PRODUCT INFORMATION

FerroOrange

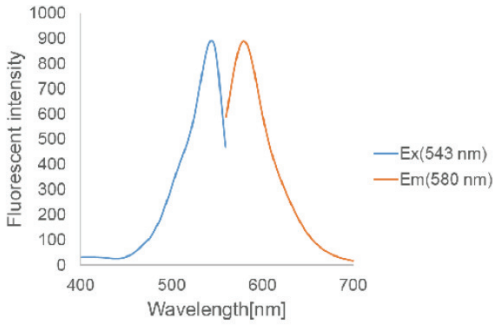
Item No. 41725

Purity: ≥90%
Ex./Em.: 540/585 nm when bound to Fe^{2+}
Supplied as: A solid
Storage: 4°C
Stability: ≥4 years
Special Conditions: Light sensitive

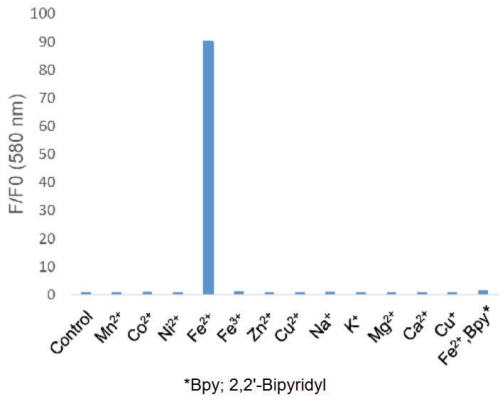
Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.

Description

FerroOrange is a cell-permeable fluorescent probe that binds irreversibly to ferrous iron (Fe^{2+}).[1] It is selective for Fe^{2+} over 12 other metal ions. When bound to Fe^{2+}, FerroOrange displays excitation/emission maxima of 540/585 nm, respectively. Monitoring iron levels in cells has high relevance to various cellular processes, especially ferroptosis, which is an iron-dependent form of cell death.[2]



Excitation and emission spectra of FerroOrange



*Bpy; 2,2'-Bipyridyl

Metal ion selectivity of FerroOrange

General Protocol

NOTE: This protocol is provided as a general method. Please optimize the staining conditions, such as the final concentration of FerroOrange and the exposure time of the stimulating agent(s).

1. Prepare solutions
   a. Prepare a 1 mM stock solution of FerroOrange in DMSO. Dissolve 24 µg of FerroOrange in 35 µl of DMSO and mix with pipetting.

      NOTE 1: DMSO can be substituted with dimethyl formamide (DMF) or ethanol. This stock solution will be stable for one month when stored at -20°C and protected from light.

      NOTE 2: The FerroOrange stock solution is a colorless solution. If any coloration is observed, it may be due to contamination. Do not use the colored solution.

   b. Immediately before labeling cells, dilute the 1 mM FerroOrange stock solution with HBSS to prepare a 1 µM FerroOrange working solution.

WARNING
THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

SAFETY DATA
This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

WARRANTY AND LIMITATION OF REMEDY
Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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NOTE 1: This working solution is unstable and should be used immediately.

NOTE 2: HBSS can be substituted with serum-free media. Serum-containing media cannot be used because it increases background.

2. Prepare, label, and image the cells

a. Prepare cells for the assay and incubate at 37°C with 5% CO_2.

NOTE: Fluorescent imaging-compatible cell culture plates should be used.

b. Discard the cell culture medium and wash the cells three times with HBSS or serum-free medium.

c. Add medium containing stimulating agents and incubate cells at 37°C with 5% CO_2.

NOTE: Optimize the incubation time according to stimulating conditions.

d. Discard the medium and wash the cells three times with HBSS or serum-free medium.

e. Add the 1 µM FerroOrange working solution and incubate for 30 minutes at 37°C with 5% CO_2.

f. Observe the cells using fluorescence microscopy with excitation and emission wavelengths of 540 and 585 nm, respectively.

Experimental Examples

Detection of intracellular Fe^{2+} in HeLa cells using FerroOrange.

1. HeLa cells (2.0×10^4 cells/well) were seeded on a µ-slide 8 well (ibidi) and cultured overnight in a 37°C incubator equilibrated with 95% air and 5% CO_2.

