

FeRhoNox™-1

Table 1. Product information

Catalog no.	Product name	Amount	Storage upon receipt	Stability
GC901	FeRhoNox™-1	50 µg × 10	≤-20°C, keep desiccated and protected from light.	1 year (when unopened and stored as described.)

1. About FeRhoNox™-1

FeRhoNox™-1 (also called as RhoNox-1) is an activatable fluorescent probe that specifically detects labile Fe²⁺ ions via orange (red) fluorescence. It irreversibly fluoresces upon reaction with Fe²⁺ ions, but does not react with other ions of physiological concentrations. FeRhoNox™-1 tends to localize in Golgi within a cell.

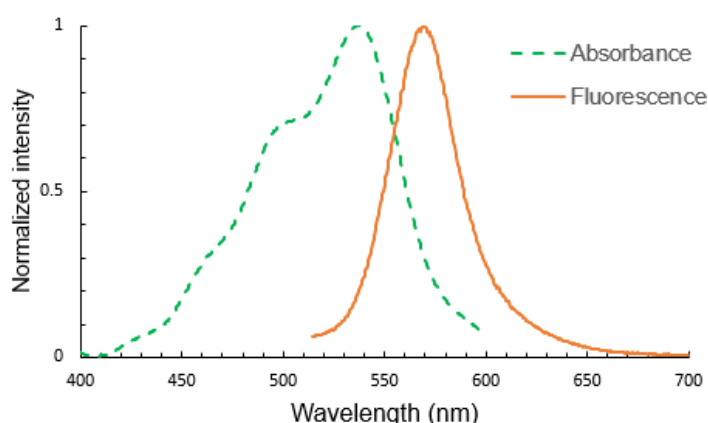


Figure 1. Absorbance/Fluorescence spectra of FeRhoNox™-1 upon reaction with Fe²⁺ ions. Maximum absorption and fluorescence are observed at 537 nm and at 569 nm, respectively.

■ Storage

This product is shipped as a dried solid in nitrogen gas-filled vials. Upon receipt, store the product desiccated and protected from light at ≤ -20°C. Storing as a solution is not recommended because the reagent degrades in solution and fluorescence background signal may increase. Please use up the solution.

2. An example of live cell imaging

■ Materials required but not provided

- Dimethyl sulfoxide (DMSO). We strongly recommend using high-quality grade named as infinity-pure or ultra-pure. DMSO bottle should be opened just before use. Storing DMSO in a deep freezer as a small aliquot is also recommended. Degraded DMSO may increase the background signal of FeRhoNox™-1.
- Observation buffer (1×PBS pH 7.4, Hank's Balanced Salt Solution (HBSS), etc.).

■ Preparation of reagent and cell imaging

1. Before opening the vial, spin down the solid to the bottom by a microcentrifuge or a desktop centrifuge.
2. Allow the vial to attain room temperature and add 109 μl of high-purity DMSO to one vial to make 1 mM solution. Use neutral buffer solution for the dilution of the DMSO solution.
3. Dilute the FeRhoNox™-1 solution with HBSS or with other neutral buffer solution to prepare 5 μM solution. (The concentration of the probe should be changed depending on the measurement conditions and samples.)
4. Remove culture medium from the cells cultured in a glass-bottom dish, rinse the cells with HBSS twice, then add the 5 μM FeRhoNox™-1 solution.
5. Incubate cells for 37°C, 5% CO₂ for 60 minutes. Incubation time should be varied depending on the conditions.
6. Rinse the cells with HBSS three times and observe cells by microscopy.
 ※ You can detect the increase of labile Fe²⁺ ions if you added Fe²⁺ in the medium. For this purpose, dissolve Fe(NH₄)₂(SO₄)₂ (FAS) to prepare a 100 mM solution just before use, then dilute the FAS solution with 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 FeRhoNox™-1 solution to detect intracellular Fe²⁺.

■ Fluorescence observation

Use green excitation filter set for Cy3 or tetramethyl rhodamine (TMR). For laser excitation, 532 nm or 543 nm laser is appropriate. Detect fluorescence around 570 nm.

Table 2. Related Products

Catalog no.	Product name	Major applications
GC902	CopperGREEN™	Detection of labile copper (I) ions (Cu ⁺).
SK2001-01	ZnAF-2	Detection of Zn ²⁺ ions.
SK2002-01	ZnAF-2DA	For live cell imaging of Zn ²⁺ ions.
SK3001-01	HPF	Detection of hydroxyl radical ($\cdot\text{OH}$) and peroxynitrite (ONOO ⁻).
SK3002-01	APF	Detection of hydroxyl radical ($\cdot\text{OH}$), peroxynitrite (ONOO ⁻) and hypochlorous acid (HOCl).
SK3003-01	NiSPY-3	Detection of peroxynitrite (ONOO ⁻).
GC3004-01	OxiORANGE™	Detection of hydroxyl radical ($\cdot\text{OH}$) and hypochlorous acid (HOCl) via orange fluorescence.
GC3006-01	HySOx	Detection and live cell imaging of hypochlorous acid (HOCl).
GC3007-01	HYDROP™	Detection of intracellular hydrogen peroxide (H ₂ O ₂).
A101-01	MAR	Detection of hypoxia.