ab284548 – Lysosomal Cytotoxicity Dual Staining Kit (Cell-Based)

For the screening of compounds that affect lysosomal function, and staning for lysosome cytotoxicity and cell dealth.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284548

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 (10X)	25 mL	-20°C
Cell Death Marker (500X)	100 µL	-20°C
Lysosomal Stain (500X)	100 µL	-20°C
Positive Control Reagent	1 vial	-20°C

Materials Required, Not Supplied

Tissue culture plates and media
Fluorescence microscope
Flow cytometer with excitation filter at 488 nm wavelength (FL1) and at 535 nm wavelength (FL2)

Reagent Preparation

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

Assay Buffer (10X): Dilute the 10X stock 1:10 in sterile water, mix well and keep on ice while in use. Keep at 4°C.

Lysosomal Stain (500X) and Cell Death Marker (500X): Warm to room temperature (RT) before use. Store at -20°C, avoid repeated freeze/thaw cycles

Positive Control Reagent: Dissolve in 100 µL of sterile PBS. Warm to room temperature before use. Aliquot and store at -20°C, avoid repeated freeze/thaw cycles.

Assay Protocol

This protocol was developed for Jurkat cells cultivated in 6-well tissue culture plates and can be adjusted for any cell type. All volumes should be adjusted accordingly for other plate formats. The assay volume is 1 mL. However, optimal conditions depend on the cell type. Reagents, buffer, and the number of cells should be adjusted accordingly for different plate formats

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**: Next day, remove the media and replace it with 2 mL of fresh aliquots containing either vehicle or test compounds at desired concentration. **For suspension cells**: Pellet the cells at 300 x g for 5 minutes at room temperature prior to media removal. **For experimental control** (Positive Control Reagent treatment): add 8 µL of Control Reagent directly into the culture media and incubate the cells for additional 24 hours at 37°C with 5% CO_2, or time required by your experimental protocol.
3. Terminate the experiment and harvest the cells. Trypsin can be used to collect adherent cells. Pellet cells at 300 x g for 5 minutes at room temperature. Wash the cells twice in 1 mL ice-cold 1X Assay Buffer. Pellet the cells at 300 x g for 5 min and remove wash buffer.
4. Re-suspend cell pellets in 1 mL of Assay Buffer and add 2 µL of 500X Lysosomal Stain, incubate 10 minutes at 37°C and 5% CO_2. Remove the supernatant by centrifuging cells at 300 x g for 5 minutes at RT.
5. Re-suspend cell pellets in 1 mL of Assay Buffer, and add 2 µL of Cell Death Marker (500X) and incubate for 2 minutes at RT. Cells are ready for analysis for lysosomal staining and cell death 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 no staining cells (Negative Control Cells). Within the main cell population, mean fluorescence intensity in FL1 (Lysosomal Staining) and FL-2 (Cell Death) 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 (lysosomal staining) and red (cell death) fluorescence respectively.

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)

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