ab234622 – Lysosomal Intracellular Activity Assay Kit

For the measurement of de-quenching substrate in cultured cells.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab234622

Storage and Stability

Store kit at -20°C, protected from light. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Buffer I	1.8 mL	-20°C
Self-Quenched Substrate	1 vial	-20°C
100X Cytochalasin D	50 µl	-20°C

PLEASE NOTE: Buffer I was previously labelled as Assay Buffer (50X). The composition has not changed.

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Cell culture medium
- 1 X PBS
- Tissue culture plates and media
- Fluorescence microscope
- Flow cytometer with excitation filter at 488 nm wavelength

Reagent Preparation

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

Analysis Buffer (50X): Dilute Analysis Buffer in 1X PBS (not supplied) to make 1X Analysis Buffer. Keep on ice while in use.

Self-quenched substrate: Resuspend with 1 ml of 1X PBS to make 1 mg/ml concentration, mix well. Aliquot and store at -20°C, avoid repeated freeze/thaw.

Cytochalasin D: Bring to room temperature before use. Aliquot and store at -20°C, avoid repeated freeze/thaw.

Assay Protocol

- This protocol was developed for U937 suspension cells, 5 x 10^5 cells/sample in 1 ml volume; however the optimal conditions may depend on the cell type. Reagents, buffer, and the number of cells should be adjusted accordingly for different size culture plates.

Sample Preparation:

1. Obtain a cell culture of desired density and culture the cells of interest for 8-12 hours in regular culture medium (10% FBS).
2. Treat the cells with a test compound or vehicle control in culture medium supplemented with 0.5% FBS.
3. Incubate the cells at 37°C with 5% CO_2 for 1 hr or time required by the tested compound.
4. To use 100X Cytochalasin D as an inhibitor control, treat cells with 1X final concentration of Cytochalasin D in culture medium with 0.5% FBS at 37°C with 5% CO_2 for 1 hr.
   Δ Note: Seed adherent cells (2-5 x10^5 cells/well) one day before starting the assay. After 8-12 hrs, remove regular culture medium (10% FBS) and treat cells with test compound or vehicle control in 500 µl tissue culture medium with 0.5% FBS. Incubate cells at 37°C with 5% CO_2 for 1 hr or desired time depending upon the test compound. To use 1X Cytochalasin D as a control, treat cells with 5 µl Cytochalasin D (final concentration 1X) in 500 µl of tissue culture medium with 0.5% FBS at 37°C with 5% CO_2 for 1 hr.
   Δ Note: Cell seeding is not required for suspension cells. Use up to 1x10^6 suspension cells/well in 500 µl of tissue culture medium with 0.5% FBS to treat with test compound or vehicle control.

Self-Quenched Substrate:

1. Prepare Self-Quenched Substrate mix for each well as following:

Item	Volume
Tissue culture medium (0.5% FBS)	492.5 µl
Self-Quenched Substrate	7.5 µl
Test compound or 1X Cytochalasin D	same conc. as in Sample Preparation-Step 4

2. Mix well. After the incubation in Sample Preparation – Step 3, spin down the plate at 400 x *g* for 5 mins and carefully remove the medium without disturbing cells. Gently add Self-Quenched Substrate mix to each well and incubate cells at 37°C with 5% CO_2 for 1 hr.

Measurement

After incubation, collect the cells and keep on ice. Wash twice in 1 ml ice-cold Buffer I containing the tested compound at the same concentration. Re-suspend cell pellet in 1 ml of 1X PBS containing the tested compound at the same concentration.

Calculation

- Cells are ready to be analyzed on flow cytometer (488 nm excitation laser).
- For flow 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 or between different cell types to distinguish different levels of release fluorescence from Self-Quenched Substrate.

Δ Notes:

a) Trypsin can be used to collect the adherent cells for performing this assay.
b) The assay can be used to measure & compare the lysosomal intracellular activity in various cell types.
c) To visualize the fluorescence level of released Self-Quenched Substrate under fluorescence microscope, centrifuge the plate at 400 x *g* for 5 min. Wash cells once with 500 µl ice-cold 1X Analysis Buffer, and replace with fresh 200 µl of 1X Analysis Buffer. Visualize cells under fluorescence microscope with a blue excitation fluorescence filter (excitation range 420-495 nm).
d) Emission is 520-525 nm.

Technical Support

For all technical or commercial enquiries please go to:
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www.abcam.co.jp/contactus (Japan)