Datasheet for ABIN2344830

CytoSelect™ 96-well Anoikis Assay (Colorimetric/Fluorometric)

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Overview

Quantity:	96 tests
Application:	Biochemical Assay (BCA)

Product Details

Brand:	CytoSelect™
Sample Type:	Cell Samples
Detection Method:	Fluorometric, Colorimetric
Characteristics:	The CytoSelect™ 96-well Anoikis Assay Kit provides a colorimetric and fluorometric format to measure anchorage-independent growth and monitoring anoikis propelled cell death. The kit contains sufficient reagents for the evaluation of 96 samples in a Hydrogel coated 96-well plate. Live cells are detected with MTT or Calcein AM. Cell death is detected with the Ethidium Homodimer (EthD-1).
Components:	1. 96-well Anchorage Resistant Plate : One 96-well Hydrogel coated plate. 2. Calcein AM (500X) : One vial - 50 µL in DMSO. 3. Ethidium Homodimer (EthD-1) (500X) : One vial - 50 µL. 4. Detergent Solution : One bottle - 25.0 mL. 5. MTT Solution : Three tubes - 1.0 mL each. 2
Material not included:	1. Cells for measuring anoikis 2. Cell culture medium 3. Inverted fluorescence/light microscope 4. Fluorometer capable of reading Calcein AM (485 nm/515 nm) and EthD-1 (525 nm/590 nm) fluorescence.

Target Details

Background:	Adhesion to the extracellular matrix (ECM) is essential for survival and propagation of many adherent cells. Apoptosis that results from the loss of cell adhesion to the ECM, or inappropriate adhesion is defined as "anoikis". Anoikis, from the Greek word for homelessness, is involved in the physiological processes of tissue renewal and cell homeostasis. A common feature of carcinoma development and growth is the ability of transformed cells to survive under "anchorage independent" or "spheroid" growth conditions. This resistance to anoikis has been shown to be involved in the loss of cell homeostasis, cancer growth, and metastasis. The inhibition of cell adhesion, spreading, and growth on the ECM is an impediment to the cellular healing process, thus making it a possible therapeutic target. Preventing anoikis and enhancing cell adhesion and spreading is a major goal in the development of cell transplantation techniques, including the therapeutic use of progenitor cells. Further studies aimed at controlling the molecular mechanisms of anoikis resistance will serve to define effective therapies for the treatment of many human malignancies.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Detect live and dead cells by microscopy, fluorescence, or flow cytometry • Quantify live cells on a standard microplate reader or fluorometer • Precoated plate included
Plate:	Pre-coated
Protocol:	Cells are cultured in Hydrogel coated plate or control plate. Cell viability is determined by MTT or Calcein AM. Anoikis propelled cell death is measured by Ethidium Homodimer (EthD-1). 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.
Assay Procedure:	1. Prepare a cell suspension containing 0.1-2.0 x 106 cells/mL in culture media. Cells can be treated with anoikis enhancing or inhibiting reagents. 2. Add 0.1 mL cell suspension to each well of the Anchorage Resistant Plate or a control 96-well cell culture plate. Culture the cells 24-72 hours at 37 °C and 5 % CO2. The time and culture conditions will depend on the cell line used and may need to be adjusted by the user. 3. Proceed with MTT Colorimetric or Calcein AM/EthD-1 Fluorometric detection. MTT Colorimetric Detection 1. Add the 10 µL of the MTT Reagent to each well of the Anchorage Resistant Plate or control 96- well plate.

Application Details

2. 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.
3. Add 100 µL of Detergent Solution to each well. Gently mix the solution by pipetting.
4. Cover the plate to protect it from light and incubate in the dark for 2-4 hours at room temperature.
5. Transfer 150 µL to a 96-well plate and measure the absorbance in each well at 570 nm in a microtiter plate reader. Calcein AM / EthD-1 Fluorometric Detection
    1. Dilute the 500X Calcein AM/EthD-1 stock solution to 100X with culture medium.
    2. Add 1 µL of the 100X Calcein AM/EthD-1 solution to each well of the 96-well Anchorage Resistant Plate or control plate to be detected.
    3. Incubate the plate 30-60 minutes at 37 °C.
    4. Monitor the cells microscopically for the presence of the green Calcein AM (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. 3

Restrictions:	For Research Use only

Handling

Storage:	4 °C/-20 °C
Storage Comment:	Store the Calcein AM and Ethidium Homodimer at -20°C. Store all other components at 4°C until their expiration dates.