Store at 4°C

FerroOrange

#36104

24 µg

Cell Signaling TECHNOLOGY®

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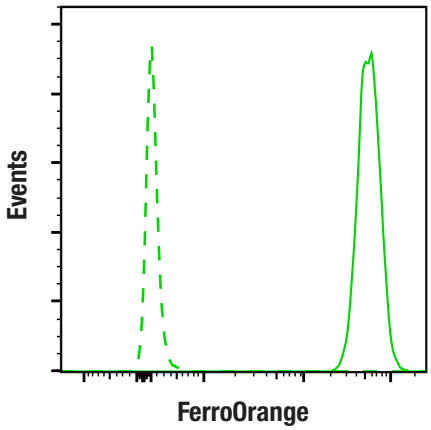
For Research Use Only. Not for Use in Diagnostic Procedures.

Description: FerroOrange is a small molecular iron sensing dye containing a fluorescent probe that selectively binds iron ions, allowing for detection of intracellular iron via live-cell fluorescent imaging. When the iron ions are bound to FerroOrange, fluorescence increases, resulting in the detection and measurement of intracellular iron. It has been shown that free iron in living cells (mostly Fe^{2+}) may be related to cellular damage or death. Iron exists in ferrous and ferric states, and FerroOrange uniquely detects the labile ferrous form, which drives ferroptosis. Ferroptosis is a distinct type of cell death defined by iron-dependent lipid peroxidation. FerroOrange aids in ferroptosis research and detection.

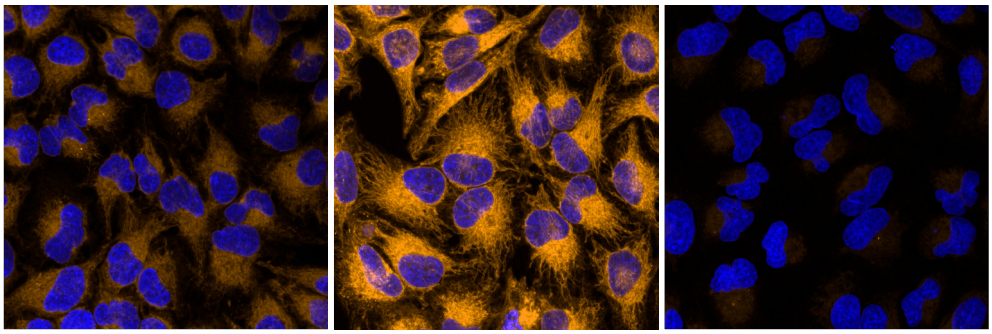
Fluorescent Properties:

Excitation maximum = 543 nm

Emission maximum = 580 nm



Flow cytometric analysis of live RAW 264.7 cells, stained with FerroOrange (solid line) or unstained (dashed line).



Confocal analysis of HeLa cells, untreated (left), treated with ammonium iron(II) sulfate (100 µM, 30 min; middle), or inhibited with 2,2'-bipyridine (100 µM) after treating with ammonium iron(II) sulfate (100 µM, 30 min; right) using FerroOrange (orange) and Hoechst 33342 #4082 (blue).

Storage: Store at 4°C desiccated and protected from light and metal. In lyophilized form, the product is stable for 12 months. Once reconstituted at a concentration of 1 mmol/L, store at -20ºC protected from light and metal, and use within 1 month.

Directions for Use:

Reagent Preparation: Material appears as a clear to whitish solid/film that is difficult to see in the tube and can consolidate within the cap. To ensure material has been contained within the vial, centrifuge the tube of lyophilized FerroOrange once it has been brought to room temperature and prior to reconstitution. To create a 1 mmol/L FerroOrange solution, combine 35 µL of DMSO with 24 µg of FerroOrange and pipette repeatedly to dissolve. To prepare the 1 µmol/L solution of FerroOrange, dilute the 1 mmol/L FerroOrange 1:1000 in HBSS. *Protect from light.*

IMPORTANT: DMF or ethanol can be used as an alternative to DMSO. Serum-free medium can be used as an alternative to HBSS. Serum-containing medium cannot be used as it will generate high background. The 1 mmol/L solution is stable for 1 month at -20°C protected from light. The 1 µmol/L solution is unstable and should be prepared and used immediately before staining the experiment.

NOTE: If working with suspension cells, it is necessary to pellet the cells by gentle centrifugation before all wash and aspiration steps.

Protocol:

1. Culture cells as appropriate, and perform treatments as needed.
2. Aspirate the supernatant.
3. Wash the cells three times with HBSS or serum-free media.
4. Add the 1 µmol/L FerroOrange working solution to the cells and incubate for 30 min.
5. Examine cells by fluorescence microscopy. Refer to the Fluorescent Properties section for excitation and emission spectra.
6. For analysis by flow cytometry, dissociate adherent cells or collect suspension cells. Analyze on a flow cytometer at desired cell density.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **FC-FP**— Flow cytometry-Fixed/Permeabilized **FC-L**— Flow cytometry-Live **E-P**—ELISA-Peptide
Species Cross-Reactivity: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse
All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.