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Product Manual

# CytoSelect™ 96-Well Anoikis Assay

Catalog Number

CBA-081

96 assays

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

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**CELL BIOLABS, INC.**  
*Creating Solutions for Life Science Research*

## **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™ 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).

## **Assay Principle**

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.

## **Related Products**

1. CBA-080: CytoSelect™ 24-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**

1. 96-well Anchorage Resistant Plate (Part No. 108101): One 96-well Hydrogel 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 -20°C. Store all other components at 4°C.

## **Assay Protocol**

1. Prepare a cell suspension containing  $0.1-2.0 \times 10^6$  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% CO<sub>2</sub>. 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**

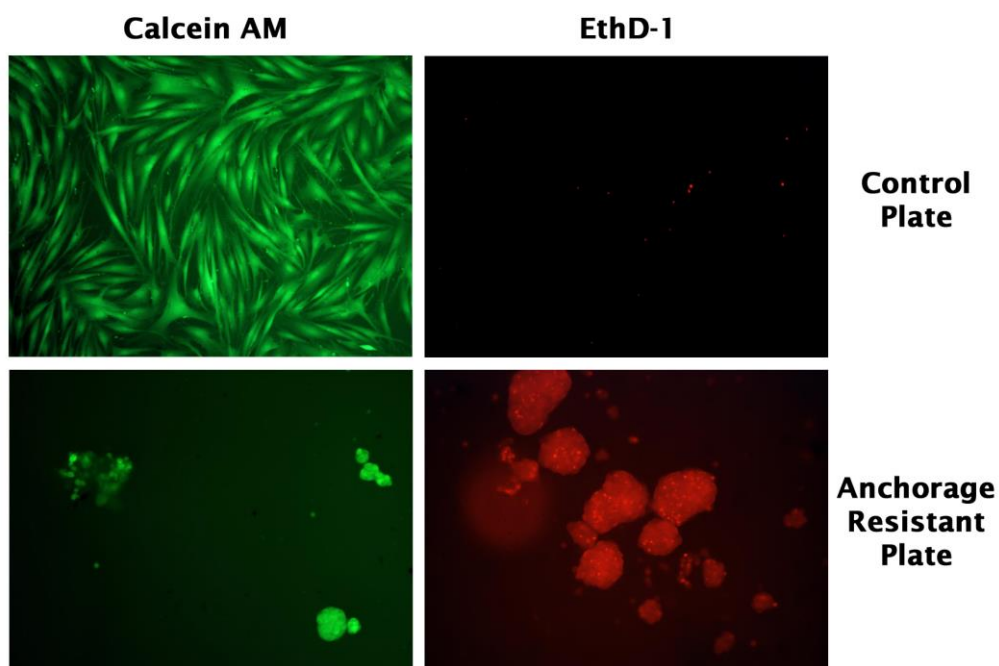
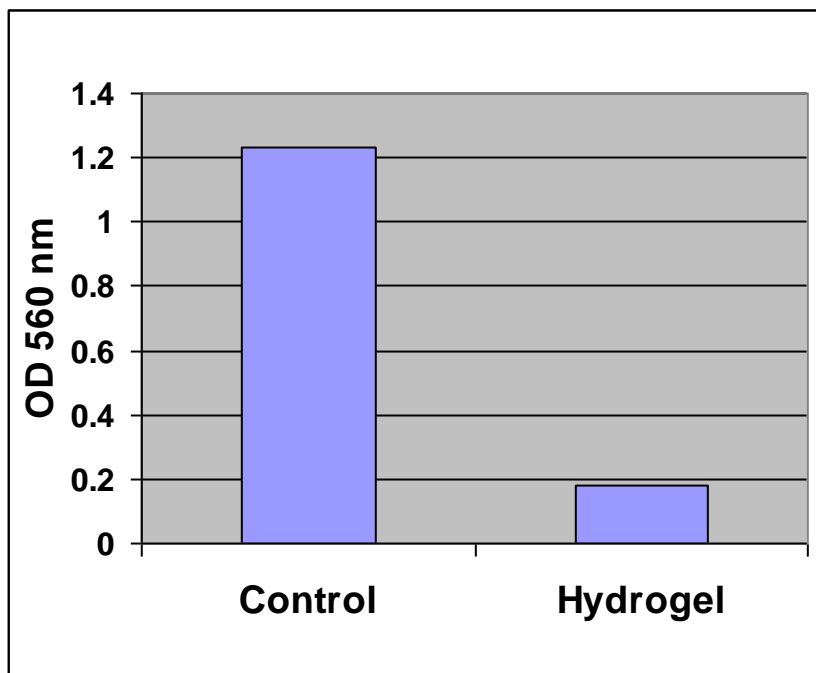
1. Add the 10 µL of the MTT Reagent to each well of the Anchorage Resistant Plate or control 96-well plate.
2. Incubate the wells 2-4 hours or overnight at 37°C. Monitor the cells occasionally with an inverted microscope for the presence of a purple precipitate.
3. Add 100 µL of Detergent Solution to 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.
2. Incubate the plate 30-60 minutes at 37°C.
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™ 96-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 40,000 cells/well in a tissue culture control plate or a Hydrogel 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**

