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CheKine™ Pro Cell Ferrous Ion (Fe^{2+}) Fluorometric Assay Kit

Cat #: KTB9060 Size: 200 T/500 T/2500 T

	Cell Ferrous Ion (Fe^{2+}) Fluorometric Assay Kit		
REF	**Cat #:** KTB9060	LOT	**Lot #:** Refer to product label
	Applicable samples: Fluorescence assays and flow cytometry on live cells		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

There is virtually no truly free, hydrated Fe^{2+} inside cells, the vast majority of iron is firmly bound to proteins (such as ferritin, heme, and iron-sulfur clusters). When we refer to intracellular Fe^{2+} concentration, we are typically referring to that small fraction of Fe^{2+} in the Labile Iron Pool (LIP) that is accessible to chelating agents and is redox-active. Precise regulation of intracellular iron homeostasis is critical for cell survival, and an imbalance in iron concentration can lead to the development of various diseases. When iron concentration is too high, ferrous ions can catalyze the production of hydroxyl radicals via the Fenton reaction, which in turn triggers lipid peroxidation, protein denaturation, and DNA damage, all of which are closely associated with major diseases such as cancer and Alzheimer's disease. Conversely, when iron concentration is too low, heme synthesis is impeded, leading to iron-deficiency anemia and resulting in clinical symptoms such as tissue hypoxia and impaired immunity.

This kit utilizes the red fluorescent probe RhoNox-4 to rapidly and sensitively detect Fe^{2+} in live cells. RhoNox-4 exhibits high selectivity and sensitivity, binding specifically to ferrous ions without interference from common metal ions such as calcium, magnesium, zinc, and copper. This probe also features excellent cell membrane permeability, low cytotoxicity, and stability, making it suitable for real-time fluorescent imaging of ferrous ions in live cells, as well as for long-term monitoring and high-throughput assays. Upon binding to ferrous ions, its fluorescent signal is significantly enhanced, enabling precise detection of free ferrous ions within live cells. RhoNox-4 is suitable for research on cellular iron metabolism, oxidative stress, and ferroptosis. After binding to ferrous ions within cells, RhoNox-4 has a maximum excitation wavelength of 545 nm and an emission wavelength of 580 nm.

Materials Supplied and Storage Conditions

Kit components	Size			Storage conditions
	200 T	500 T	2500T	
Assay Buffer	20 mL	50 mL	100 mL×2+50 mL	4℃
RhoNox-4 (1000X)	20 μL	50 μL	250 μL	-20℃, protected from light
Hoechst 33342 (1000X)	20 μL	50 μL	250 μL	-20℃, protected from light

Note: Based on the 200T package size, 100 μL of RhoNox-4 Staining Solution per sample for fluorescence microscopy staining yields 200 tests; 500 μL of RhoNox-4 Staining Solution per sample for flow cytometry yields 40 tests; For microplate reader assays, 100 μL of RhoNox-4 Staining Solution per sample allows for 200 tests.

Materials Required but Not Supplied

- Fluorescence microscope (Ex/Em=545 nm/580 nm), flow cytometer (PE), microplate reader (Ex/Em=545 nm/580 nm)
- 96-well/24-well/12-well/6-well cell plates
- Centrifuges, adjustable pipettes, and pipette tips
- Deionized water, PBS

Reagent Preparation

Assay Buffer: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

RhoNox-4 (1000X): Ready to use as supplied; Equilibrate to room temperature before use; Store at -20℃, protected from light.

Hoechst 33342 (1000X): Ready to use as supplied; Equilibrate to room temperature before use; Store at -20℃, protected from light.

RhoNox-4 Staining Solution: Add 1 μL of RhoNox-4 (1000×) and 1 μL of Hoechst 33342 (1000×) to each 1 mL of Assay Buffer, mix well. (Hoechst 33342 staining is optional and may be performed as needed. Hoechst 33342 emits blue fluorescence with a maximum excitation wavelength of 346 nm and a maximum emission wavelength of 460 nm)

Assay Procedure

1. Fluorescence Microscopy Analysis：

1.1 For adherent cells：

(1) Culture or induce the cells under the appropriate conditions, then proceed with subsequent experiments.

(2) After aspirating the culture medium, wash the cells twice with PBS.

(3) Add an appropriate volume of RhoNox-4 Staining Solution to the cells. The recommended volumes are 100 μL per well for a 96-well plate, 300 μL per well for a 24-well plate, 500 μL per well for a 12-well plate, and 1 mL per well for a 6-well plate. Incubate at 37℃ in the dark for 0.5-2 h.

