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Lysosomal Cytotoxicity Dual Staining Kit (Cell-Based)

(Catalog # BN00696; 50 assays; Store at -20°C)

I. Introduction:

Lysosomes are membrane-bound organelles important for various cellular processes. They contain hydrolytic enzymes utilized in the metabolism of some biomolecules. The extracellular cargo (e.g. nutrients, toxins) binds to the cell membrane and is subsequently transported into membrane-bound endosomes for further degradation by lysosomes while intracellular components are transported to lysosomes through autophagy. Lysosomal dysfunction is associated with many human conditions such as aging and neurodegenerative disease. Assay Genie has developed the Lysosomal Cytotoxicity Dual Staining Kit (cell-based) which contains two probes; membrane permeable, selective Lysosomal Stain and Cell Death Marker. We also include a Positive Control Reagent, which increases lysosome activity and staining, thus serves as an experimental control. This easy-to-use, non-radioactive kit allows studying the regulation of lysosome and cytotoxicity at the cellular level by using Fluorescence Microscopy and Flow Cytometry in cultured cells.

II. Applications:

- Staining for lysosome cytotoxicity and cell death.
- Screening for compounds that affect lysosomal function.

III. Sample Type:

- Suspension or adherent cells cultures

IV. Kit Contents:

Components	BN00696	Cap Code	Part Number
Assay Buffer (10X)	25 ml	NM	BN00696-1
Lysosomal Stain (500X)	100 µl	Orange	BN00696-2
Cell Death Marker (500X)	100 µl	Red	BN00696-3
Positive Control Reagent	1 vial	Blue	BN00696-4

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **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.

VII. Lysosomal Cytotoxicity Dual Staining 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.

1. Sample Preparation:

a. 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.

b. **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.

c. 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.

d. Re-suspend cell pellets in 1 ml of Assay Buffer and add 2 µl of 500X Lysosomal Stain, incubate 10 min at 37°C and 5% CO_2. Remove the supernatant by centrifuging cells at 300 x *g* for 5 min at RT.

e. 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*.

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2. FACS and Fluorescence microscope analysis:

a. **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.

b. **Fluorescence Microscope analysis:** Observe the cells under the fluorescence microscope for green (lysosomal staining) and red (cell death) fluorescence respectively.

A. **B.**

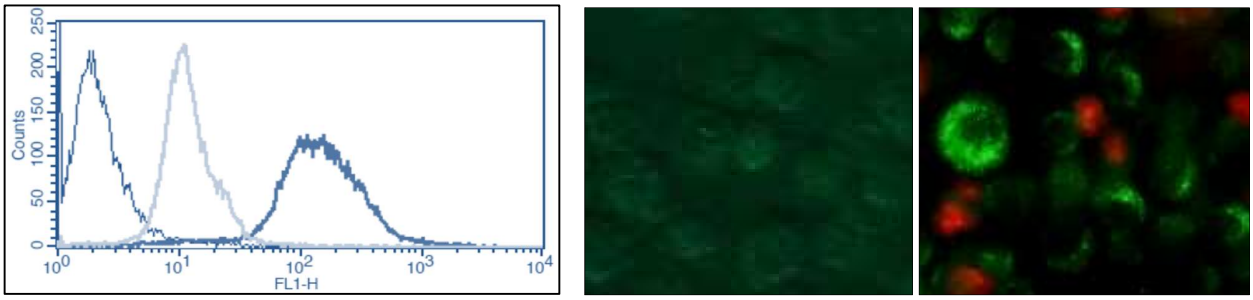

Figure: Lysosomal Staining in Jurkat cells. 1x10^6 Jurkat cells were treated with vehicle or Positive Control Reagent for 24 hr. After treatment, cells were incubated in 1 ml of Assay Buffer with 1X Lysosomal Staining for 10 minutes at 37°C, followed by stain with 1X Cell Death Marker according to kit's protocol. **Panel A:** Comparison of histograms from FACS analysis showing lysosomal staining. Unstained cells (black); Control cells stained with 1X Lysosomal Staining (green); Cells treated with Positive Control Reagent staining (blue). **Panel B:** Cells treated with vehicle (left panel; basal level of lysosomal staining), or Positive Control Reagent (right panel; green fluorescence). Increased green fluorescence compared to the basal levels confirms accumulation of Lysosomal Stain inside activated lysosomes. Low levels of red fluorescence resulting from Cell Death Marker shows low levels of Lysosomal Stain toxicity.

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