Version 1 Last updated 29 May 2018

ab234622 Lysosomal Intracellular Activity Assay Kit

For the measurement of lysosomal intracellular activity in suspension or adherent cells.

This product is for research use only and is not intended for diagnostic use.

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1. 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 Cytochalasin D, a cell-permeable inhibitor of endocytosis that serves as an experimental control. This easy-to-use, non-radioactive kit allows imaging and accurate measurement of de-quenching substrate in cultured cells.

Prepare suspension or adherent cells.

Replace media with fresh medium containing either vehicle (positive control) or the test compound. Incubate for 1 hour, or time required by your experimental protocol, at 37°C with 5% CO_2.

Add Self-Quenched Substrate into the positive control, experimental control and tested compound cells. Incubate for 1 hour, or the time required for your specific cell line, at 37°C with 5% CO_2.

Wash the cells twice in 1 ml ice-cold 1X Assay Buffer containing the tested compound at the same concentration.

Analyze by FACS of Fluorescence microscopy.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer (50X)	1.8 mL	-20°C	-20°C
Self-Quenched Substrate	1 vial	-20°C	-20°C
Cytochalasin D (100X)	50 µL	-20°C	-20°C

3. Materials Required, Not Supplied

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

- 1X PBS.
- Fluorescence microscope.
- Flow cytometer with excitation filter at 488 nm wavelength.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Assay Buffer (50X)

1. Dilute 50X Assay Buffer 50 times in 1X PBS to obtain a 1X Assay Buffer.
2. Keep on ice while in use.

5.2 Self-Quenched substrate

1. Re-constitute the vial with 1 mL of 1X PBS. Mix well.
2. Aliquot and store at -20°C, avoid repeated freeze/thaw.

5.3 Cytochalasin D

1. Warm to room temperature before use.
2. Aliquot and store at -20°C, avoid repeated freeze/thaw.

6. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay.

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. Obtain suspension or adherent cell culture of desired density.
2. Incubate the cells for 8-12 hours in appropriate medium supplemented with 10% FBS at 37°C with 5% CO_2.

7. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

7.1 For adherent and suspension cells:

1. Next day, remove the media and replace with fresh complete medium containing either vehicle (positive control) or the test compound at desired concentration.
2. For experimental control (Cytochalasin D treatment): dilute the 100X Cytochalasin D stock directly into the media to obtain the 1X final concentration.
3. Incubate the cells for 1 hour, or time required by your experimental protocol, at 37°C with 5% CO_2.

   Δ Note: For suspension cells: Pellet the cells at 300 x *g* for 5 minutes at room temperature prior to media removal.

7.2 For adherent and suspension cells:

1. 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 7.1.
2. For experimental control: add Cytochalasin D to 1X final concentration.
3. Add 15 µl of Self-Quenched Substrate per 1 mL of media into the positive control, experimental control and tested compound cells.
4. Incubate the cells for 1 hour, or the time required for your specific cell line, at 37°C with 5% CO_2.

   Δ Note: For suspension cells: Pellet the cells at 300 x *g* for 5 minutes at room temperature prior to media removal.

7.3 For adherent and suspension cells:

1. Terminate the experiment and harvest the cells.
2. Wash the cells twice in 1 mL ice-cold 1X Assay Buffer containing the tested compound at the same concentration as in step 7.1.

Δ Note: For suspension cells: Pellet the cells at 300 x *g* for 5 minutes at room temperature prior to media and washes removal.

7.4 For adherent and suspension cells:

1. 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).

7.5 FACS acquisition and analysis:

1. Select the main cell population in the FSC vs SSC plot to exclude dead cells and cellular debris.
2. 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 released fluorescence from Self-Quenched Substrate.

   Δ Note: Trypsin can be used to collect the adherent cells prior to FACS analysis.

   Δ Note: The assay can be used to measure and compare the lysosomal intracellular activity in various cell types.

7.6 Fluorescence microscope analysis:

1. To visualize the fluorescence of released Self-Quenched Substrate, observe the cells under fluorescence microscope with 488 nm excitation filter.

8. FAQs / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

9. Typical Data

Data provided **for demonstration purposes only.**

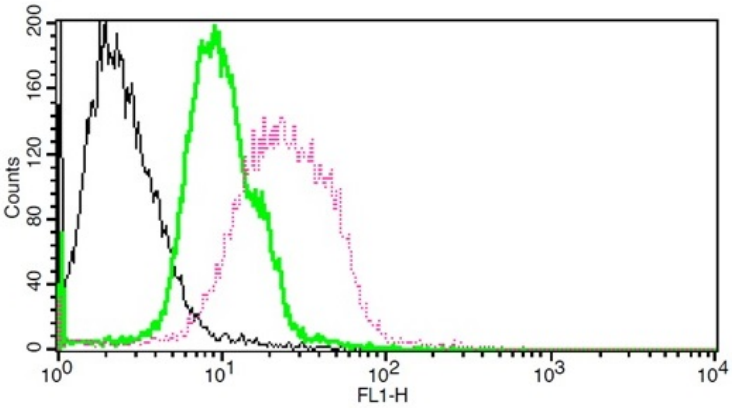

Figure 1. Release of self-Quenched Substrate in U937 cells. 1x10^6 U937 cells were pretreated with vehicle or 1X Cytochalasin D for 1 hour. After pre-treatment, cells were incubated with Self-Quenched Substrate and the same concentration of Cytochalasin D for additional hour in medium supplemented with 0.5% FBS according to kit's protocol. Comparison of histograms from flow analysis showing the inhibition of De-Quenching of Substrate by Cytochalasin D. Unstained cells (black); experimental control (green) in the presence of 1X Cytochalasin D; Positive control (pink) without 1X Cytochalasin D.

