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

Catalog No：E-BC-F086

Specification：96T

Measuring instrument：Flow Cytometry

Elabscience® Cellular Senescence 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：

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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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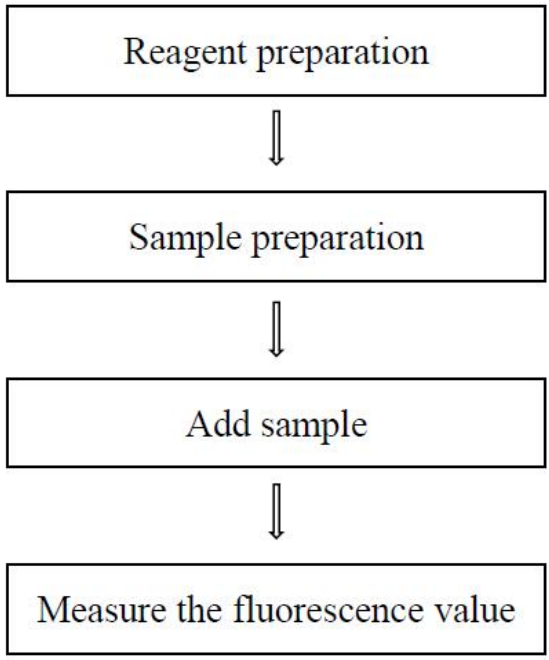
Assay summary



Intended use

This kit can be used to measure the SA-β-gal activity in the cellular senescence.

Detection principle

The senescence-associated β-galactosidase (SA-β-gal) is a key biomarker of cellular senescence. Studies have shown that the activity of this enzyme significantly increases in senescent cells and is related to abnormal lysosomal function. It is commonly used to detect the state of cellular senescence and study the mechanism of aging.

This kit can detect the activity of SA-β-gal in cells through a simple fluorescence method. The probe can enter the cell through the cell membrane, and SA-β-gal hydrolyzes the probe to emit fluorescence. The fluorescence intensity can be detected using a flow cytometer to determine the activity of SA-β-gal.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Bafilomycin A_1	0.05 mL × 1 vial	-20℃, 12 months, shading light
Reagent 2	Probe	0.05 mL × 1 vial	-20℃, 12 months, shading light

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 (FITC), 5% CO_2 Incubator

Reagents:

Complete medium,1 × PBS(0.01M PBS，pH 7.4)

Consumptive material:

0.22 μm filter membrane

Reagent preparation

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

② The preparation of theC working solution:
Before testing, please prepare sufficient bafilomycin A_1 working solution. For example, prepare 5810 μL of bafilomycin A_1 working solution (mix well 10 μL of bafilomycin A_1 and 5800 μL of complete medium). It is recommended to filter the bafilomycin A_1 working solution through a 0.22 μm filter membrane. The prepared solution should be used up within 8 h protected from light.

③ The dilution of the probe working solution:
Before testing, please prepare sufficient probe working solution. For example, prepare 20010 μL of probe working solution (mix well 10 μL of probe and 20000 μL of complete medium). It is recommended to filter the probe working solution through a 0.22 μm filter membrane. The prepared solution should be used up within 8 h protected from light.

The key points of the assay

① Cell experiments are conducted in a sterile environment.

② When handling adherent cells, the operation should be carried out slowly and gently to minimize cell loss.

③ It is recommended to conduct a preliminary experiment before the formal experiment to obtain the best experimental results.

Operating steps

Parameter setting of instrument	
Flow Cytometry	Ex/Em: 488/538 nm; FITC

Suspension cells: The cells can be cultured and treated according to the experimental needs and ensure that the cells are healthy and do not grow excessively. Collect cells and centrifuge at 500×g for 3 min at 4°C to remove supernatant. Resuspend the cells in complete medium. It is recommended that the cell density be 2×10^5/mL. For example, 2×10^5 cells are resuspend in 1 mL of complete culture medium. Set up negative control tubes and sample tubes, add 500 μL of cell suspension to each tube.

① Negative control tube/Sample tube: Add 20 μL of bafilomycin A_1 working solution to the tubes.

② Incubate at 37°C, 5% CO_2 incubator for 30-60 min (The incubation time can be adjusted according to the number and type of cells).

③ Negative control tube and sample tube: Centrifuge at 500 × g for 3 min to remove supernatant. Resuspend the cells with 500 μL of probe working solution.

④ Incubate at 37°C, 5% CO_2 incubator for 30-60 min (The incubation time can be adjusted according to the number and type of cells).

⑤ Negative control tube and sample tube: Centrifuge at 500×g for 3 min to remove supernatant. Wash once the cells with 500 μL of 1 × PBS(0.01M PBS，pH 7.4).

⑥ Negative control tube and sample tube: Centrifuge at 500×g for 3 min to remove supernatant. Resuspend the cells with 300 μL of 1 × PBS(0.01M PBS，pH 7.4) .

⑦ Flow cytometry detect: The number of cell samples should be at least

1×10^4 cells. The flow cytometer is set to detect using the FITC channel.

Adherent cells: Take a 24-well plate as an example. It is recommended that the cell density be 1 ×10^5/mL. After inoculating the cells, they should be incubated overnight in a 37°C, 5% CO_2 incubator.

① Negative control tube and sample tube: Add 20 μL of bafilomycin A_1 working solution to the wells.

② Incubate at 37°C, 5% CO_2 incubator for 30-60 min (The incubation time can be adjusted according to the number and type of cells).

③ Negative control tube and sample tube: Remove the medium carefully and add 500 μL of probe working solution slowly.

④ Incubate at 37°C, 5% CO_2 incubator for 30-60 min (The incubation time can be adjusted according to the number and type of cells).

⑤ Negative control tube and sample tube: Remove the medium carefully, wash once the cells with 500 μL of 1 × PBS(0.01M PBS，pH 7.4).

⑥ Remove 1 × PBS(0.01M PBS，pH 7.4) carefully, add the complete medium after digesting the cells. Centrifuge at 500 × g for 3 min to remove supernatant. Resuspend the cells with 300 μL of 1 × PBS(0.01M PBS，pH 7.4) .

⑦ Flow cytometry detect: The number of cell samples should be at least 1×10^4 cells. The flow cytometer is set to detect using the FITC channel.

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.