Anoikis Assay Kit (#BN00918)

(Catalog # BN00918; 100 assays; Store at -20°C)

I. Introduction:

Anoikis is a form of cellular apoptosis induced by the inability of normal cells to attach to the extracellular matrix (ECM). Incorrect attachment or no ECM attachment could affect cell growth and differentiation in normal cells. However, research studies has determined that metastatic cells can undergo Anoikis without affecting their ability to migrate and invade surrounding or distant organs. Assay Genie's Anoikis assay kit quantifies live cells using fluorometric (Calcein AM) reagents and a proprietary coated chamber that mimics Anoikis conditions. Our assay is easy to use, sensitive and compatible with fluorimeters.

II. Application:

- Measure anoikis in cells
- Screen compounds that influence/induce anoikis

III. Sample Type:

- Metastatic cell lines

IV. Kit Contents:

Components	BN00918	Cap Code	Part number
Assay Buffer	100 ml	NM	BN00918-1
Anoikis Chamber	1 each	Plate	BN00918-2
Chamber Control (Uncoated)	1 each	Plate	BN00918-3
Calcein AM Dye	1 vial	Green	BN00918-4

V. User Supplied Reagents & Equipment:

- Plate Reader
- Cell Culture Media
- Anhydrous, sterile DMSO

VI. Storage and Reagents Preparation:

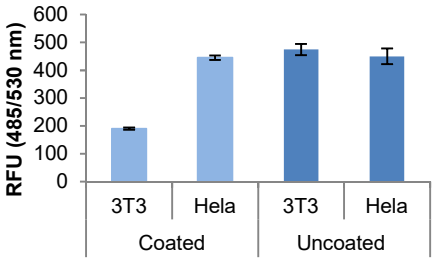
Store kit at -20°C, protected from light. Components should be opened and kept under sterile conditions. Briefly centrifuge small vials prior to opening. Assay is performed under sterile conditions. Read entire protocol before performing the experiment.

- **Anoikis Chamber:** Ready to use. Keep at Room temperature.
- **Calcein AM:** Resuspend in 220 µl anhydrous DMSO (not provided). Aliquot and store -20°C. Use within 2 months.
- **Assay Buffer:** Ready to use. Keep at 4°C.

VII. Anoikis Assay Protocol:

1. Cell Growth: Grow cells of interest in desired media and culture conditions to ~80% confluency. Harvest cells and centrifuge at 1,000 x g, for 5 min. to pellet cells. Resuspend cell pellet in media and count the number of cells using hemocytometer or automated cell counter. Resuspend cells at 1 x 10^6 cells/ml in culture media. Open both the Anoikis Chamber and the Chamber Control (Uncoated) in a laminar flow hood and wash with 100 µl sterile Assay Buffer twice before use. Add 100 µl cell suspensions to each well of the Anoikis Chamber and the Chamber Control (Uncoated).

Notes: Appropriate incubation time depends on the individual cell type and cell concentrations used. Therefore, it is recommended to determine the optimal incubation time for each experiment.



2. Treatment: Treat cells with Anoikis enhancing or inhibiting reagents. For background control, treat the cells with vehicle. Culture the cells 24-72 hr. at 37ºC and 5% CO_2. The incubation time and culture conditions will depend on the cell line used and may need to be adjusted by the user.

3. Data collection and Analysis: After the desired incubation with inducers/inhibitors, carefully remove the plate cover. Add 2 µl of the Calcein AM dye to each well of the 96-well Anchorage Resistant Plate or control plate to be detected. Incubate the plate 30-60 min. at 37ºC. Monitor the cells microscopically for the presence of Calcein AM (Green Channel) fluorescence. The fluorescence can be quantitatively measured with a fluorescence microplate reader (Ex: 485 nm and Em: 530 nm). Subtract the background control from the treated samples.

Figure 1: Anoikis Assay: 3T3-NIH and Hela Cells were used. 3T3 Cells undergo Anoikis (Apoptosis) in the Anoikis Chamber (coated) plates, whereas cancer cells overcome Anoikis and are able to grow in absence of Anchorage (Chamber Control, Uncoated). No change was observed in cell growth in uncoated plates.

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