BioTracker™ FerroOrange Live Cell Dye

Live Cell Dye

Cat. # SCT210-35nmol

pack size: 35 nmol

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Store at -20ºC

Data Sheet

Background

Iron, the most abundant transition metal in our bodies, is involved in several biologically important processes such as respiration, oxygen transport, and energy production in collaboration with oxygen. In living cells, iron exists mainly as ferrous (Fe2+) and ferric (Fe3+) ionic forms. As a major contributor to oxidative damage of cells, Fe2+ is implicated in serious diseases such as cancers and neurodegenerative disorders, because of its ability to produce harmful reactive oxygen species via contact with oxygen, superoxide, and hydrogen peroxide (H_2O_2).

BioTracker™ FerroOrange Live Cell Dye is an orange fluorescent probe that specifically detects labile iron (II) ions (Fe2+) only. The intensity of fluorescence does not increase in the presence of iron (III) ions (Fe3+) or bivalent metal ions other than iron. It also does not react to chelated iron in ferritin and other substances. FerroOrange is suitable for live-cell imaging because it is highly cell-permeable and has low cell toxicity.

Storage

Store BioTracker™ FerroOrange Live Cell Dye at -20°C, desiccate and protect from light

Note: Centrifuge vial briefly to collect contents at bottom of vial before opening.

Spectral Properties

Absorbance maximum: 542 nm
Emission maximum: 572 nm

Quality Control

Purity: ≥ 95% confirmed by LC.

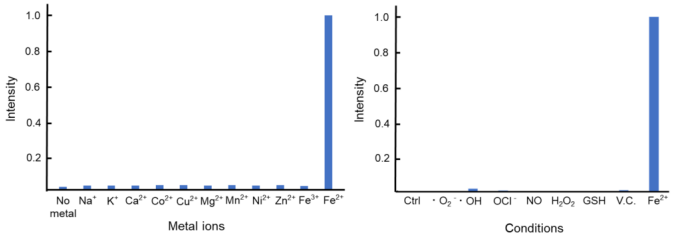

Figure 1: Specificity. Reactivity of BioTracker FerroOrange live cell dye to various metal ions, reactive oxygen species, or reducing agents. The dye shows strong fluorescence increases only upon reaction with Fe^{2+}. Relative fluorescent intensities compared to that when reacted with Fe^{2+} are shown.

Protocol

Materials required but not provided

1. Dimethyl Sulfoxide (DMSO)
2. Appropriate washing and observation buffer (PBS pH 7.4, HBSS, etc.). It should be a solution without phenol red

Reagent Preparation

1. Before opening the vial, warm the vial to room temperature. Then spin down the solid to the bottom by a microcentrifuge.
2. Add 35 µl of DMSO to 1 vial (35 nmol) to prepare 1 mM concentration. Finally, dissolve the solid entirely by pipetting for more than 5 times. The dye solution is almost colorless (faint blue).
3. Use neutral buffer or culture medium for dilution of the DMSO solution and use immediately upon dilution. The dye could be oxidized in acidic solutions.

Example of Cell Staining

Observation of labile iron (II) ions (Fe2+) in HepG2 cells

1. Seed HepG2 cells in a glass bottom dish and culture overnight.
2. Remove the culture medium from the dish and rinse cells gently twice with washing buffer to remove extracellular Fe2+.
3. Dilute 1mM stock solution of FerroOrange in HBSS to prepare a staining solution with a final concentration of 1µM.
4. Add the staining solution to the culture vessel and incubate at 37°C for 30 minutes.
5. After the staining, rinse the stained cells twice with washing buffer, replace it with observation buffer.
6. Observe the cells with a fluorescent microscope

Note: You can detect the increase of labile Fe2+ ions as a positive control, if you added Fe2+ in HBSS or serum-free medium. For this purpose, dissolve $Fe(NH_4)_2(SO_4)_2$ (FAS) in pure water to prepare a 100 mM solution just before use and dilute it with serum-free cell culture medium to a final concentration of 100 µM with HBSS. After cells were cultured in the FAS solution for 30 minutes, wash the cells to remove extracellular FAS and add the FerroOrange solution to detect intracellular Fe2+. After incubating the cells in this solution for 30 minutes, intracellular Fe2+ concentration rises. Wash extracellular Fe2+ with washing buffer and add the dye solution.

Note: Do not use the solutions with serum. Because FerroOrange reacts with Fe2+ in serum before it reacts with Fe2+ in the cells, the intracellular Fe2+ cannot be detected correctly.

Note: We recommend optimizing dye concentrations and incubation time in your conditions. In our experience, incubation in 1 µM dye at 37oC for 30 minutes gave good results for HeLa cells. If cells tend to come off from the dish easily, usage of poly-L-lysine or other coating materials before seeding the cells is recommended.

