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

Catalog No：E-BC-F101

Specification：96T

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

Elabscience® Cell Ferrous Iron (Fe^{2+})

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
Tel: 1-832-243-6086
Fax: 1-832-243-6017
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

This kit can be used to measure ferrous irons (Fe^{2+}) content in alive cell sample.

Detection principle

Studies have confirmed that iron is the most abundant transition metal element in living organisms, and it is involved in a variety of physiological activities. In recent years, free iron in cells have attracted more attention due to their high reactivity and their correlation 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: 542 nm emission wavelength: 575 nm).

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer	50 mL ×2 vials	-20 ℃, 12 months, shading light
Reagent 2	2 mmol/L Probe	0.15 mL ×1 vial	-20 ℃, 12 months, shading light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

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=542 nm/575 nm), Fluorescence microscope (Ex/Em=542 nm/575 nm), Flow cytometry(Ex/Em=542 nm/570-620 nm)

Reagent preparation

① Equilibrate all the reagents to room temperature before use. Aliquot 2 mmol/L probe stored at -20℃, and avoid repeated freeze/thaw cycles is advised.

② The preparation of buffer working solution：
Mix the buffer, double distilled water at a ratio of 1:9 fully. Buffer working solution can be replaced by serum-free medium.

③ The preparation of probe working solution：
Dilute the 2 mmol/L probe with buffer working solution, the recommended working concentration is 2-10 μmol/L. The probe working solution should be prepared on spot. Keep it protected from light and use within 2 hours.

The key points of the assay

① If use buffer working solution washing and incubation cell, prepare sufficient amount of the buffer working solution before the experiment.

② The 2 mmol/L probe avoid repeated freezing and thawing. Before use, it is necessary to fully melt, centrifuge until the liquid reaches the bottom of the tube and then open the cap. The probe working solution should be prepared on spot.

③ The fluorescent product is easy to quench, and it is best to measure within 2 h after incubation to prevent fluorescence weakening.

Operating steps

Detection of culture cell sample

Instrument parameter	
Fluorescence microplate reader	Ex/Em = 542 nm/575 nm
Flow cytometry	Ex/Em = 542 nm/575 nm (with PE channel)
Fluorescence microscope	Ex/Em = 542 nm/570-620 nm

1. Suspension cells:

① Centrifuge the sample at 300×g for 5~10 min and wash with buffer working solution for 2~3 times. Centrifuge and collect the cell precipitation for fluorescence detection.

② Add 1 mL of probe working solution to 10^6 cells. Incubate at 37°C for 30~60 min protected from light.

③ Centrifuge at 300×g for 5 minutes to remove supernatant.

④ Wash with buffer working solution for 2~3 times.

⑤ Re-suspend collected cells with 0.2-0.5 mL of buffer working solution for detection by fluorescence microplate reader or flow cytometry.

2. Adherent cells:

① Culture the adherent cells on a sterile cover glass.

② Remove the cover glass from the medium and absorb excess medium.

③ Add 1 mL of probe working solution to 10^5 cells and shake gently it to completely cover the cells. Incubate at 37℃ for 30~60 min protected from light.

④ Remove the probe working solution, and wash the cells with buffer working solution for 2~3 times. Observe the cells by fluorescence microscope.

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.