(4) After incubation, proceed directly to fluorescence microscopy analysis (Ex/Em = 545 nm/580 nm).

Note: Incubation times and the working concentration of RhoNox-4 should be adjusted appropriately for different cell types and states; these can be adjusted as needed within the range of 0.5× to 2.5× based on actual conditions.

1.2 For suspension cells:

(1) Culture or induce the cells under the appropriate conditions, then proceed with subsequent experiments.

(2) Centrifuge at 300 g for 5 minutes to collect the cells (5×10^5-1×10^6), then wash twice with PBS.

(3) Add 100 μL of RhoNox-4 Staining Solution to the cells and incubate at 37℃ in the dark for 0.5-2 h.

(4) Suspend the stained cells on a glass slide, cover the cells with a coverslip, and observe under a fluorescence microscope (Ex/Em = 545 nm/580 nm).

Note: Washing after staining may cause the signal to fade; therefore, it is recommended to proceed directly to detection without washing after incubation to ensure the accuracy of the results.

2. Flow Cytometry Analysis:

(1) Culture or induce the cells under the appropriate conditions, then proceed with subsequent experiments.

(2) For suspension cells, centrifuge at 300 g for 5 minutes to collect the cells, wash twice with PBS, and discard the PBS. For adherent cells, first digest the cells with trypsin (without EDTA), then centrifuge at 300 g for 5 minutes to collect the cells, wash twice with PBS, and discard the PBS.

(3) Add 500 μL of RhoNox-4 Staining Solution to the cells and incubate at 37℃ in the dark for 0.5-2 h.

(4) After incubation, proceed directly to flow cytometry analysis (PE channel).

Note: Incubation times and the working concentration of RhoNox-4 should be adjusted appropriately for different cell types and states; these can be adjusted as needed within the range of 0.5× to 2.5× based on actual conditions.

3. Fluorescent Microplate Reader Assay:

3.1 For adherent cells：

(1) It is recommended to seed cells in a 96-well, fully black cell culture plate, culture or induce the cells under the appropriate conditions, and then proceed with subsequent experiments.

(2) After aspirating the culture medium, wash the cells twice with PBS.

(3) Add 100 µL of RhoNox-4 Staining Solution to the cells and incubate at 37℃ in the dark for 0.5-2 h.

(4) After incubation, proceed directly to detection using a fluorescence microplate reader (Ex/Em = 545 nm/580 nm, top-reading).

Note: If using a 96-well black-bottomed cell culture plate, set the microplate reader to bottom-reading mode.

3.2 For suspension cells:

(1) Culture or induce the cells under the appropriate conditions, then proceed with subsequent experiments.

(2) Centrifuge at 300 g to collect the cells (5×10^5-1×10^6) and wash them twice with PBS.

(3) Add 100 µL of RhoNox-4 Staining Solution to the cells and incubate at 37℃ in the dark for 0.5-2 h.

(4) After incubation, proceed directly to detection using a fluorescence microplate reader (Ex/Em = 545 nm/580 nm).

4. Treatment of Acute Iron Overload Cell Model

To induce changes in intracellular ferrous ion concentrations, transferrin, ferroptosis inducers such as Erastin, and ferrous sulfate can be used. Ferrous sulfate can be used to rapidly regulate changes in intracellular ferrous ion concentrations. The specific procedure is as follows:

(1) Reagent Preparation: Prepare a 20 mM ferrous sulfate solution using deionized water, when ready for use, dilute it with serum-free medium to obtain a 100-200 µM ferrous sulfate treatment solution.

(2) Cell Treatment: Treat the cells to be analyzed with a ferrous sulfate solution and incubate them in a cell culture incubator at 37℃ for 2 hours. The incubation time may be adjusted appropriately for different cell types and conditions. After incubation, wash the cells twice with PBS, then stain them using RhoNox-4 Staining Solution.

Recommended Products

Catalog No.	Product Name
KTB1114	CheKine™ Mirco Cell Total Iron Content Assay Kit
KTB1116	CheKine™ Mirco Cell Ferrous Ion Content Assay Kit
KTB1050	CheKine™ Micro Lipid Peroxidation (MDA) Assay kit
KTB9050	CheKine™ Pro Malondialdehyde (MDA) Fluorometric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.