2. The cells were washed with serum-free medium (200 µl) three times. Then, serum-free medium (200 µl) was added to the cells.

3. Ammonium iron (II) sulfate (10 mmol/L) was prepared with purified water.

4. Ammonium iron (II) sulfate (2 µl) was added to wells (The final concentration:100 µmol/l). To mix Ammonium iron (II) sulfate and serum-free medium, the entire medium was pipetted up from wells and then immediately pipetted back one time.

   NOTE 1: Please do not disturb the cells during pipetting.

   NOTE 2: When adding 10 mmol/L Ammonium iron (II) sulfate to well, please exactly follow step 4 as described. Do not add pre-prepared 100 µmol/L Ammonium iron (II) sulfate to cells. It may result in precipitation of Ammonium iron (II) sulfate during the experiment due to a vortex or a pipetting.

5. The cells were incubated for 30 minutes in a 37°C incubator equilibrated with 95% air and 5% CO_2, and the cells were washed with HBSS (200 µl) three times.

6. FerroOrange (1 µmol/l) and 2,2'-bipyridyl (Bpy) (100 µmol/l) were added to the cells as HBSS solution (200 µl), and then cells were incubated for 30 min in a 37°C incubator equilibrated with 95% air and 5% CO_2.

7. The cells were observed under a confocal fluorescence microscope.

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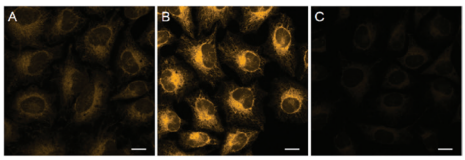

Detection of intracellular Fe^{2+} in HeLa cells using FerroOrange.

A. Control
B. Ammonium iron(II) sulfate treated
C. Ammonium iron(II) sulfate and 2,2'-Bipyridyl (Bpy) treated

Ex/Em = 561 nm/ 570-620 nm
Scale bars = 20 µm

The fluorescence intensity of FerroOrange was increased in HeLa cells treated with Ammonium iron(II) sulfate compared with the findings in untreated cells; conversely, its fluorescence intensity was decreased in cells treated with Bpy. Therefore, FerroOrange reacted with intracellular Fe^{2+}.

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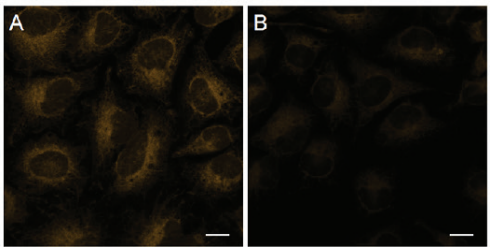
1. HeLa cells (2.0×10^4 cells/well) were seeded on a µ-slide 8 well (ibidi) and cultured overnight in a 37°C incubator equilibrated with 95% air and 5% CO_2.

2. The cells were washed with HBSS (200 µl) three times.

3. FerroOrange (1 µmol/l) and Bpy (100 µmol/L) were added to the cells as HBSS solution (200 µl), and the cells were incubated for 30 minutes in a 37°C incubator equilibrated with 95% air and 5% CO_2.

4. The cells were observed under a confocal fluorescence microscope.



Detection of intracellular Fe^{2+} in HeLa cells using FerroOrange.

A. Control
B. 2,2'-Bipyridyl (Bpy) treated

Ex/Em = 561 nm/ 570-620 nm
Scale bars = 20 µm

The fluorescence intensity of FerroOrange was decreased in HeLa cells treated with Bpy compared with that in untreated cells. Therefore, FerroOrange reacted with intracellular Fe^{2+}.

References

1. Grubwieser, P., Brigo, N., Seifert, M., *et al.* Quantification of macrophage cellular ferrous Iron (Fe^{2+}) content using a highly specific fluorescent probe in a plate reader. *Bio. Protoc.* **14(3)**, e4929 (2024).
2. Angeli, J.P.F., Miyamoto, S., and Schulze, A. Ferroptosis: The greasy side of cell death. *Chem. Res. Toxicol.* **32**, 362-369 (2019).

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