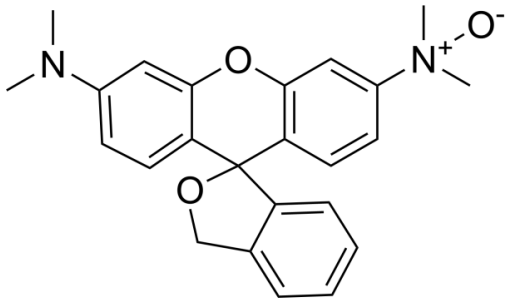
Product Data Sheet

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 Ex/Em = 550/575 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

accumulation, and lysosomal Fe 2+ 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 μM. 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 μM HMRhoNox-M at 37°C for 30 min. 3.2.1.2 Detection of endogenous Fe2+ baseline[1]: Incubate with 1 μM HMRhoNox-M at 37°C for 30 min. 3.2.1.3 Detection of exogenous Fe2+[1]: First add 100 μM Fe2+ to the cells, incubate at 37°C for 30 min, then incubate with 1 μM HMRhoNox-M at 37°C for 30 min. 3.2.1.4 Monitoring of transferrin-induced iron uptake[1]: First add 5 μM holoTf to the cells, incubate at 37°C for 30 min, then incubate with 1 μM HMRhoNox-M at 37°C for 30 min. 3.2.1.5 Ferroptosis imaging studies[4]: Incubate with 1 μM HMRhoNox-M at 37°C for 1 h. 3.2.2 BMDMs[3]: First pre-incubate the cells with 100 μM 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 μM HMRhoNox-M and 1 mM 2,2'-bipyridine (Bpy) simultaneously at 37°C for 30 min. 4.1.2 First add 5 μM holoTf + 25 μM apoTf to the cells, incubate at 37°C for 30 min, then incubate with 1 μM HMRhoNox-M at 37°C for 30 min. 4.1.3 First add 5 μM holoTf to the cells, incubate at 4°C for 30 min, then incubate with 1 μM HMRhoNox-M at 37°C for 30 min. 4.1.4 First add 5 μM holoTf + 1 mM NaN3 to the cells, incubate at 37°C for 30 min, then incubate with 1 μM 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

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.

Caution: Product has not been fully validated for medical applications. For research use only.
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