For research use only

FerroOrange

Table 1 Product information

CAT. No.	Product name	Size	Storage upon receipt	Stability
GC904-01	FerroOrange	35 nmol × 5	≤−20°C, keep desiccated and protected from light.	1 year (when unopened and stored as described.)
GC904-02		35 nmol × 1		

1. About FerroOrange

FerroOrange is an orange fluorescent probe for cell imaging designed to detect specifically labile iron (II) ions (Fe^{2+}) localized in endoplasmic reticulum (ER). This reagent reacts specifically to Fe^{2+} by irreversibly changing to an orange fluorescent substance. Therefore, this probe can be used to selectively distinguish Fe^{2+} ions from other metal ions.

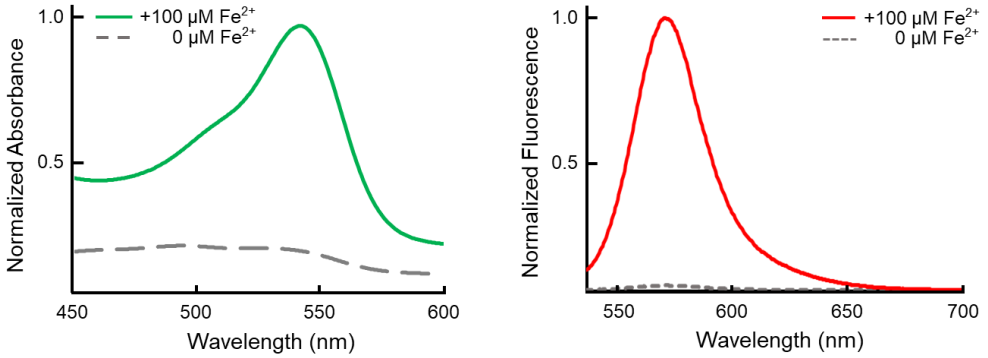
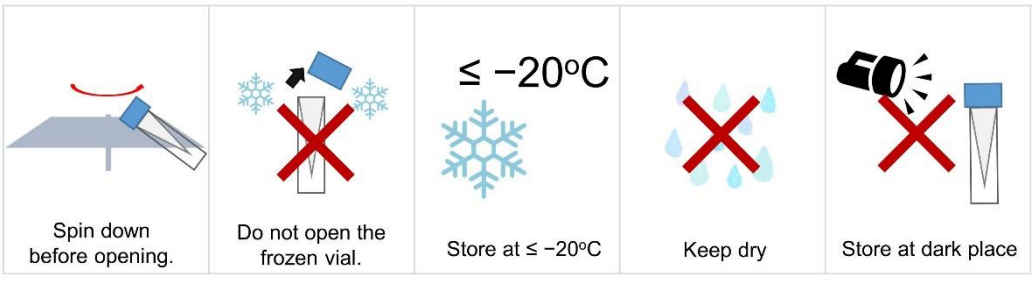


Fig.1 Absorption spectrum and fluorescence spectrum of FerroOrange in 0.05 M HEPES buffer (pH 7.4). The absorption maximum is observed at 542 nm, and the fluorescence maximum is observed at 572 nm.

■ Storage

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



Spin before opening | Open at room temperature | Store in a freezer | Keep dry | Store in a dark place

2. Preparation of reagent

■ Materials required but not provided

・Dimethyl Sulfoxide (DMSO)

・Appropriate washing and observation buffer (PBS pH 7.4, HBSS, etc.). It should be a solution without phenol red.

■ Preparation of reagent

FerroOrange is a colorless powder. Return the vial to room temperature taking care not to absorb moisture, place it into a microcentrifuge, collect the powder adhering to the lid and walls on the bottom and open the vial.

Add 35 µL of DMSO to 1 vial (35 nmol) and repeat pipetting 5 or more times to completely dissolve the reagent. The resulting solution is 1mM. The FerroOrange solution is almost colorless. Use neutral buffer or culture medium for dilution of the DMSO solution and use immediately upon dilution. FerroOrange could be oxidized in acidic solutions.

3. Example of Cell Staining

Observation of labile iron (II) ions (Fe^{2+}) 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 Fe^{2+}.
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.

※ You can detect the increase of labile Fe^{2+} ions as a positive control, if you added Fe^{2+} 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 FerroOrange solution to detect intracellular Fe^{2+}.

※ After incubating the cells in this solution for 30 minutes, intracellular Fe^{2+} concentration rises. Wash extracellular Fe^{2+} with washing buffer and add FerroOrange.

※ Do not use the solutions with serum. Because FerroOrange reacts with Fe^{2+} in serum before it reacts with Fe^{2+} in the cells, the intracellular Fe^{2+} cannot be detected correctly.

※ We recommend optimizing dye concentrations and incubation time in your conditions. In GORYO Chemical, incubation in 1 µM dye at 37°C for 30 minutes gave good results for HeLa cells (human cervical cancer cell line). 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.

■ 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.

* We are grateful to Dr. Hideko Nagasawa and Dr. Tasuku Hirayama (Gifu Pharmaceutical University) with their support and advices for the commercialization of FerroOrange.

Table 2. Related products

Catalog no	Material	Usage
GC901	FeRhoNox™-1	To detect Fe^{2+} localized in Golgi.
GC902	CopperGREEN™	To detect Cu^{+}
GC903-01	FerroFarRed™	To detect Fe^{2+} localized in ER. Deep red fluorescent reagent.
SK2001-01	ZnAF-2	To detect Zn^{2+}
SK2002-01	ZnAF-2 DA	To detect intracellular Zn^{2+}
SK3001-01	HPF	To detect hydroxyl radicals ($^{\cdot}OH$) and peroxynitrite ($ONOO^{-}$).
SK3002-01	APF	To detect hydroxyl radical ($^{\cdot}OH$), peroxynitrite ($ONOO^{-}$), and hypochlorous acid (HClO).
SK3003-01	NiSPY-3	To detect peroxynitrite ($ONOO^{-}$).
GC3004-01	OxiORANGE™	To detect hydroxyl radicals ($^{\cdot}OH$) 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).
A101-01	MAR	To detect hypoxic response of cells.

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