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ab270790

Autophagy Assay Kit (Red)

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Autophagy Assay Kit datasheet:

www.abcam.com/ab270790

(use www.abcam.cn/ab270790 for China, or www.abcam.co.jp/ab270790 for Japan)

For the detection of Cathepsin B activity in cell culture media.

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

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1. Overview

Autophagy Assay Kit (Red) enables researchers to detect and monitor the *in vitro* development of autophagy in living cells. Autophagy Probe, Red is a cell-permeant aliphatic molecule that fluoresces brightly when inserted in the lipid membranes of autophagosomes and autolysosomes. Autophagy Probe, Red can be readily detected by flow cytometry with optimal excitation at 590 nm and peak emission at 620 nm.

2. Materials Supplied and Storage

Store kit components at the indicated temperatures immediately on receipt.

Δ Note: Once reconstituted with DMSO, use Autophagy Red probe immediately, or store at $\leq -20^{\circ}\text{C}$ for up to 6 months, protected from light and thawed no more than twice during that time.

Item	Quantity 50 tests	Quantity 200 Tests	Storage temperatur e
Autophagy Probe, Red	1 vial	1 vials	-20°C
Fixative	1 x 6 mL	1 x6 mL	+4°C
10X Cellular Assay Buffer	1 bottle (15 mL)	1 Bottle (60mL)	+4°C

3. Materials Required, Not Supplied

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

- DMSO, 100 μ L per vial to reconstitute Autophagy Probe, Red
- DiH₂O, 135-540 μ L per bottle to dilute 10X Cellular Assay Probe
- Phosphate buffered saline (PBS) pH 7.4, 400 μ L per vial to dilute Autophagy Probe, Red
- Cultured cells treated with the experimental conditions ready for staining
- Cultured cells treated with the experimental conditions ready to be analyzed
- Reagents to induce autophagy such as Rapamycin, and to inhibit autophagy such as Chloroquine
- Hemocytometer
- Centrifuge at $<200 \times g$
- FACS tubes (for flow cytometry analysis)
- 15 mL polypropylene centrifuge tubes (1 per sample)
- Autophagy Probe, Red reagent optimally excites at 590 nm and has a peak emission at 620 nm (use a green/yellow laser for flow cytometry).

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. Experimental Preparation

- 5.1** Staining cells with Autophagy Probe, Red can be completed within a few hours. However, Autophagy Probe, Red is used with living cells, which require periodic maintenance and cultivation several days in advance. In addition, once the proper number of cells has been cultivated, time must be allotted for the experimental treatment, which may vary. The recommended sample size is 0.5 mL cells at 5×10^5 cells/mL.
- 5.2** Create cell populations, such as:
- Cells that were exposed to the experimental treatment.
 - A negative control population of cells that received a placebo treatment.
- 5.3** Culture cells to a density optimal for the specific experimental protocol. Cell density should not exceed 10^6 cells/mL. Cells cultivated in excess of this concentration may begin to naturally enter apoptosis. An initial experiment may be necessary to determine how much Autophagy Probe, Red reagent to use.

6. Controls

Create experimental samples and control cell populations:

- 6.1 **Treated experimental population(s):** cells exposed to the experimental condition(s).
- 6.2 **Positive control for Autophagy Probe, Red (cells induced to initiate autophagy):** Numerous treatments exist for activating autophagy (such as starvation in Earl's Balanced Salt Solution, or exposure to Rapamycin which mimics starvation by inhibiting mTOR) or inhibiting fusion of autophagosome with lysosomes and lysosomal degradation (exposure to Chloroquine).
- 6.3 **Negative control for Autophagy Probe, Red (untreated cells):** cells mock-treated with DMSO.

Δ Note: A common pool of cells should be used to generate the positive and negative control populations for Autophagy Probe, Red, and should contain similar quantities of cells. For example, if labeling with Autophagy Probe, Red, make 4 populations:

Control #	
1 and 2	Unlabeled: Induced and untreated cells
3 and 4	Autophagy Red probe-labeled: induced and untreated cells

7. Preparation of Autophagy Probe, Red

- 7.1 Autophagy Probe, Red is supplied as a lyophilized powder that may be slightly visible as an iridescent purple sheen inside the vial. Protect from light and use gloves when handling. Once diluted in aqueous buffer, Autophagy Probe, Red solution must be used immediately; prepare it just before staining.
- 7.2 Reconstitute the vial of Autophagy Probe, Red with 100 μ L DMSO to form the 250X stock solution. The stock solution should be bluish-purple. Once reconstituted in DMSO, it may be aliquoted and stored at $\leq -20^{\circ}\text{C}$ for 6 months protected from light. Avoid repeated freeze/thaw cycles.
- 7.3 Immediately prior to addition to the samples and controls, dilute Autophagy Probe, Red reagent 1:5 by adding 400 μ L PBS to each vial to form the 50X Autophagy Probe, Red solution. Use 50X Autophagy Probe, Red within 30 minutes of dilution into aqueous buffers.
- 7.4 These amounts are recommendations, however, the sample size and Autophagy Probe, Red staining concentration needed may vary based on the experimental conditions used. Each investigator should adjust the amount of Autophagy Probe, Red to accommodate the particular cell line and research conditions.