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Fluorescence observation

The fluorescence can be observed in a fluorescence microscope with a general G excitation filter such as for Cy3. 532 nm, 514 nm or 561 nm lasers are often used for excitation in laser microscopes and flow cytometers. Excitation with 488 nm laser is also capable. The fluorescence emission is 572 nm. For analysis by flow cytometer, a filter used for phycoerythrin (PE) is appropriate.

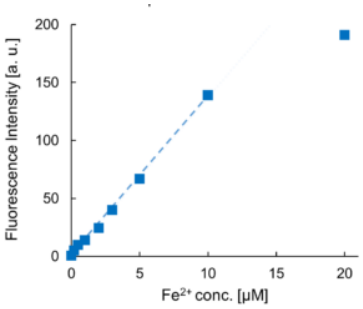

Figure 2. Reactivity of 2 μM of FerroOrange to various concentrations of $Fe(SO_4)_2(NH_4)_2$. It shows linear fluorescence increase to about 5 times concentrations of Fe^{2+}.

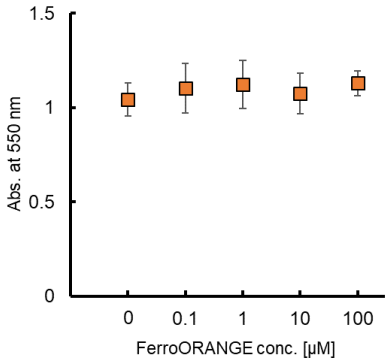

Figure 3: Cytotoxicity was not detected in 100 times concentration of usual use (100 μM). Metabolic activity of HepG2 cells in various concentrations of FerroOrange. At different concentration, the dye (with 1% DMSO as cosolvent) was added and the metabolic activity of cells were measured by MTT assay after 24 hours culture (n = 3, error bar indicates standard deviation).

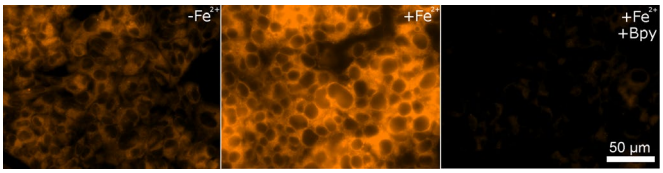

Figure 4: Different fluorescence intensities depending on the Fe^{2+} concentrations. Here we demonstrate an imaging example of HepG2 cells. Left panel indicates the physiological level of intracellular Fe^{2+}. Center panel indicates the increased Fe^{2+} to which Fe^{2+} was overloaded by adding to the culture medium. When Fe^{2+} chelator was added (right), intracellular Fe^{2+} level was greatly decreased.

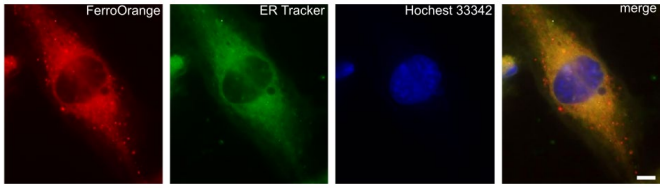

Figure 5: Fluorescence signal of FerroOrange mainly localizes in endoplasmic reticulum. FerroOrange (red), ER Tracker (green), and Hoechist 3342 (blue) were loaded to HT-1080 cells and observed. FerroOrange localizes mainly in endoplasmic reticurrum. Bar indicates 10 μm. Cell line distributed by JCRB Cell Bank was used.

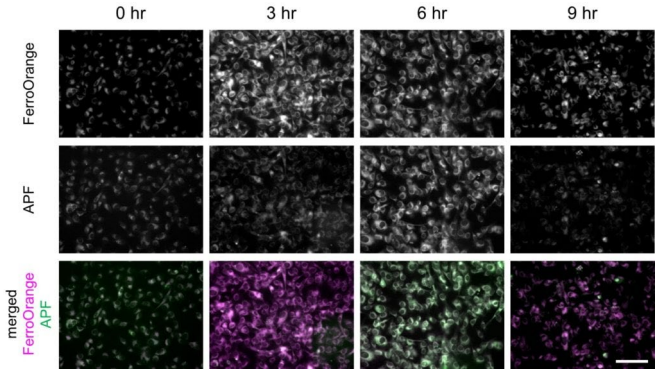

Figure 6: Fluorescence intensity of FerroOrange and APF in erastin-applied HT-1080 cells (upper and middle rows) were shown. Images were overlayed as pseudocolor images (bottom). Magenta indicates fluorescence of FerroOrange, green indicates that of APF. Bar indicates 100 μm. Erastin was applied to HT-1080 cells to induce ferroptosis, and intracellular labile Fe^{2+} and ROS were imaged after 3, 6, 9 hours after the application, using the fluorescent probes. Fluorescence signal of FerroOrange which indicates labile Fe^{2+} was maximum at 3 hours after the induction.

References

Tomita K et al. *MiR-7-5p is a key factor that controls radioresistance via intracellular Fe2+ content in a clinically relevant radioresistant cells.* Biochem. and Biophy. Res. Commun. 2019. Oct; 518(4): 712-718.