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

Catalog No：E-BC-F108

Specification：48T/96T

Measuring instrument：Fluorescence Microplate reader, Fluorescence Microscope, Flow Cytometry

Elabscience® Cell Ferrous (Fe^{2+}) Fluorometric Assay Kit

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

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
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Email: techsupport@elabscience.com
Website: www.elabscience.com

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

The kit is suitable for detecting ferrous (Fe^{2+}) level in alive cell sample.

Detection principle

Studies have confirmed that iron is the most abundant transition metal in living organisms, playing a crucial role in numerous physiological processes. In recent years, cellular free iron has attracted increasing attention due to its high reactivity and its association with cell damage and death. Free iron exist in the cell as stable Fe^{2+} and Fe^{3+}. Considering the reducing environment, metal transporter and water solubility of Fe^{2+}, the behavior of Fe^{2+} is more important than that of Fe^{3+}.

This kit provides a fluorescent probe that can specifically bind to Fe^{2+}, and the probe can enter the cell interior well, which is suitable for the detection of Fe^{2+} in alive cells. When the probe reacts with Fe^{2+}, an irreversible orange (red) fluorescent product is generated (excitation wavelength: 530 nm emission wavelength: 580 nm).

Kit components & storage

Item	Component	Size 1 (48 T)	Size 2(96 T)	Storage
Reagent 1	2 mmol/L Probe	0.075 mL ×1 vial	0.15 mL ×1 vial	-20°C, 12 months, shading light
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before

use to prevent loss.

Instruments

Fluorescence microplate reader (Ex/Em=530 nm/580 nm), Fluorescence microscopy and Flow cytometry (PE Channel)

Materials required but not provided

PBS (0.01 M，pH = 7.4) or phenol red-free basal medium

Reagent preparation

① Equilibrate all the reagents to 25°C before use. Aliquot 2 mmol/L Probe stored at -20°C, and avoid repeated freeze/thaw cycles is advised.

② The preparation of Probe Working Solution：

Dilute the 2 mmol/L Probe with PBS (0.01 M, pH = 7.4). Recommended preparation concentration: 20–100 μmol/L(final concentration in the system: 1–5 μmol/L). The Probe Working Solution should be freshly prepared before use. Keep it protected from light and use within 8 h.

The key points of the assay

① PBS (0.01 M, pH 7.4) may be substituted with phenol red-free basal medium.

② Avoid repeated freeze-thaw cycles of Probe. Before use, ensure it is fully dissolved, and centrifuge it until all liquid is collected at the bottom of the tube before opening. The Probe Working Solution is recommended to be prepared and used immediately.

③ After staining, it is recommended to perform detection or observation within 2 h.

④ If the signal is weak when detected by fluorescence microscope or fluorescence microplate reader, the incubation time of the Probe can be appropriately extended before detection.

⑤ The flow cytometer has high detection sensitivity. The optimal final concentration of the Probe is recommended to be screened and determined at around 1 μmol/L.

⑥ No washing is required after Probe incubation, directly perform detection or observation. Washing will lead to a decrease in the fluorescence signal.

Operating steps

Detection of culture cell sample

Instrument parameter	
Fluorescence microplate reader	Ex/Em = 530 nm/580 nm
Flow cytometry	PE
Fluorescence microscope	PE

1. Suspension cells:

After treating the cells according to the experimental design, wash them with PBS (0.01 M, pH = 7.4), then resuspend the cells in the same PBS. A recommended cell density is 2 × 10^5 cells/mL; for example, resuspend 2 × 10^5 cells in 1 mL of PBS. Set up blank tubes, control tubes, and experimental tubes, and add 200 μL of the corresponding cell suspension to each tube.

① Blank tubes: Add 10 μL of PBS.

Control tubes: Add 10 μL of Probe Working Solution.

Experimental tubes: Add 10 μL of Probe Working Solution.

② Incubate the tubes in a 37°C incubator for 10–60 min (the optimal incubation time varies for different cell types. An initial incubation duration of 10 min is recommended, with subsequent optimization of the incubation time tailored to the cell type used for the best results). After incubation, perform detection using a flow cytometer (PE channel) or a fluorescence microplate reader (Ex/Em=530 nm/580 nm).

2. Adherent cells:

Taking a 24-well plate as an example, seed the cells according to the experimental requirements to ensure that the cells are healthy and not overgrown. After treating the cells according to the experimental design,

wash them with PBS, then add 500 μL of PBS to each well. Set up blank wells, control wells, and experimental wells.

① Blank wells: Add 25 μL of PBS.

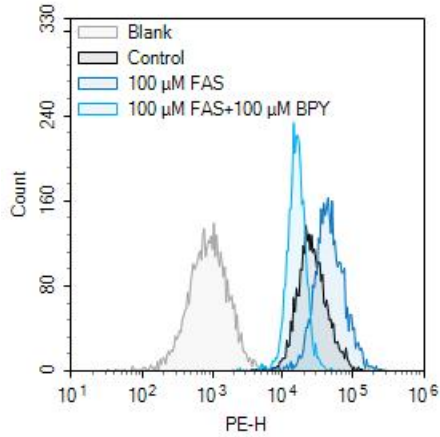
Control wells: Add 25 μL of probe working solution.

Experimental wells: Add 25 μL of probe working solution.

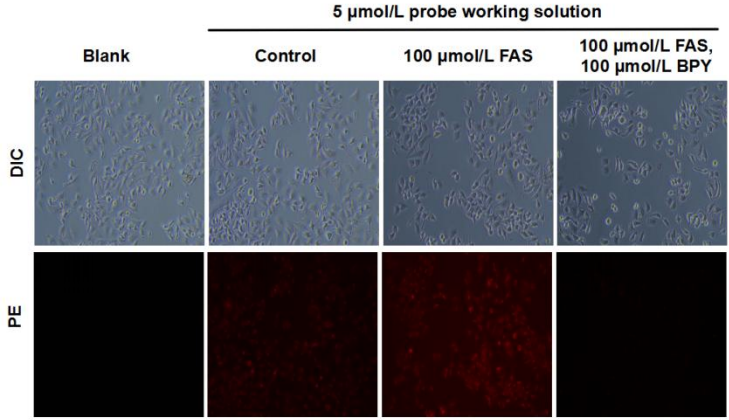
② Incubate the plate in a 37 °C incubator in the dark for 10–60 min (the optimal incubation time varies for different cell types. An initial incubation duration of 10 min is recommended, with subsequent optimization of the incubation time tailored to the cell type used for the best results). After incubation, observe the cells under a fluorescence microscope. Alternatively, digest and collect the cells before performing detection using a flow cytometer (PE channel) or a fluorescence microplate reader (Ex/Em=530 nm/580 nm).

Appendix I Performance Characteristics

① Flow cytometry results of jurkat cells



② Fluorescence microscopy results of Hela cells



In the control group, weak red fluorescence was observed in normal cells after probe addition, indicating the presence of endogenous ferrous ions (Fe^{2+}) in the cells. After treatment with 100 μmol/L ammonium ferrous sulfate (FAS) for 30 min, the intracellular ferrous ion content increased, and the red fluorescence was significantly enhanced after probe staining.

Furthermore, after additional treatment with 100 μmol/L 2,2'-bipyridine (BPY) for 30 min, BPY bound to ferrous ions and prevented their reaction with the probe, resulting in a significant decrease in red fluorescence. Actual results can vary depending on the experimental setup and equipment used. The figure illustrates a typical outcome for reference purposes.

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.