BioTracker™ Far-red Labile Fe^{2+} Live Cell Dye

Live Cell Dye

Cat. # SCT037

pack size: 50 nmol x 5

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 far-red labile Fe2+ dye is a fluorescent probe that specifically detects labile iron (II) ions (Fe2+). This probe is designed to selectively react with only Fe2+ separately from other metal ions and irreversibly turns into a far-red fluorescent substance. It mainly localizes inside endoplasmic reticulum (ER) and/or golgi bodies. It can also be used with a flow cytometer equipped with a red laser. The dye shows almost no cytotoxicity at concentrations up to 100 µM (~20 times that of generally used concentrations).

Storage

Store BioTracker™ far-red labile Fe^{2+} 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: 646 nm
Emission: 662 nm

Quality Control

Purity: ≥ 98% confirmed by LC.

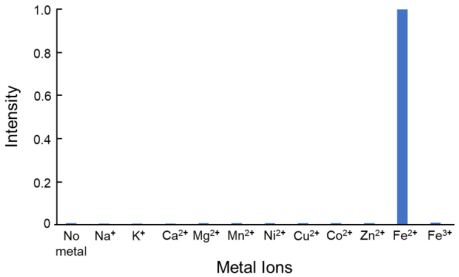

Figure 1: Reactivity of the dye with various metal ions. A pronounced increase in fluorescence occurs only in presence of Fe^{2+}. Relative fluorescence intensity to that upon reaction with Fe^{2+}.

Protocol

Materials required but not provided

1. Dimethyl sulfoxide (DMSO)
2. Appropriate observation buffer (PBS pH 7.4, HBSS, etc.). It should be a solution without phenol red. · Serum-free cell culture medium (D-MEM, etc.)

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 50 µl of DMSO to 1 vial (50 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).

Example of Cell Staining

Observation of labile Iron (II) ions (Fe2+) in HeLa Cells

1. Seed the cells in a glass bottom dish and culture overnight.
2. Remove the culture medium from the dish and rinse twice gently with the observation buffer.
3. Dilute 1 mM stock solution of the dye in a serum-free cell culture medium to prepare a staining solution with a final concentration of 5 µM.
4. Add the staining solution to the dish and incubate for 1 hour at 37°C.
5. After the staining, wash once with the observation buffer and replace with the observation buffer.
6. Observe the cells with a fluorescence microscope.

Note: Optimal concentration of FerroFarRed and reaction time may vary on cell type and condition. In our experience, incubating HepG2 cells with the dye under the same condition, gave good results.

Note: If cells are easily detached from the dish, usage of poly-L-lysine or other coating materials is recommended.

Note: You can detect the increase of labile Fe2+ if you added Fe2+ in the medium. For this purpose, dissolve $Fe(NH_4)_2(SO_4)_2$ (FAS) to prepare a 100 mM solution just before use, then dilute the FAS solution with serum-free cell culture medium to prepare 100 µM FAS solution. After cells were cultured in the FAS solution for 30 minutes, wash the cells to remove extracellular FAS and add the dye solution to detect intracellular Fe2+. In our experience, dilution of FAS and the dye with HBSS, instead of serum-free medium, gave good results. Do not use the solutions with serum. Under this condition, intracellular Fe2+ cannot be detected correctly because the dye reacts with Fe2+ in the serum before reacting with intracellular Fe2+.

