Anoikis Assay Kit

ARG83599 Anoikis Assay Kit is a detection kit for the quantification of Anoikis in cell.

Catalog number: ARG83599

Package: 96 wells

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Phone: +886 (3) 621 8100

Fax: +886 (3) 553 0266.

Email: info@arigobio.com

PRINCIPLE OF THE ASSAY

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.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, store **500X Calcein AM** and **500X EthD-1** at -20°C, all other components store at 4°C. Use the kit before expiration date.

Component	Quantity	Storage information
Hydrogel-coated microplate	96 wells	4°C
500X Calcein AM	50 µl	-20°C
500X EthD-1	50 µl	-20°C
Detergent Solution	25 ml	4°C
MTT	1 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 485/525 nm
- Pipettes and pipette tips
- Cell and Cell culture medium
- Deionized or distilled water
- Microscope

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, store **500X Calcein AM** and **500X EthD-1** at -20°C, all other components store at 4°C. Use the kit before expiration date.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Change pipette tips between the addition of different reagent or samples.

ASSAY PROCEDURE

Cell culture

1. Prepare a cell suspension containing 1-20 x 10^5 cells/ml in culture media.
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 at 5% CO_2.
    - The time and culture conditions depend on the cell line used and may need to be adjusted by the user.

MTT Colorimetric Detection

1. Add the **10 µl** of the **MTT Reagent** into each well of Hydrogel-coated microplate.
2. Incubate the wells 2-4 hours or overnight at 37°C.
   - Monitor the cells with an inverted microscope for the presence of a purple precipitate.
3. Add **100 µl** of **Detergent Solution** into each well. Gently mix the solution by pipetting.
4. Incubate for **2-4 hours** at **RT** in the dark
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 Detection

1. Prepare a mixture of **Calcein AM**, **EthD-1**, and **culture medium** at a 1:1:3 ratio.
2. Add **100 µl** of the mix **Calcein AM** / **EthD-1 solution** to all wells and incubate for **30-60 minutes** at **37°C**.
3. Monitor the cells microscopically for the presence of the green **Calcein AM** (Ex/Em: 485 / 515 nm) or **EthD-1** (Ex/Em: 525 / 590 nm) fluorescence. The fluorescence can be quantitatively measured with a fluorescence microplate reader.