

Product Data Sheet

Product Name: HMRhoNox-M

Cat. No.: GC80338

Chemical Properties

Cas. No. 1616953-95-7

Formula $C_{24}H_{24}N_2O_3$

M.Wt 388.46

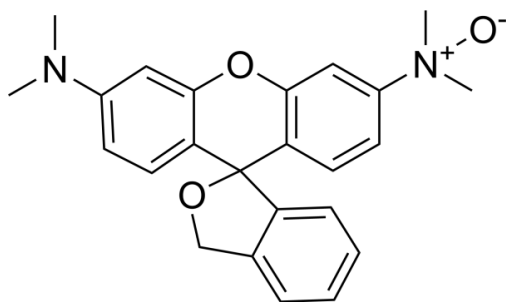
Solubility

Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37°C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution: ship with blue ice. All other available size: ship with RT, or blue ice upon request.

Structure



Background

HMRhoNox-M (LysoRhoNox) is a selective fluorescent probe for detecting Fe^{2+} (ferrous iron ions), with excitation/emission wavelengths of $\text{Ex/Em} = 550/575 \text{ nm}$. HMRhoNox-M exists in a non-fluorescent spirocyclic closed conformation under physiological pH conditions. Fe^{2+} triggers the deoxidation of its N-oxide group, shifting the equilibrium toward the fluorescent quinone-type (TMHMR) open conformation. HMRhoNox-M shows selectivity for Fe^{2+} over other metal ions, as well as biologically relevant reactive oxygen species, reactive sulfur species, reactive nitrogen species and reducing agents. HMRhoNox-M can penetrate cell membranes and localize to lysosomes, enabling the imaging of intracellular Fe^{2+} fluctuations, transferrin-mediated labile iron

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accumulation, and lysosomal Fe²⁺ changes associated with ferroptosis [1] [2] [3] [4].

In Vitro, Guidelines (The following is a recommended experimental protocol for guidance only, and adjustments are required based on your specific needs)

1. Stock Solution Preparation
 - 1.1 Solvents: DMF; DMSO.
 - 1.2 Recommended concentration: 1 mM.
2. Working Solution Preparation
 - 2.3 Diluents: 50 mM HEPES buffer (pH 7.4); cell culture medium; Hank's Balanced Salt Solution (HBSS) without phenol red, calcium chloride, and magnesium chloride.
 - 2.2 Working concentration: 1-3 μ M.
 - 2.3 Notes: Adjust the working solution concentration as needed; prepare and use immediately.
3. Staining Procedures
 - 3.1 Sample Type Description
 - 3.1.1 Adherent cells^{[1][2][4]}: Including HepG2, SH-SY5Y neuroblastoma cells, and human fibrosarcoma (HT1080) cells.
 - 3.1.2 Bone marrow-derived macrophages (BMDMs, suspension cells)^[3].
 - 3.2 Incubation Conditions
 - 3.2.1 Adherent cells:
 - 3.2.1.1 Incubate with 1-3 μ M HMRhoNox-M at 37°C for 30 min.
 - 3.2.1.2 Detection of endogenous Fe²⁺ baseline^[1]: Incubate with 1 μ M HMRhoNox-M at 37°C for 30 min.
 - 3.2.1.3 Detection of exogenous Fe²⁺^[1]: First add 100 μ M Fe²⁺ to the cells, incubate at 37°C for 30 min, then incubate with 1 μ M HMRhoNox-M at 37°C for 30 min.
 - 3.2.1.4 Monitoring of transferrin-induced iron uptake^[1]: First add 5 μ M holoTf to the cells, incubate at 37°C for 30 min, then incubate with 1 μ M HMRhoNox-M at 37°C for 30 min.
 - 3.2.1.5 Ferroptosis imaging studies^[4]: Incubate with 1 μ M HMRhoNox-M at 37°C for 1 h.
 - 3.2.2 BMDMs^[3]: First pre-incubate the cells with 100 μ M ammonium iron citrate for 2 h, then stain with HMRhoNox-M to detect lysosomal Fe²⁺.
 - 3.3 Washing Steps
 - 3.3.1 Adherent cells^[4]: Wash the cells twice with HBSS; for Fe (II) uptake assays, first wash with FBS-free MEM, then wash twice with HBSS.
4. Control Setup
 - 4.1 Negative Controls
 - 4.1.1 Incubate cells with 1 μ M HMRhoNox-M and 1 mM 2,2'-bipyridine (Bpy) simultaneously at 37°C for 30 min.
 - 4.1.2 First add 5 μ M holoTf + 25 μ M apoTf to the cells, incubate at 37°C for 30 min, then incubate with 1 μ M HMRhoNox-M at 37°C for 30 min.
 - 4.1.3 First add 5 μ M holoTf to the cells, incubate at 4°C for 30 min, then incubate with 1 μ M HMRhoNox-M at 37°C for 30 min.
 - 4.1.4 First add 5 μ M holoTf + 1 mM NaN₃ to the cells, incubate at 37°C for 30 min, then incubate with 1 μ M HMRhoNox-M at 37°C for 30 min.
 - 4.1.5 MCOLN1-knockout BMDMs serve as the negative control for TRPML1-mediated lysosomal Fe²⁺ release.
 - 4.2 Positive Controls
 - 4.2.1 WT BMDMs serve as the positive control for lysosomal Fe²⁺ release^[3].
 - 4.3 Blank Control, used to exclude reagent fluorescence interference^[4].
 - 4.3.1 For ferroptosis imaging studies, set up vehicle-only control group, Erastin -treated control group, and Erastin + Deferoxamine mesylate co-treated control group^[4].
5. Detection and Analysis
 - 5.1 Instrument Types: Fluorescence

