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Lysosomal Intracellular Activity Assay Kit (Cell-Based)

Rev 01/20

(Catalog # K448-50; 50 assays; Store at -20°C)

I. Introduction:

Lysosomes are membrane-bound organelles important for various cellular processes. They contain acid-activated hydrolytic enzymes that are utilized in the degradation of proteins and other biomolecules. The extracellular cargo (e.g. nutrients, toxins or cell debris) binds to the cell membrane and is subsequently sequestered into membrane-bound endosomes for further degradation by the lysosomes, while intracellular components are transported to lysosomes through autophagy. Lysosomal dysfunction is associated with many inherited metabolic, neurodegenerative and aging-related diseases. Although the intracellular activity of lysosome is difficult to measure in living cells, BioVision has developed 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 by fluorescence microscopy and/or flow cytometry. **BioVision's Lysosomal Intracellular Activity Assay Kit (Cell-Based)** includes Bafilomycin A1, a cell-permeable inhibitor of the lysosomal membrane V-ATPase proton pump, which serves as an anti-lysosomal experimental control. This easy-to-use, non-radioactive kit allows imaging and accurate measurement of lysosomal protease activity in cultured cells.

II. Applications:

- Measurement of lysosomal intracellular activity.
- Elucidation of the mechanisms of endocytic pathway in living cells.
- Screening compounds with anti-lysosomal intracellular activity.

III. Sample Type:

- Suspension or adherent cells cultures

IV. Kit Contents:

Components	K448-50	Cap Code	Part Number
Assay Buffer (50X)	1.8 ml	Brown	K448-50-1
Self-Quenched Substrate	1 vial	Orange	K448-50-2
Bafilomycin A1 (1000X)	50 µl	Yellow	K448-50-3

V. User Supplied Reagents and Equipment:

- 1X PBS
- Tissue culture plates and media
- Fluorescence microscope (with FITC/GFP filter set)
- Flow cytometer with excitation filter at 488 nm wavelength

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 (50X):** Dilute 50X Assay Buffer 50 times in 1X PBS to obtain a 1X Assay Buffer. Keep on ice while in use.
- **Self-quenched Substrate:** Re-constitute the vial with 1 ml of 1X PBS. Mix well. Aliquot and store at -20°C, avoid repeated freeze/thaw cycles.
- **Bafilomycin A1:** Warm to room temperature before use. Aliquot and store at -20°C, avoid repeated freeze/thaw.

VII. Lysosomal Intracellular Activity Assay Protocol:

This protocol was developed for U937 suspension cells and can be adjusted for any cell type. The cell culture density was 1 x 10^6 cells/ml and an assay volume of 1 ml; however, optimal conditions depend on the cell type. Reagents, buffer, and the number of cells should be adjusted accordingly for different plates.

1. Sample Preparation:

a. Obtain suspension or adherent cell culture of desired density and incubate the cells for 8-12 hrs 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 with fresh complete medium containing either vehicle (positive control) or the test compound at desired concentration. For experimental control (lysosome inhibition by Bafilomycin A1 treatment): dilute the 1000X Bafilomycin A1 stock directly into the media to obtain the 1X final concentration. Incubate the cells for 3 hours, or time required by your experimental protocol, at 37°C with 5% CO_2. **For suspension cells:** Pellet the cells at 300 x *g* for 5 minutes at room temperature prior to media removal**.**

c. **For adherent and suspension cells:** Upon completion, remove the media and replace with fresh aliquots supplemented with 0.5% FBS. Add vehicle (positive control) or test compound at the same concentration as in step 1b. For experimental control: add Bafilomycin A1 to 1X final concentration. Add 15 µl of Self-Quenched Substrate per 1 ml of media into the positive control, experimental control and tested compound cells. Incubate the cells for 1 hour, or the time required for your specific cell line, at 37°C with 5% CO_2. **For suspension cells:** Pellet the cells at 300 x *g* for 5 minutes at room temperature prior to media removal**.**

d. **For adherent and suspension cells:** Terminate the experiment and harvest the cells. Wash the cells twice in 1 ml ice-cold 1X Assay Buffer containing the tested compound at the same concentration as in step 1b. **For suspension cells:** Pellet the cells at 300 x *g* for 5 minutes at room temperature prior to media and washes removal**.**