Measurement of Fe2+ in HepG2 Cells Using a Flow Cytometer

1. Seed the cells in a multi-well plate and culture overnight.

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2. Remove the culture medium from the plate and rinse twice gently with the observation buffer.
3. Dilute 1 mM stock solution of the dye in a serum-free cell culture medium to prepare a staining solution with a final concentration of 5 µM.
4. Add the staining solution to the plate and incubate for 1 hour at 37°C.
5. After the staining, wash once with PBS and then add 0.25% trypsin-EDTA solution to detach the cells from the multi-well plate.
6. Dilute 0.25% trypsin-EDTA solution with PBS on ice and centrifuge the cell suspension at 500 x g for 5 minutes to precipitate the cells.
   Note: Do not neutralize trypsin with serum. In the case of neutralizing with serum, intracellular Fe2+ cannot be detected correctly because the dye reacts with Fe2+ in the serum before reacting with intracellular Fe2+.
7. Discard the supernatant and resuspend the cells in PBS.
8. Filter the cell suspension through a cell strainer (40 µm nylon mesh) to remove debris.
9. Analyze the sample using a flow cytometer.
   Note: Optimal concentration of the dye and reaction time may vary on cell type and condition.
   Note: You can detect the increase of labile Fe2+ if you added Fe2+ in the medium. For this purpose, dissolve $Fe(NH_4)_2(SO_4)_2$ (FAS) to prepare a 100 mM solution just before use, then dilute the FAS solution with serum-free cell culture medium to prepare 100 µM FAS solution. After cells were cultured in the FAS solution for 30 minutes, wash the cells to remove extracellular FAS and add the dye solution to detect intracellular Fe2+.

Fluorescence observation

For laser excitation, wavelength around 635 nm is appropriate. The fluorescence could be detected at around 660 nm. For observation by fluorescent microscopes, use red excitation filter set for Cy5. For analysis by flow cytometer, filter used for Allophycocyanin (APC) is appropriate.

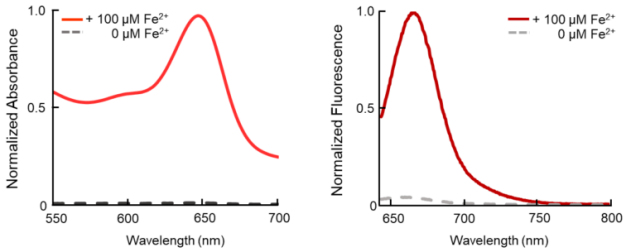

Figure 2: Absorption (left) and fluorescence (right) spectrum. Reaction with Fe2+ significantly increases the fluorescence intensity.

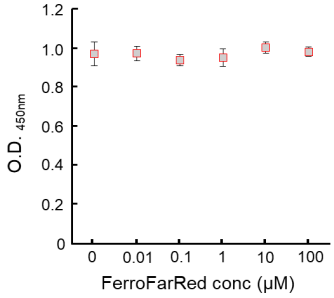

Figure 3: Metabolic activity of HeLa cells in various concentrations of the dye measured by the CCK-8 assay (n = 3; ±S. D.). The dye shows almost no cytotoxicity at concentrations up to 100 µM (~20 times of used concentration).

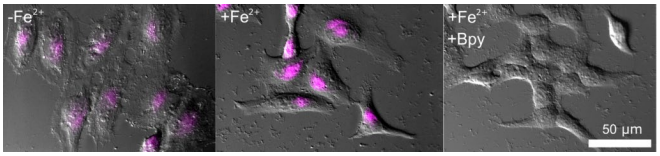

Figure 4. Fluorescence signal was increased in HeLa cells treated with Fe^{2+} (*middle*) compared with control cells (*left*). The fluorescence signal was decreased by adding Bpy (2,2′-Bipyridyl), Fe^{2+} chelator, in addition to Fe^{2+} (*right*). The magenta pseudo color shows the fluorescent image of BioTracker dye. Magenta pseudo color was overlaid to grayscale images of DIC.

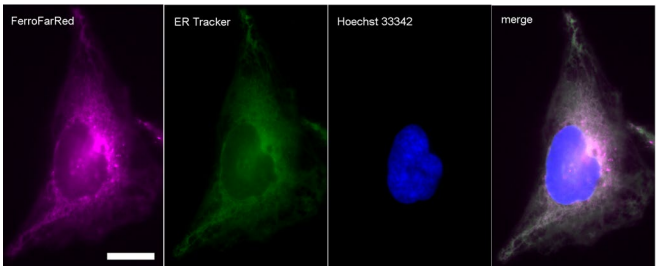

Figure 5: HeLa cells stained with ER Tracker (green) and Hoechst 33342 (blue) was reacted with BioTracker far-red labile Fe2+ dye (magenta). The BioTracker dye localizes in endoplasmic reticulum.

References

Hirayama T et al. *A universal fluorogenic switch for Fe (II) ion based on N-oxide chemistry permits the visualization of intracellular redox equilibrium shift towards labile iron in hypoxic tumor cells.* Chem Sci. 2017. Jul 1;8(7): 4858-4866.