Lysosomal Intracellular Activity Assay Kit ab234622

Overview

Product name: Lysosomal Intracellular Activity Assay Kit
Detection method: Fluorescent
Sample type: Adherent cells, Suspension cells
Assay type: Cell-based
Species reactivity: Reacts with: Other species, Mammals

Product overview:
Lysosomal Intracellular Activity Assay Kit (ab234622) provides a proprietary Lysosome-Specific Self-Quenched Substrate which has low background fluorescence, high signal to background ratio and is pH insensitive. The substrate, acting as endocytic cargo, can be taken up by cells and degraded within an endo-lysosomal vesicle. The fluorescent signal is recovered from the Self-Quenched Substrate. The fluorescence signal, generated by degradation, is proportional to the intracellular lysosomal activity and can be measured using a fluorescence microscopy and/or flow cytometry. The kit includes Bafilomycin A1, a cell-permeable inhibitor of the lysosomal membrane V-ATPase proton pump, that serves as an anti-lysosomal experimental control. This easy-to-use, non-radioactive kit allows imaging and accurate measurement of de-quenching substrate in cultured cells.

Notes:
Lysosomes are membrane-bound organelles important for various cellular processes. They contain hydrolytic enzymes that are 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.

Platform:
Flow cytometer, Fluorescence microscope

Properties

Storage instructions: Store at -20°C. Please refer to protocols.

Components | 50 tests
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Assay Buffer (50X) | 1 x 1.8ml
Self-Quenched Substrate | 1 vial
Bafilomycin A1 (1000X) | 1 x 50µl
1x10^6 U937 cells were pretreated with vehicle or 1X Bafilomycin A1 for 3 hours. After pretreatment, cells were incubated with Self-Quenched Substrate and the same concentration of Bafilomycin A1 for an additional hour in medium supplemented with 0.5% FBS according to kit’s protocol. Comparison of histograms from flow cytometric analysis (FL1/FITC channel), showing the inhibition of Substrate De-quenching following treatment with lysosome-depolarizing agent: unstained cells (purple curve), untreated positive control cells (green curve) and experimental control cells treated with 1X Bafilomycin A1 (yellow curve).

1x10^6 U937 cells were pretreated with vehicle or 1X Bafilomycin A1 for 1 hour. After pre-treatment, cells were incubated with Self-Quenched Substrate and the same concentration of Bafilomycin A1 for additional hour in medium supplemented with 0.5% FBS according to kit’s protocol. Images of U937 cells obtained using fluorescence microscope. Top: positive control cells treated Self-quenched substrate only. Bottom: negative control cells treated with 1X Bafilomycin A1. U937 cells showing the release of Self-quenched substrate in the endocytotic vesicle (punctured pattern).

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