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

Catalog No：E-BC-F073

Specification：48T/96T

Measuring instrument：Fluorescence Microplate reader

(Ex:490 nm/440 nm;Em:535 nm)

Elabscience® Intracellular Acidification Rate 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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Intended use

This kit can be used to measure intracellular acidification rate of cell samples.

Detection principle

Intracellular acidification rate refers to the degree of change in the pH of the intracellular environment, which is an important parameter for the study of cell function. Constant intracellular pH level is an indispensable condition for maintaining normal cell metabolism and function. Most physiological and biochemical reactions in the cell, such as cell growth, calcium regulation, enzyme activity, receptor mediated signal transduction, ionic action, endocytosis, and cell adhesion, etc. Therefore, there is a close relationship between intracellular pH regulation and metabolism, and accurate detection of intracellular pH fluctuations is an important way to understand intracellular metabolism. At the same time, studying the interaction between intracellular pH regulation and metabolism will help to better understand the biochemical processes in cells and provide new ideas and methods for the treatment of related diseases.

The probe used in this test box has the characteristics of dual excitation wavelength. The probe has the maximum absorption at 490 nm and an equal absorption point at 440 nm. By calculating the ratio of the two excitation wavelengths, the fluctuation of intracellular pH can be detected.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Saline Solution	12 mL × 1 vial	24 mL × 1 vial	-20°C, 12 months
Reagent 2	Probe	Liquid × 1 vial	Liquid × 2 vials	-20°C, 12 months shading light
	Black 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:

Fluorescence microplate reader (Ex:490 nm&440 nm;Em:535 nm),

Incubator (37℃)

Reagents:

DMSO

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of high concentration probe stock solution:
Dilute one vial of probe with 108 μL of DMSO, mix well to dissolve.
Aliquoted storage at -20°C for 1 month.

③ The preparation of working solution:
Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 500 μL of working solution (mix well 495 μL of saline solution and 5 μL of high concentration probe stock solution). The working solution should be prepared on spot (the probe is unstable in saline solution, and the working solution is effective within 1 h after preparation, it is recommended to prepare the working solution after cell pretreatment).

Operating steps

The preparation of sample:

Suspension cells: Cell culture is performed according to the experimental design to ensure that the cells are healthy and do not overgrow. Centrifuge at 500×g for 5 min at 4℃ and discard the supernatant.

Adherent cells: Cell culture is performed according to the experimental design to ensure that the cells are healthy and do not overgrow. Remove the cell culture medium, digest the cells with trypsin, add serum-containing medium to stop digestion, use normal saline (0.9% NaCl) to wash and collect the cells, centrifuge at 500×g at 4℃ for 5 min, discard the supernatant.

① Resuspension cells with working solution, the recommended cell density is 2.5×10^6 /mL.

② Blank well: add 200 μL working solution to the wells.
Sample well: add 200 μL cell suspension to the wells.

③ Mix fully for 3 s with fluorescence microplate reader. Measure the fluorescence intensity at the excitation wavelength of 490 nm and the emission wavelength of 535 nm, as $F_{1(490)}$. Measure the fluorescence intensity at the excitation wavelength of 440 nm and the emission wavelength of 535 nm, as $F_{1(440)}$.

④ Incubate at 37℃ for 30 min protected from light, the incubation time can be adjusted between 10 and 60 min, and the incubation time is related to the cell type and probe concentration.

⑤ Mix fully for 3 s with fluorescence microplate reader. Measure the fluorescence intensity at the excitation wavelength of 490 nm and the emission wavelength of 535 nm, as $F_{2(490)}$. Measure the fluorescence intensity at the excitation wavelength of 440 nm and the emission wavelength of 535 nm, as $F_{2(440)}$.

Calculation

The sample:

$$\text{intracellular acidification rate (\%)} = \frac{\Delta F1_{(490)} / \Delta F1_{(440)} - \Delta F2_{(490)} / \Delta F2_{(440)}}{\Delta F1_{(490)} / \Delta F1_{(440)}} \times 100\%$$

[Note]

$\Delta F1_{(490)}$: The absolute fluorescence value at the excitation wavelength of 490 nm before incubation, $F1_{sample\ (490)} - F1_{black\ (490)}$.

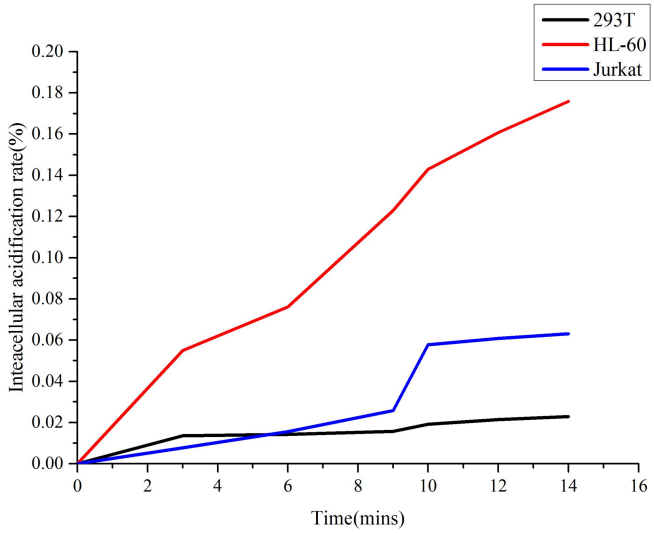
$\Delta F1_{(440)}$: The absolute fluorescence value at the excitation wavelength of 440 nm before incubation, $F1_{sample\ (440)} - F1_{black\ (440)}$.

$\Delta F2_{(490)}$: The absolute fluorescence value at the excitation wavelength of 490 nm after incubation, $F2_{sample\ (490)} – F2_{black\ (490)}$.

$\Delta F2_{(440)}$: The absolute fluorescence value at the excitation wavelength of 440 nm after incubation, $F1_{sample\ (440)} - F1_{black\ (440)}$.

Appendix I Performance Characteristics

1. Intracellular acidification rate of 293T, HL-60, Jurkat (same cell volume)



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.