Datasheet for ABIN1000239

Cell Viability Assay Kits

Go to Product page

Overview

Quantity:	10000 tests
Application:	Cellular Assay (CA)

Product Details

Characteristics:	Safe. Non-radioactive assay (cf. 3 H-thymidine incorporation assay). Sensitive and accurate. As low as 100 cells can be accurately quantified. Time efficient. High-throughput assay in 96-well and 384-well plates allows simultaneous processing ten of thousands of samples per day. Homogeneous and convenient. A single reagent and mix-incubate- measure type assay. No wash and reagent transfer steps are involved. Robust and amenable to HTS: Z' factors of 0.6 to 0.9 are routinely observed in 96-well and 384-well plates. Can be readily automated on HTS liquid handling systems.

Target Details

Background:	Homogeneous assay for cell viability, proliferation, cytotoxcity, high-throμghput screening for anticancer agents. Fluorimetric method (530nm/590nm). This homogeneous assay involves simply adding a single reagent, the reagent, to the cell culture and measuring the fluorescence intensity (excitation wavelength = 530 - 570 nm, emission wavelength = 590 - 620 nm) after an incubation step. The CellQuanti- Blue TM reagent, like other resazurin-based assays such as the Alamar Blue reagent, utilizes the redox dye resazurin which is not fluorescent, but upon reduction by metabolically active cells is converted into a highly fluorescent product (resorufin). Living cells can readily reduce this non-

Target Details

	toxic reagent and the resulting increase in fluorescence intensity can be conveniently monitored using a fluorescence spectrophotometer or plate reader. Nonviable cells have no metabolic capacity and, thus, will not reduce the dye. Therefore, the fluorescence intensity observed in this assay is a true measure of the viable cells. The reagent has been optimized for maximum sensitivity, reproducibility and long shelf-life. The homogeneous cell-based assay can be performed in multi-well plates. The reagent is compatible with all culture media and with all liquid handling systems for high-throughput screening applications in 96-well and 384-well plates. Applications include cell proliferation, cytotoxicity and apoptosis.

Application Details

Application Notes:	Cell Proliferation: effects of cytokines, growth factor, nutrients. Cytotoxicity and Apoptosis: evaluation of toxic compounds, anti-cancer antibodies, toxins, environmental pollutants etc. Drug Discovery: high-throughput screening for anticancer drugs.
Comment:	Incubation time. The incubation time is dependent on the cell line. Some cell lines exhibit strong metabolic activity and, thus, require shorter incubation time than less metabolically active cell lines. The incubation time can be easily determined by reading the plate multiple times e.g. every 30 minutes after adding the reagent. In general, incubation for 1 to 5 hours is sufficient. Extensive incubation (such as >18 hours) may result in non-linear fluorescence response at high cell numbers. Cell number. Generally the optimized reagent shows a broad range linear fluorescence response to the number of culturing cells. It is recommended to determine the number of cells per well that gives a highest signal:noise ratio. The optimal cell number can be easily determined by serial dilution of cells. Controls. A positive control that is either cytotoxic or promotes cell proliferation can be run although it is not required. Saponin is a cytotoxic detergent that is available from BioAssay Systems (see Figure 2 in Technical Notes). A blank control, i.e., culture medium without cells or cells containing 0.1% saponin, should be done for each assay. The blank control determines background fluorescence that must be subtracted for data analysis. BioAssay Systems CQBL004.pdf 2007 • by BioAssay Systems 3191 Corporate Place, Hayward, CA 94545, USA • Website: www.bioassaysys.com Tel: 510-782-9988, Fax: 510-782-1588 • Email: order@bioassaysys.com, info@bioassaysys.com Page 2 of 2 Cell Viability Assay Kits Non-radioactiveFluorescentAssayforCellProliferationandCytotoxicity
Protocol:	The assay is based on the conversion of the non- fluorescent reagent to fluorescent product by metabolically active cells. For most cells this reductive reaction takes 1 to 5 hours. The

fluorescence intensity of the product is then quantified on a fluorescent microplate reader. Although most culture media contain phenol red, phenol red does not interfere with the assay. All data in Technical Notes were obtained in culture media containing phenol red.

Procedure using 96-well plate:

1. Plate and culture cells (80 µL) in black 96-well tissue culture plates. Typical culture medium contains DMEM, 10% fetal bovine serum and antibiotics (penicillin/ strepto-mycin, gentamycin, etc), amino acids and other nutrients. Assays can be performed on either adherent cells or cells in suspension. The number of cells can vary from 100 to 80,000 per well. The volume can vary from 50 to 150 µL, although 80 µL is used in this protocol. In addition to the test samples, control wells of culture medium containing no cells or cells treated with a toxic reagent such as 0.1% saponin should be included.
2. Add test compounds and controls and incubate cells for the desired period of time (typically overnight). It is recommended that assays be run in duplicate or triplicate. Compounds and controls (20 µL) can be added in phosphate buffered saline (PBS) or culture medium. The Control reagent can be conveniently reconstituted with 5 mL PBS (1% saponin).
3. Equilibrate the Reagent to room temperature. Add 10 µL (per 100 µL of cell culture) of the reagent per well. The volume of the reagent can be adjusted depending on the volume of cell culture. Tap plate to mix cells with compounds. Incubate for 1 to 5 hours at 37°C.
4. Measure fluorescent intensity for each well on a fluorescence plate reader. If a Molecular Devices LJL Analyst is used, use the rhodamine filter sets (530nm excitation filter, 590nm emission filter and 570nm dichroic mirror).

Procedure using 384-well plate:

1. Plate and culture cells (40 µL) in black 384-well tissue culture plates. The number of cells can vary from 100 to 20,000 per well. The volume can vary from 25 to 60 µL, although 40 µL is used in this protocol. In addition to the test samples, control wells of culture medium containing no cells or cells treated with a toxic reagent such as 0.1% saponin should be included.
2. Add test compounds and controls and incubate cells for the desired period of time. It is recommended that assays be run in duplicate or triplicate and that compounds be added in PBS or culture medium with a volume of 10 µL.
3. Equilibrate Reagent to room temperature. Add 5 µL Reagent (per 50 µL of cell culture). Tap plate lightly to mix Reagent with cells. Incubate for 1 to 5 hours at 37°C.
4. Measure fluorescent intensity for each well on a fluorescence plate reader. If a Molecular Devices LJL Analyst is used, use the rhodamine filter sets (530nm excitation filter, 590nm emission filter and 570nm dichroic mirror).

Application Details

Reagent Preparation:	Important: bring reagent to room temperature before use.
Restrictions:	For Research Use only

Handling

Storage:	4 °C