ab284938 – Anoikis Assay kit

For the measurement of Anoikis in metastatic cell lines.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284938

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer	100 mL	-20°C
Anoikis Chamber	1 each	-20°C
Chamber control (uncoated)	1 each	-20°C
Calcein AM Dye	1 vial	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Plate reader
- Cell culture media
- Anhydrous, sterile DMSO

Reagent Preparation

Store the kit at -20°C. Protect from light. Read the entire protocol before performing the assay.

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.

Assay Protocol

Cell Growth:

1. Grow cells of interest in desired media and culture conditions to ~80% confluency.
2. Harvest cells and centrifuge at 1,000 x g, for 5 min. to pellet cells.
3. Resuspend cell pellet in media and count the number of cells using hemocytometer or automated cell counter.
4. Resuspend cells at 1 x 10^6 cells/ml in culture media.
5. 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.
6. Add 100 µL cell suspensions to each well of the Anoikis Chamber and the Chamber Control (Uncoated).

Δ 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.*

Treatment:

1. Treat cells with Anoikis enhancing or inhibiting reagents. For background control, treat the cells with vehicle.
2. Culture the cells 24-72 hr at 37°C 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. After the desired incubation with inducers/inhibitors, carefully remove the plate cover.
4. Add 2 µL of the Calcein AM dye to each well of the 96-well Anchorage Resistant Plate or control plate to be detected.
5. Incubate the plate 30-60 min. at 37°C.

Measurement:

1. Monitor the cells microscopically for the presence of Calcein AM (Green Channel) fluorescence.
2. The fluorescence can be quantitatively measured with a fluorescence microplate reader at Ex/Em: 485/530 nm.

Calculation:

Subtract the background control from the treated samples.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)