（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F202

Specification：48T/96T

Measuring instrument：Flow Cytometry, Fluorescence microscope

Elabscience® Lysosomal Activity Fluorometric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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Intended use

This kit can be used to measure the activity of proteases in cellular lysosomes.

Detection principle

Lysosomes are the "digestive organs" of the cell, responsible for breaking down macromolecules (such as proteins, lipids, and polysaccharides) and clearing damaged organelles. The assessment of their activity typically encompasses multiple aspects, including acidification capacity, enzymatic activity, and degradative function.

This kit utilizes a fluorescent probe based on BSA-conjugated dye to detect lysosomal activity. The probe enters cells via endocytosis and is subsequently hydrolyzed by proteases within the lysosomes, thereby emitting fluorescence, which can be detected using flow cytometry.

Kit components & storage

Item	Component	Size1 (48 T)	Size2 (96 T)	Storage
Reagent 1	Probe Stock Solution	0.083 mL × 1vial	0.166 mL × 1 vial	-20˚C, 12 months, shading light
	Black Clear-bottom Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Flow Cytometry, Incubator

Reagents & Consumables:

Phenol Red-Free Basal Medium, 0.22 μm filter

Reagent preparation

① Equilibrate all the reagents to 25˚C before use.

② The preparation of Probe Working Solution:

Prepare the required volume of Probe Working Solution immediately before use.

Example: To prepare 1,000 μL of a Probe Working Solution, add 10 μL of Probe Stock Solution to 990 μL of Phenol Red-Free Basal Medium and mix thoroughly. Filter through a 0.22 μm filter membrane, and then place it in a 37°C incubator for 5 minutes before use. The Probe Working Solution is stable for 24 h when stored at 2-8°C protected from light.

The key points of the assay

① Avoid repeated freeze-thaw cycles of the Probe Stock Solution; the Probe Stock Solution can be aliquoted and stored at -20°C.
② Use phenol red-free basal medium for detection.
③ When working with adherent cells, pipette gently to minimize cell loss.

Operating steps

Suspension cells:

① Collect the cells by centrifugation and discard the supernatant. Resuspend the cells in phenol red-free basal medium according to the experimental groups. The cell density should not be less than 2×10^5 cells/mL; for example, resuspend 2×10^5 cells in 1 mL of phenol red-free basal medium. Add 100 µL of the cell suspension to the corresponding EP tubes.
② Add 150 µL of the Probe Working Solution.Incubate at 37°C in a 5% CO_2 incubator for 1-4 hours (the optimal incubation time varies for different cell types. Use 60 minutes as the initial incubation time and optimize appropriately based on the specific cells used to achieve the best results). After incubation, transfer 200 µL of the sample to a well of a microplate for subsequent detection.
③ Flow cytometry detection: Acquire at least 1×10^4 cells per sample using the PE-Texas Red channel.

Adherent cells:

① Using a 96-well plate as an example, set up the cell culture plate according to the experimental requirements. It is recommended that the cell seeding density be no less than 2 × 10^4 cells/mL. After seeding, culture the cells overnight in a 37°C, 5% CO_2 incubator.

② Aspirate the culture medium and wash the cells twice with phenol red-free basal medium, then discard the supernatant.

③ Add 150 μL of the probe working solution and incubate. Incubate in a 37°C, 5% CO_2 incubator for 1-4 hours (the optimal incubation time varies for different cell types. Use 60 minutes as the initial incubation time and optimize appropriately based on the cells used to achieve the best results).

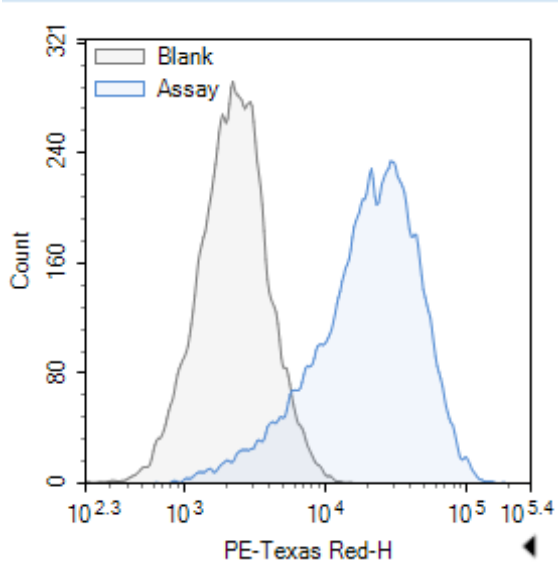
④ After incubation, decide whether to detach the cells based on their adherence: carefully aspirate the supernatant, add 0.05 mL of trypsin to each well, and digest for 5 minutes. Then, add 0.1 mL of phenol red-free basal medium to resuspend the cells.

⑤ Flow cytometry analysis: The sample volume should contain at least 1 × 10^4 cells. Use the PE-Texas Red channel for detection on the flow cytometer.

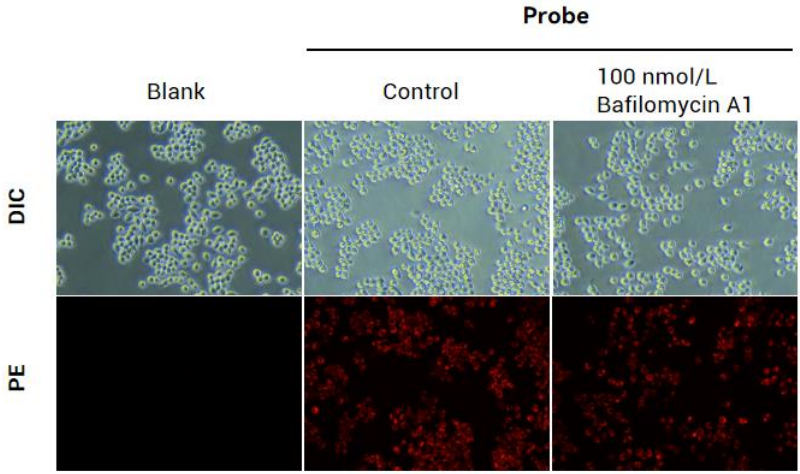
Fluorescence Microscopy: After staining, aspirate the medium, wash the cells twice with phenol-red-free basal medium, and discard the supernatant. Add fresh phenol-red-free basal medium and observe under a fluorescence microscope. Confocal Microscopy: Ex: 488 nm, Em: 580 – 630 nm. For conventional fluorescence microscopy, use a PE filter.

Appendix I Performance Characteristics

1. The RAW264.7 cell samples were incubated with the Probe Working Solution for 4 hours, and the results detected by flow cytometry are as follows:



2. The RAW264.7 cell samples were incubated with the Probe Working Solution for 4 hours, and the results detected by fluorescence microscope are as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.