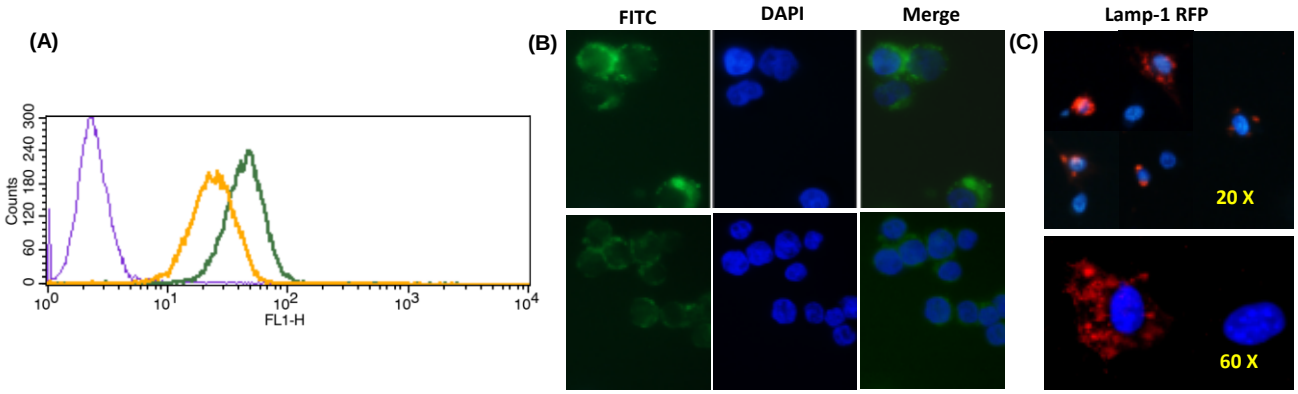
e. Re-suspend cell pellets in 1 ml of 1X PBS containing the tested compound at the same concentration as in step 1b. Cells are ready to be analyzed on flow cytometer (488 nm excitation laser) or imaged by fluorescence microscopy (FITC/GFP filter set).

2. **FACS Acquisition and Analysis:** select the main cell population in the FSC vs SSC plot to exclude dead cells and cellular debris. Within the main cell population, mean fluorescence intensity in FL1 can be quantified and compared between untreated cells and cells treated with test compounds (including Bafilomycin A1) or between different cell types to distinguish different levels of released fluorescence from Self-Quenched Substrate.

   Notes:

   - Trypsin can be used to collect the adherent cells prior to FACS analysis.
   - The assay can be used to measure and compare the lysosomal intracellular activity in various cell types.

3. **Fluorescence Microscope Analysis:** To visualize the fluorescence of released Self-Quenched Substrate, observe the cells under fluorescence microscope with 488 nm excitation filter (FITC/GFP filter cube).



Figures: Release of Self-Quenched Substrate in U937 cells. 1x10^6 U937 cells were pretreated with vehicle or 1X Bafilomycin A1 for 3 hrs. 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. **Panel A:** 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). **Panel B:** Fluorescence microscopy images of U937 cells showing the release of Self-quenched Substrate in the endocytotic vesicles (punctate pattern). *Top:* positive control cells, treated with Self-quenched Substrate only. *Bottom:* cells treated with 1X Bafilomycin A1. **Panel C:** Lysosomal staining with fluorophore-tagged Lysosomal Associated Membrane Protein 1 (Lamp-1 RFP, a lysosomal marker). Cells were stained with nuclear dye for 10 min, washed with 1X PBS and mounted on the slide. Images were taken using a Nikon TE2000 inverted fluorescence microscope with a 60X objective lens.

VIII. Related Products:

Glucose Uptake Colorimetric Assay Kit (K676)
Glucose Colorimetric/Fluorometric Assay Kit (K606)
Glucose and Sucrose Colorimetric/Fluorometric Assay Kit (K616)
PicoProbe™ Glucose-6-Phosphate Fluorometric Assay Kit (K687)
Glucose Dehydrogenase Activity Colorimetric Assay Kit (K786)
Glucose-1-Phosphate (G1P) Colorimetric Assay Kit (K697)
GluTracker™ Glucose Uptake Assay Kit (Cell-Based) (K681)
Glucose AGE-BSA-II (2223)
GPI, human recombinant (7822)
SGLT-1 Antibody (3711)
Glucose Uptake Fluorometric Assay Kit (K666)
SGLT-2 Antibody (3690)
Maltose and Glucose Colorimetric/Fluorometric Assay Kit (K618)
PicoProbe™ Glucose Fluorometric Assay Kit (K688)
Glucose Oxidase Activity Colorimetric/Fluorometric Assay Kit (K788)
Glucose-6-Phosphate Colorimetric Assay Kit (K657)
2-NBDG (2760)
G6PD, Human Recombinant (7532)
GLUT4 Antibody (3945)
Self-Quenched BODIPY$_{FL}$ Conjugate of BSA (Green) (7632)

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