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microscope; fluorescence imaging system. 5.2 Excitation/Emission Wavelengths: Excitation wavelengths are 532-554 nm, 550 nm, or 555 nm; emission wavelengths are 572-642 nm, 575 nm. 5.3 Result Analysis 5.3.1 Changes in Fluorescence Intensity: 5.3.1.1 When intracellular Fe²⁺ levels increase, significant fluorescence turn-on enhancement is observed; in cells where Fe²⁺ is chelated or endocytosis is inhibited, fluorescence intensity decreases to the basal level^[1]. 5.3.1.2 Compared with wild-type cells, lysosomal fluorescence intensity is elevated in VPS35-knockdown cells; treatment with R55 reduces the fluorescence intensity of VPS35-knockdown cells^[2]. 5.3.1.3 HMRhoNox-M fluorescence intensity decreases in LPS-stimulated WT BMDMs, indicating lysosomal Fe²⁺ release; co-treatment with NAC inhibits this decrease in fluorescence intensity^[3]. 5.3.1.4 Fluorescence intensity increases when labile Fe (II) levels rise^[4]. 5.3.1.5 In Erastin-induced ferroptosis, lysosomal fluorescence intensity increases at 3 h and 5 h after treatment, and this phenomenon is inhibited by co-treatment with Deferoxamine mesylate^[4]. 5.3.2 Fluorescence Localization: Shows a punctate staining pattern, localized to lysosomes; lysosomal membrane^{[1][2][3][4]}. 5.3.3 Color Change: Yellow fluorescence emission; orange-yellow fluorescence^{[1][2][4]}.

References:

- [1]. Niwa M, et al. A new class of high-contrast Fe(II) selective fluorescent probes based on spirocyclized scaffolds for visualization of intracellular labile iron delivered by transferrin. *Organic & biomolecular chemistry*. 2014 Sep 14;12(34):6590-7.
- [2]. Hirayama T, et al. A Golgi-targeting fluorescent probe for labile Fe(ii) to reveal an abnormal cellular iron distribution induced by dysfunction of VPS35. *Chemical science*. 2019 Feb 07;10(5):1514-1521.
- [3]. Xing Y, et al. Lysosomes finely control macrophage inflammatory function via regulating the release of lysosomal Fe²⁺ through TRPML1 channel. *Nat Commun*. 2025 Jan 24;16(1):985.
- [4]. Hirayama T, et al. Organelle-specific analysis of labile Fe(ii) during ferroptosis by using a cocktail of various colour organelle-targeted fluorescent probes. *Metallomics: integrated biometal science*. 2019 Jan 23;11(1):111-117.

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