (500X) (Part No. 108002): One vial – 50 µL in DMSO.
4. Ethidium Homodimer (EthD-1) (500X) (Part No. 108003): One vial – 50 µL.
5. Saponin (100X) (Part No. 124001): One vial – 100 µL

Materials Not Supplied

1. Cells for measuring viability
2. Cell culture medium
3. Microtiter plate reader
4. 24-well or 96-well black-walled fluorescence microtiter cell culture plates.
5. Fluorometer capable of the green Calcein AM (Ex: 485 nm and Em: 515 nm) or red EthD-1 (Ex: 525 nm and Em: 590 nm) fluorescence.

Storage

Store the Calcein AM and Ethidium Homodimer at -20ºC. Store all remaining kit components at 4ºC.

Preparation of Reagents

Allow all reagents to warm to room temperature before use. Centrifuge all vials briefly prior to opening.

- Calcein AM/EthD-1 Solution: Prepare a 1X Calcein AM/EthD-1 solution by diluting the provided stocks 1:500 in medium. For example, to prepare a 5.0 mL solution, add 10 µL of Calcein AM and 10 µL of EthD-1 to 4.980 mL of medium. Vortex thoroughly.

  Note: Prepare only the amount necessary for the immediate application. Do not store diluted solutions of Calcein AM or Ethidium Homodimer.

- 1X Saponin Solution: Prepare a 1X Saponin solution by diluting the provided stock 1:100 in media. Vortex thoroughly. Store the diluted solution at 4°C.

Assay Protocol

Note: This protocol is written for use with adherent cell lines. Non-adherent cell lines may also be used by incubating cells in a 96-well or 24-well plate. Washes can be done in a test tube or microcentrifuge tube.

1. Prepare a cell suspension containing 0.5-2.0 x 10^6 cells/ml in medium.
2. Add 10,000 to 50,000 cells per well to a 96-well cell culture plate or 50,000 to 100,000 cells per well to a 24-well cell culture plate. Culture the cells 12-24 hours at 37°C and 5% CO_2. The time and culture conditions will depend on the cell line used and may need to be adjusted.
3. Gently remove the media from the wells.

4. Wash each well with three times with medium to remove loosely attached and dead cells.
5. (optional) Add 1X Saponin solution to control wells to initiate cell death (100 µL/well in a 96-well plate or 250 µL/well in a 24-well plate). Incubate the plate for 10 minutes at room temperature. Carefully remove the media from the wells. Wash each well once with medium.
   Note: Saponin treated cells are very loosely attached to the plate. Gentle washing is essential to minimize cell loss.

Detection Protocol

Cell viability may be determined using either of the following methods.

I. MTT Colorimetric Detection

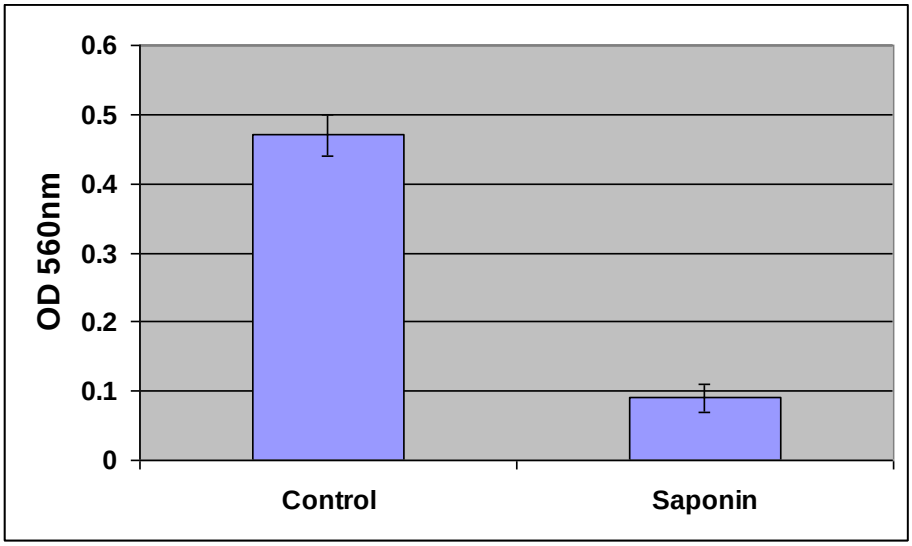
1. Add media to each well as follows: 100 µL/well in a 96-well plate or 250 µL/well in a 24-well plate.
   Note: Include blank control wells of medium only for absorbance readings.
2. Add the MTT Reagent to each well at a 1:10 ratio. For example, add 10 µL/well for a 96-well plate or 25 µL/well for a 24-well plate.
3. Incubate the wells 2-4 hours or overnight at 37°C. Monitor the cells occasionally with an inverted microscope for the presence of a purple precipitate.
4. Once the precipitate is clearly visible, add 100 µL of Detergent Solution for every 10 µL of MTT Reagent added to each well (100 µL/well for a 96-well plate or 250 µL/well for a 24-well plate). Gently mix the solution by pipetting.
5. Cover the plate to protect it from light and incubate in the dark for 2-4 hours at room temperature.
6. Remove the plate cover and measure the absorbance in each well at 570 nm in a microtiter plate reader. For samples run in a 24-well plate, transfer 150 µL to a 96-well plate and measure the absorbance in each well at 570 nm in a microtiter plate reader.
7. If the values appear to be low, incubate the plate longer in the dark.

