Product Manual

# CytoSelect™ 24-Well Anoikis Assay

**Catalog Number**

| | |
|---|---|
| CBA-080 | 24 assays |

**FOR RESEARCH USE ONLY**
**Not for use in diagnostic procedures**

CELL BIOLABS, INC.
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## Introduction

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.

The CytoSelect™ 24-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 assay of 24 samples in a Poly-Hema coated 24-well plate. Live cells are detected with MTT or Calcein AM. Cell death is detected with the Ethidium Homodimer (EthD-1).

## Assay Principle

Cells are cultured in poly-Hema 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.

## Related Products

1. CBA-081: CytoSelect™ 96-Well Anoikis Assay
2. CBA-230: Cellular Senescence Detection Kit (SA-β-Gal Staining)
3. CBA-231: 96-Well Cellular Senescence Assay (SA β-Gal Activity)
4. CBA-232: Quantitative Cellular Senescence Assay (SA β-Gal)
5. CBA-240: CytoSelect™ Cell Viability and Cytotoxicity Assay

## Kit Components (shipped at room temperature)

1. Anchorage Resistant Plate (Part No. 108001): One 24-well Poly-Hema coated plate.
2. Calcein AM (500X) (Part No. 108002): One vial – 50 µL in DMSO.
3. Ethidium Homodimer (EthD-1) (500X) (Part No. 108003): One vial – 50 µL.
4. Detergent Solution (Part No. 108004): One bottle – 25.0 mL.
5. MTT Solution (Part No. 113502): Three tubes – 1.0 mL each.

## Materials Not Supplied

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.

## Storage

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

## Assay Protocol

1. Prepare a cell suspension containing 0.1-2.0 x $10^6$ cells/ml in culture media. Cells can be treated with anoikis enhancing or inhibiting reagents.
2. Add 0.5 mL cell suspension to each well of the Anchorage Resistant Plate or a control 24-well cell culture plate. Culture the cells 24-72 hours at 37ºC and 5% $CO_2$. 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

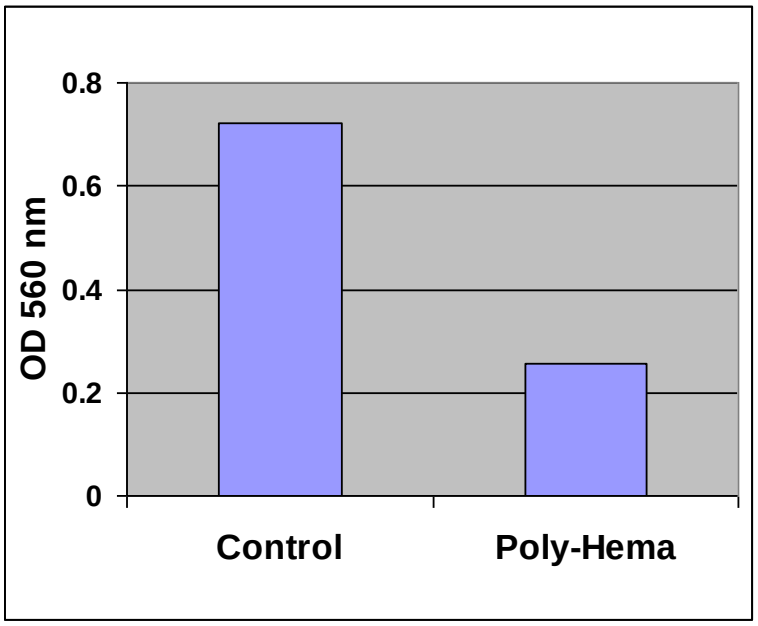
1. Add the 50 µL of the MTT Reagent to each well of the Anchorage Resistant Plate or control 24-well plate.
2. Incubate the wells 2-4 hours or overnight at 37ºC. Monitor the cells occasionally with an inverted microscope for the presence of a purple precipitate.
3. Add 500 µL of Detergent Solution to 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 200 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. Add 1 µL of Calcein AM (500X) and 1 µL of Eth-D1 (500X) to each well of the 24-well Anchorage Resistant Plate or control plate to be detected.
2. Incubate the plate 30-60 minutes at 37ºC.
3. 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.

## Example of Results

The following figures demonstrate typical results with the CytoSelect™ 24-well Anoikis Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



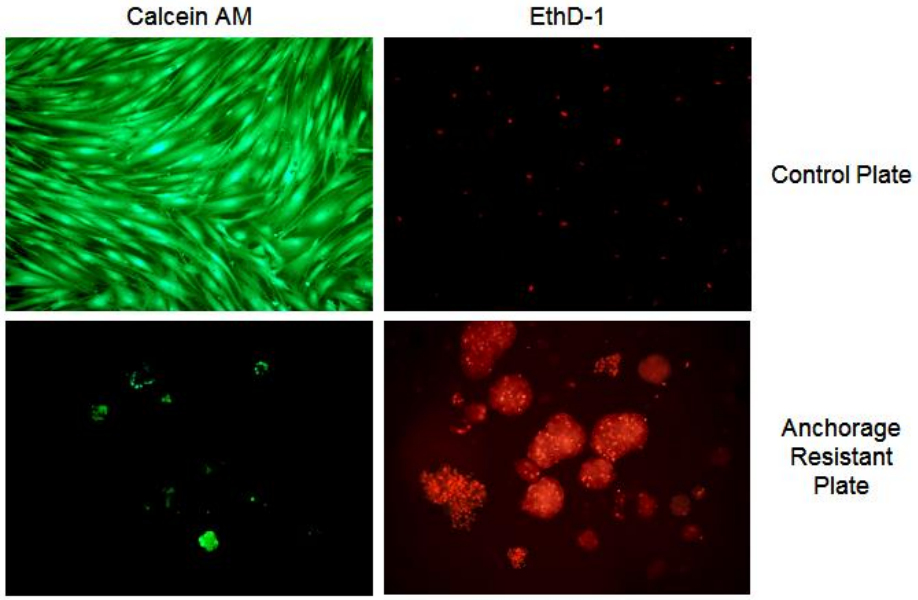




**Figure 1. Anoikis Assay of Human Foreskin Fibroblast BJ-TERT Cells.** BJ-TERT cells were seeded at 50,000 cells/well in a tissure culture control plate or a Poly-Hema coated plate. Cells were allowed to culture for 24 hours. Cell viability was determined by MTT and Calcein AM, while anoikis-like cell death was stained with EthD-1.

## References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## Recent Product Citations


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