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

Catalog No：E-BC-F100

Specification：96T

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=490 nm/525 nm)

Elabscience® Fluo-4 Calcium 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 calcium in alive cell samples.

Detection principle

As an important metal ion in biological organism, calcium ion not only participates in the transmission of intracellular signals, but also plays an important role in many life activities of cells. Therefore, the detection of calcium ion concentration is of great significance in experimental research.

This assay kit utilizes the cell-membrane-permeable calcium fluorescent probe Fluo-4 AM. The probe is non-fluorescent outside cells. Once inside cells, it is hydrolyzed by intracellular esterases to Fluo-4, which becomes trapped within the cytoplasm. Free Fluo-4 exhibits very weak fluorescence, but its fluorescence intensity increases significantly upon binding to Ca^{2+} . The probe shows maximum excitation at approximately 490 nm and maximum emission at 525 nm, and the fluorescence intensity is directly proportional to the intracellular calcium level.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Saline Solution	60 mL × 1 vial	-20℃, 12 months
Reagent 2	Solubility Enhancer	16 mL × 1 vial	-20℃, 12 months
Reagent 3	Probe	Liquid × 2 vials	-20℃, 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/Em= 490 nm/525 nm), Fluorescence Microscope, Flow Cytometry, Incubator

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of working solution：

Before testing, please prepare sufficient working solution according to the sample wells (solubility enhancer : probe = 99 : 1). The working solution should be prepared on spot. The amount of working solution can be referred to the table below for the detection of the fluorescence microplate reader (the concentration of working solution can be adjusted according to the experimental results in the actual experiment, and the volume of probe can be appropriately increased).

	1 well	10 wells	50 wells	100 wells
Solubility enhancer (μL)	99	990	4950	9900
Probe (μL)	1	10	50	100
Working solution（μL）	100	1000	5000	10000

The key points of the assay

① The sample should be living cells.

② The probe should be aliquoted storage and avoid repeated freeze/thaw cycles.

③ The probe is easy to quench in water, and should be used as soon as possible after preparing the working solution. Samples should be measured within 2 hours after incubation with working solution to prevent fluorescence weakening.

Operating steps

Instrument parameter Settings	
Fluorescence Microplate Reader	Ex/Em = 490 nm/525 nm
Flow Cytometry	Ex/Em = 490 nm/525 nm， The detection can be set with the parameters of FITC.

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 collect the cell precipitation.

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 for 2 min, add serum-containing medium to stop digestion, wash and collect the cells, centrifuge at 500×g at 4℃ for 5 min, discard the supernatant and collect the cell precipitation.

① Detection of fluorescence microplate reader: Resuspension cells with saline solution, the recommended cell density is 1×10^4 /μL, such as 1×10^6 cells, resuspension with 100 μL saline solution (cell density

can be adjusted according to cell type and cell activity).

Detection of Flow cytometry: Resuspension cells with working solution, the recommended cell density is 0.2×10^4 - 0.5×10^4 /μL, such as 1×10^6 cells, resuspension with 200-500 μL working solution (cell density can be adjusted according to cell type and cell activity).

② Detection of fluorescence microplate reader: add 50 μL cell suspension to the black microplate and then add 100 μL working solution. It is recommended to set the control well at the same time: add 50 μL cell suspension to the black microplate and then add 100 μL saline solution.

Detection of Flow cytometry: add 150 μL cell suspension to the black microplate.

③ Incubate at 37℃ for 30 min with shading light. (The incubation time is dependent on cell type and probe concentration, and the incubation time can be adjusted between 10-60 min).

④ Measure the fluorescence intensity at the excitation wavelength of 490 nm and the emission wavelength of 525 nm with fluorescence microplate reader. Measure the fluorescence intensity at the excitation wavelength of 490 nm and the emission wavelength of 525 nm with flow cytometry.

Fluorescence Microscope(only use for adherent cells):

① Cell culture: In the cell culture plate, lay the cell slide and add the cells. Cell culture is performed according to the experimental design to ensure that the cells are healthy and do not overgrow.

② Set up the control well (use saline solution instead of working solution).

③ Remove the cell culture medium, and wash the cells once with the saline solution (Note: do not blow directly on the cells to avoid the cells falling off).

④ Add the appropriate volume (to adequately cover the cells) of the working solution, usually not less than 1 mL for one well of the six-well plate.

⑤ Incubate at 37℃ for 30 min with shading light. (The incubation time is dependent on cell type and probe concentration, and the incubation time can be adjusted between 10-60 min).

⑥ Remove the working solution and wash the cells 2-3 times with the saline solution to fully remove the probes which did not enter the cells.

⑦ Taking out the cell slides and put it on a glass slide for direct detection under a fluorescence microscope.

⑧ Detection wavelength setting: Set the excitation wavelength of 490 nm, set the exposure time according to the fluorescence intensity, and measure all samples under the same parameter.

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.