GORYO CHEMICAL

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For research use only

FerroFarRed™

Table 1 Product Information

Catalog no.	Product name	Amount	Storage upon receipt	Stability
GC903-01	FerroFarRed	50 nmol x 5	≤−20°C, keep desiccated and protected from light.	1 year (when unopened and stored as described.)

1. About FerroFarRed

FerroFarRed (also known as SiRhoNox-1 or ER-SiRhoNox) is a fluorescent probe that specifically detects labile iron (II) ions (Fe^{2+}) via far-red fluorescence. This probe could react with only Fe^{2+} separately from other metal ions and irreversibly turns into a far-red fluorescent substance. It mainly localizes inside endoplasmic reticulum (ER)

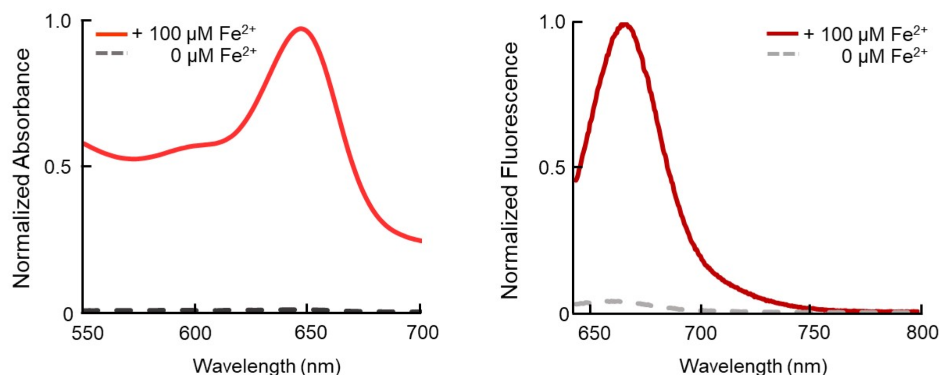
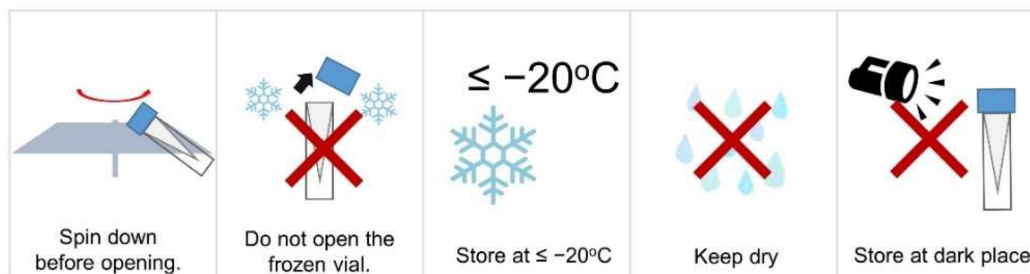


Fig. 1 Absorption spectra (left) and fluorescent spectra (right) of FerroFarRed under 0.05 M HEPES buffer (pH 7.4).

Spectrum of FerroFarRed with 100 μM Fe^{2+} (solid lines) and without Fe^{2+} (dotted lines). The absorption maximum is 646 nm, whereas the maximum fluorescence wavelength is 662 nm.

■ Storage

Upon receipt, store the product desiccated and protected from light at ≤ −20°C. Storing as a solution is not recommended.



2. Preparation of reagent

■ Materials required but not provided

· Dimethyl sulfoxide (DMSO)
· 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.)

■ Preparation of reagent

FerroFarRed is a blue solid. Before opening the cap, warm the vial to the room temperature. Then, use a microcentrifuge to spin down the solid that might adhere on the cap.

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 FerroFarRed solution is almost colorless (faint blue).

3. Example of Cell Staining

Observation of labile Iron (II) ions (Fe^{2+}) 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 FerroFarRed 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.
   ※ Optimal concentration of FerroFarRed and reaction time may vary on cell type and condition. In GORYO Chemical, Inc., incubating HepG2 cells with the dye under the same condition, gave good results. If cells are easily detached from the dish, usage of poly-L-lysine or other coating materials is recommended.
   ※ You can detect the increase of labile Fe^{2+} if you added Fe^{2+} 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 FerroFarRed solution to detect intracellular Fe^{2+}.
   ※ In GORYO Chemical, Inc., dilution $Fe(NH_4)_2(SO_4)_2$ and FerroFarRed with HBSS, instead of serum-free medium, gave good results.
   ※ Do not use the solutions with serum. Under the condition, intracellular Fe^{2+} cannot be detected correctly because FerroFarRed reacts with Fe^{2+} in the serum before reacting with intracellular Fe^{2+}.

Measurement of Fe^{2+} in HepG2 Cells Using a Flow Cytometer

1. Seed the cells in a multi-well plate and culture overnight.
2. Remove the culture medium from the plate and rinse twice gently with the observation buffer.
3. Dilute 1 mM stock solution of FerroFarRed 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.
   ※ Do not neutralize trypsin with serum. In the case of neutralizing with serum, intracellular Fe^{2+} cannot be detected correctly because FerroFarRed reacts with Fe^{2+} in the serum before reacting with intracellular Fe^{2+}.
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.
   ※ Optimal concentration of FerroFarRed and reaction time may vary on cell type and condition.
   ※ You can detect the increase of labile Fe^{2+} if you added Fe^{2+} 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 FerroFarRed solution to detect intracellular Fe^{2+}.

■ 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.

■ References

See also the publications below, for other usages and protocols.

T. Hirayama, A. Miki, H. Nagasawa (2018) *Metallomics*. in press DOI: 10.1039/c8mt00212f

K. Sakamoto, T. Suzuki, K. Takahashi, T. Koguchi, T. Hirayama, A. Mori, T. Nakahara, H. Nagasawa, K. Ishii (2018) *Exp. Eye Res.* **171**: 30-36 DOI: 10.1016/j.exer.2018.03.008

T. Hirayama, H. Tsuboi, M. Niwa, A. Miki, S. Kadota, Y. Ikeshita, K. Okuda, H. Nagasawa (2017) *Chem. Sci.* **8**: 4858-4866 DOI: 10.1039/c6sc05457a

Table 2. Related Products

Catalog no	Material	Usage
GC901	FeRhoNox™-1	To detect Fe^{2+} localized in Golgi.
GC902	CopperGREEN™	To detect Cu^{+}
SK2001-01	ZnAF-2	To detect Zn^{2+}
SK2002-01	ZnAF-2DA	To detect intracellular Zn^{2+}
GC3004-01	OxiORANGE™	To detect hydroxyl radicals (˙OH) and hypochlorous acid (HClO). Orange fluorescent reagent.
GC3006-01	HySOx	To detect intracellular hypochlorous acid (HClO).
GC3007-01	HYDROP™	Fluorescence probe to detect only intracellular hydrogen peroxide (H_2O_2).
GC3008-01	HYDROP-EX™	Fluorescence probe to detect only hydrogen peroxide (H_2O_2).
SK3001-01 SK3001-02	HPF	To detect hydroxyl radicals (˙OH) and peroxynitrite ($ONOO^-$).
SK3002-01 SK3002-02	APF	To detect hydroxyl radical (˙OH), peroxynitrite ($ONOO^-$), and hypochlorous acid (HClO).
SK3003-01	NiSPY-3	To detect peroxynitrite ($ONOO^-$).
A101-01	MAR	To detect hypoxic response of cells.