8. Preparation of 1Xcellular Assay Buffer

- Cellular Assay Buffer is an isotonic solution used to stabilize cells when staining with Autophagy Red probe. It contains mammalian proteins to stabilize cells, and sodium azide to retard bacterial growth (1X Cellular Assay Buffer contains 0.01% w/v sodium azide).
 - Alternative solutions including cell culture media containing FBS and other additives may be used to stain cells instead of the 1X Cellular Assay Buffer.
- 8.1** 10X Cellular Assay Buffer may form precipitates during cold storage. If this happens, gently warm it until all crystals have dissolved. Do not boil.
- 8.2** Dilute 10X Cellular Assay Buffer 1:10 in diH₂O. For example, add 15 mL 10X Cellular Assay Buffer to 135 mL diH₂O for a total of 150 mL; add 60 mL 10X Cellular Assay Buffer to 540 mL diH₂O for a total of 600 mL.
- 8.3** 1X Cellular Assay Buffer may be stored at 4°C and used within 1 week or frozen and used within 6 months.

9. Fixative

Fixative is a formaldehyde solution designed to cross-link and aggregate intracellular components. If the stained cell populations cannot be evaluated immediately after labeling with Autophagy Probe, Red, add Fixative at a ratio of 1:5-1:10. For example, to use Fixative at 1:10, add 100 μ L Fixative to 900 μ L cells. Never add Fixative until all the staining and final wash steps have been completed. Fixed cells may be stored on ice or at 4°C for up to 16 hours, protected from light. The kit Fixative will not interfere with the red fluorescent label. Do not use absolute ethanol- or methanol-based fixatives, as they may inactivate the Autophagy Probe, Red label.

ΔNote: Fixative contains formaldehyde <10% and methanol <5% and is harmful. Avoid contact with skin, eyes, and clothing by wearing lab coat, gloves, and safety glasses. In case of exposure, immediately flush eyes or skin with water. See SDS for further information.

10. Flow cytometry Analysis

The recommended sample size is 0.5 mL. The recommended cell concentration is $3\text{-}5 \times 10^5$ cells/mL.

- 10.1 Expose cells to the experimental or control conditions. Set aside two populations to create positive and negative instrument controls with Autophagy Probe, Red (Section 6).
- 10.2 Optional wash step: depending on the experimental conditions used to induce autophagy, it may be necessary to wash cells and then resuspend them in fresh cell culture medium prior to staining.
- 10.3 Transfer 490 μL cells into fresh tubes.
- 10.4 Add 10 μL 50X Autophagy Probe, Red solution, forming a final volume of 500 μL . Autophagy Probe, Red can be added directly to the cell culture medium for the staining process. If different cell volumes were used, add Autophagy Probe, Red at a ratio of 1:50. Mix by gently flicking the tubes. The amount of Autophagy Probe, Red should be optimized for each cell line and experimental condition.
- 10.5 Incubate cells at 37°C protected from light. The incubation period may range from 30 minutes to several hours and should be optimized for each cell line and experimental condition. As cells may settle on the bottom of the tubes, gently resuspend them by swirling cells every 20 minutes to ensure an even distribution of Autophagy Probe, Red throughout the staining process.
- 10.6 Centrifuge at $200 \times g$ for 10 minutes at room temperature.
- 10.7 Carefully remove and discard supernatants. Resuspend in 0.5 mL of 1X Cellular Assay Buffer and mix gently. If desired, cells can be washed using fresh cell culture medium or alternate buffers such as PBS containing BSA. Take care that the wash steps are completed quickly so as not to cause unanticipated cellular stress by prolonged exposure to nutrient-deprived buffers.
- 10.8 Repeat wash steps until samples have been washed 3 times. After final centrifugation, resuspend the pellet in 0.5 mL 1X Cellular Assay Buffer.
- 10.9 Cells are ready for analysis. An optional fixation step may be performed at this time. Add the provided Fixative at a v/v ratio of 1:5-1:10. For example, add 50 μL of Fixative to 450 μL of

sample. To analyze the samples, measure fluorescence using a green/yellow laser equipped with an appropriate filter.

11. Notes

Technical Support

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