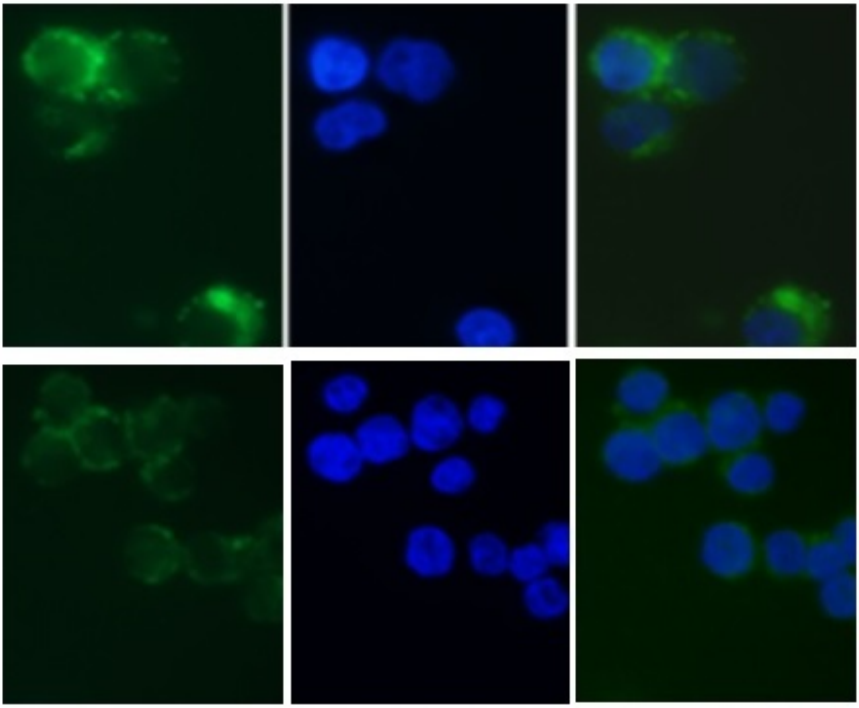

Figure 2. Release of self-Quenched Substrate in U937 cells. 1x10^6 U937 cells were pretreated with vehicle or 1X Cytochalasin D for 1 hour. After pre-treatment, cells were incubated with Self-Quenched Substrate and the same concentration of Cytochalasin D for additional hour in medium supplemented with 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 Cytochalasin D. U937 cells showing the release of Self-quenched substrate in the endocytotic vesicle (punctured pattern).

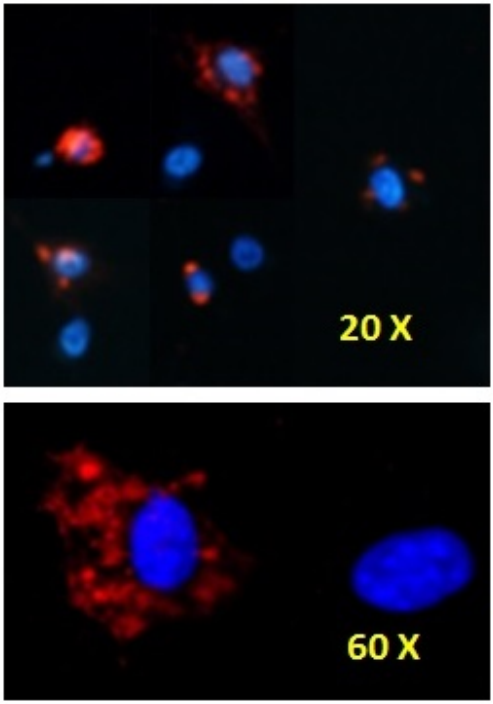

Figure 3. Release of self-Quenched Substrate in U937 cells. $1x10^6$ U937 cells were pretreated with vehicle or 1X Cytochalasin D for 1 hour. After pre-treatment, cells were incubated with Self-Quenched Substrate and the same concentration of Cytochalasin D for additional hour in medium supplemented with 0.5% FBS according to kit's protocol. Lysosomal staining with Lysosomal Associated Membrane Protein 1 (Lamp-1 RFP, a lysosomal marker). Cells were stained with nuclear dye for 10 minutes, washed with 1X PBS and mounted on the slide. Images were taken using a fluorescence microscope with a 60X objective lens.

10. Notes

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | 400 921 0189 / +86 21 2070 0500
Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abc.com | +64-(0)9-909-7829