II. Calcein AM/EthD-1 Fluorometric Detection

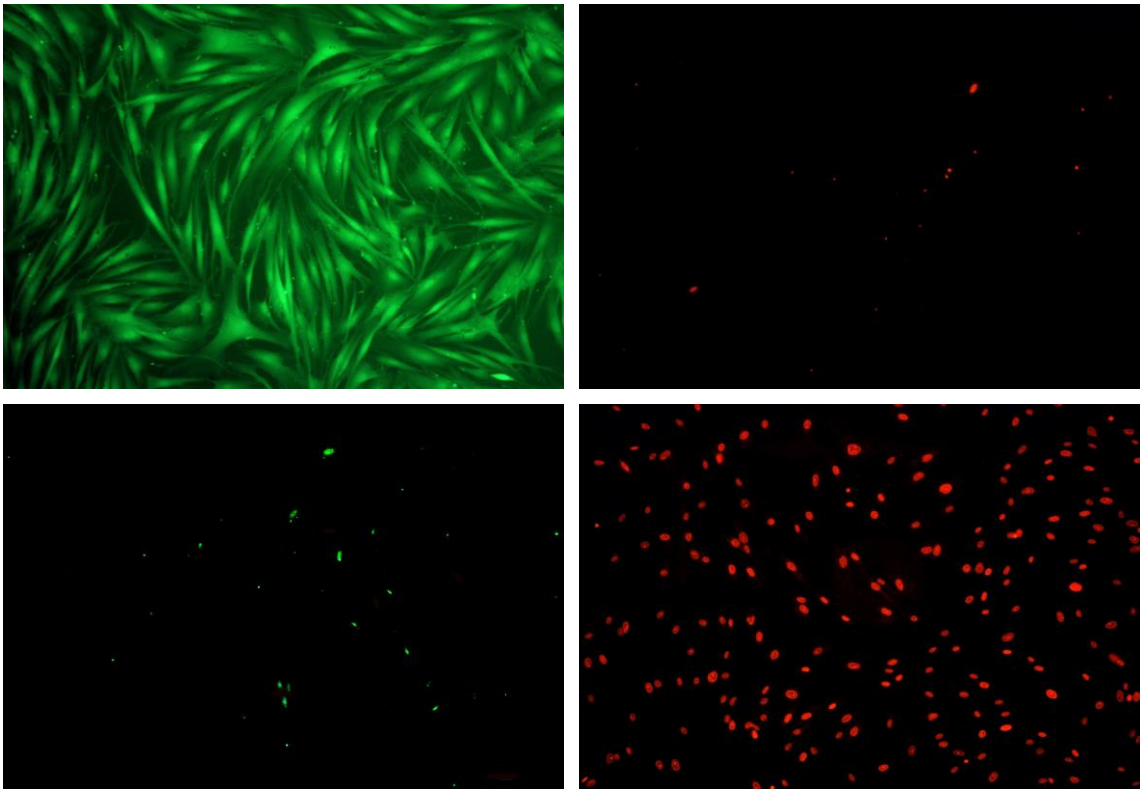
1. Add Calcein AM/EthD-1 solution to each well as follows: 100 µL/well in a 96-well plate or 400 µL in a 24-well plate.
   Note: Include blank control wells of medium only for background fluorescence readings.
2. Incubate the plate 30 minutes at 37°C
3. Remove the Calcein AM/EthD-1 solution. Wash wells 2 times with medium.
4. After the last wash, add enough medium to cover the cells.
5. Monitor the cells microscopically for the presence of the green Calcein (Ex: 485 nm and Em: 515 nm) or red EthD-1 (Ex: 525 nm and Em: 590 nm) fluorescence. The fluorescence can be quantitatively measured with a fluorescence microplate reader.

Example of Results

The following figures demonstrate typical results with the CytoSelect™ Cell Viability and Cytotoxicity Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



Calcein AM EthD-1



- Saponin

+ Saponin

Figure 1. Human Foreskin Fibroblast BJ-TERT Cell Viability. BJ-TERT cells were seeded at 50,000 cells/well and allowed to culture for 24 hours. Cells were then treated with and without Saponin. Cell samples were then treated with MTT, Calcein AM or EthD-1.

Calculation of Results

Relative numbers of live and dead cells can be expressed as percentages or as absolute numbers of cells. The absolute number of viable cells in a sample can be obtained by creating a standard curve of cell number versus MTT absorbance or Calcein fluorescence at 515 nm. Likewise, the absolute number of dead cells in a sample can be obtained by creating a standard curve of cell number versus EthD-1 fluorescence at 590nm after Saponin treatment. The absorbance reading or fluorescence intensity is linearly related to the number of cells in the sample.

References

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Recent Product Citations

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Warranty

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