1. Bates RC, Buret A, van Helden DF, Horton MA, Burns GF. (1994) *J Cell Biol* **125**, 403-415.
2. Frisch SM, Francis H. (1994) *J Cell Biol* **124**, 619-626.
3. Frisch SM, Screaton RA. (2001) *Curr Opin Cell Biol* **13**, 555-562.
4. Meredith JE, Jr Fazeli B, Schwartz MA. (1993) *Mol Biol Cell* **4**, 953-961.
5. Rak J, Mitsuhashi Y, Erdos V, Huang SN, Filmus J, Kerbel RS. (1995) *J Cell Biol* **131**, 1587-1598.

## **Recent Product Citations**

1. Huang, C.P. et al. (2022). Overexpression of multiple epidermal growth factor like domains 11 rescues anoikis survival through tumor cells-platelet interaction in triple negative breast Cancer cells. *Life Sci.* **299**:120541. doi: 10.1016/j.lfs.2022.120541.
2. Tsai, Y.F. et al. (2021). Interleukin 17A promotes cell migration, enhances anoikis resistance, and creates a microenvironment suitable for triple negative breast cancer tumor metastasis. *Cancer Immunol Immunother.* doi: 10.1007/s00262-021-02867-x.
3. González-Llorente, L. et al. (2019). Overexpression of Mitochondrial IF1 Prevents Metastatic Disease of Colorectal Cancer by Enhancing Anoikis and Tumor Infiltration of NK Cells. *Cancers (Basel)*. **12**(1). pii: E22. doi: 10.3390/cancers12010022.
4. Miyake, N. et al. (2019). Targeting ROR1 in combination with pemetrexed in malignant mesothelioma cells. *Lung Cancer*. doi: 10.1016/j.lungcan.2019.10.024.
5. Kozak, J. et al. (2019). Interactions between microRNA-200 family and Sestrin proteins in endometrial cancer cell lines and their significance to anoikis. *Mol Cell Biochem.* doi: 10.1007/s11010-019-03547-2.
6. Wu, J.I. et al. (2019). Crabp2 Promotes Metastasis of Lung Cancer Cells via HuR and Integrin  $\beta$ 1/FAK/ERK Signaling. *Sci Rep.* **9**(1):845. doi: 10.1038/s41598-018-37443-4.
7. Matsumoto, Y. et al. (2018). SALL4 - KHDRBS3 network enhances stemness by modulating CD44 splicing in basal-like breast cancer. *Cancer Med.* **7**(2):454-462. doi: 10.1002/cam4.1296.

## **Warranty**

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