ab284545 – Calcium Detection Kit (Cell-Based)

For the detection of calcium.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284545

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 (50X)	2 x 1.8 mL	-20°C
Calcium Probe	1 vial	-20°C
Experimental Control (1000X)	40 µL	-20°C

Materials Required, Not Supplied

- Tissue culture plates, 1X PBS, Fetal Bovine Serum and cell culture media
- Fluorescence microscope
- Flow cytometer with excitation filter at 488 nm wavelength (FL1)

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Assay Buffer (50X): Dilute each vial (50X) into 88.2 ml of sterile 1X PBS, mix well and keep on ice while in use. Keep at 4°C.

Calcium Probe: In a tissue culture hood prepare a 250X stock solution by adding 78 µL of dry DMSO to Calcium Detector vial, mix Store at -20°C, avoid repeated freeze/thaw cycles, use within two months.

Experimental Control (1000X): Warm to room temperature before use. Aliquot and store at -20°C, avoid repeated freeze/thaw cycles, use within two months.

Assay Protocol

This protocol was developed using Jurkat cells and HeLa cells cultivated in 6-well tissue culture plates and can be adjusted for any cell type. The assay volume is 1 mL and it should be adjusted accordingly for different plate formats. Optimal conditions depend on the cell type and test compound, therefore we suggest an initial test of several concentrations of Calcium Probe and Positive Control reagent to find the best conditions for your experimental design.

Sample Preparation

1. Obtain suspension or adherent cell culture of desired density and incubate the cells for 8-12 hours in appropriate medium supplemented with 10% FBS at 37°C with 5% CO_2.
2. **For adherent and suspension cells**: Include appropriate controls: Negative control – cells not exposed to the Calcium Probe or treatment, Positive control –cells to be incubated with 1X Calcium Probe only, Experimental control – cells incubated in presence of 1X Experimental Control reagent. Remove the media and replace it with 1 mL of culture media with 0.5% FBS containing either vehicle, 1X Experimental Control reagent or test compounds at desired concentration. Incubate the cells for additional 30 minutes at 37°C with 5% CO_2, or time required by your experimental protocol. Pellet the suspension cells at 400 x g for 5 minutes at 4°C temperature and remove media; use these settings throughout the entire protocol.
3. Terminate the experiment. Trypsin can be used to collect adherent cells. Centrifuge detached adherent and/or suspension cells at 400 x g for 5 minutes at 4°C. Wash the cell pellets twice in 500 µL of ice-cold 1X Assay Buffer. Centrifuge the cells between washes as above and discard the supernatant.
4. Re-suspend cell pellets in 250 µL of medium with 0.5% FBS and add 1 µL of 250X Calcium Probe to Test, vehicle, Positive and Experimental Control cells. Incubate 45 min at 37°C and 5% CO_2. Remove the supernatant after centrifuging cells at 400 x g for 5 min at 4°C.
5. Re-suspend cell pellets in 1 mL of Assay Buffer and incubate for 2 minutes at RT. Cells are ready for analysis for calcium staining by flow cytometer and fluorescence microscope.

FACS and Fluorescence microscope analysis

FACS acquisition: select the main cell population in the FSC vs. SSC plot to exclude dead cells and cellular debris from Negative Control Cells. Within the main cell population, mean fluorescence intensity in FL1 (Calcium Staining) can be quantified and compared between untreated cells and cells treated with test compounds or between different cell types.

Fluorescence Microscope analysis: Observe the cells under the fluorescence microscope for green (Calcium staining) and Blue (Nuclear Staining) fluorescence respectively.

Technical Support

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