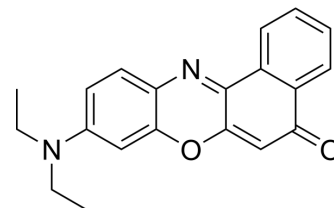


## Nile Red

Cat. No.:	HY-D0718
CAS No.:	7385-67-3
Molecular Formula:	C <sub>20</sub> H <sub>18</sub> N <sub>2</sub> O <sub>2</sub>
Molecular Weight:	318.37
Target:	Fluorescent Dye
Pathway:	Others
Storage:	4°C, protect from light * In solvent : -80°C, 2 years; -20°C, 1 year (protect from light)



### SOLVENT & SOLUBILITY

In Vitro	DMSO : 2 mg/mL (6.28 mM; ultrasonic and warming and heat to 60°C)																								
	Ethanol : 1 mg/mL (3.14 mM; ultrasonic and warming and heat to 60°C)																								
	Preparing Stock Solutions	<table border="1"> <thead> <tr> <th rowspan="2">Solvent</th> <th rowspan="2">Mass</th> <th colspan="3">Concentration</th> </tr> <tr> <th>1 mg</th> <th>5 mg</th> <th>10 mg</th> </tr> </thead> <tbody> <tr> <td>1 mM</td> <td>3.1410 mL</td> <td>15.7050 mL</td> <td>31.4100 mL</td> </tr> <tr> <td>5 mM</td> <td>0.6282 mL</td> <td>3.1410 mL</td> <td>6.2820 mL</td> </tr> <tr> <td>10 mM</td> <td>---</td> <td>---</td> <td>---</td> </tr> </tbody> </table>	Solvent	Mass	Concentration			1 mg	5 mg	10 mg	1 mM	3.1410 mL	15.7050 mL	31.4100 mL	5 mM	0.6282 mL	3.1410 mL	6.2820 mL	10 mM	---	---	---			
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Please refer to the solubility information to select the appropriate solvent.																									
In Vivo	<ol style="list-style-type: none"> <li>Add each solvent one by one: 10% DMSO &gt;&gt; 40% PEG300 &gt;&gt; 5% Tween-80 &gt;&gt; 45% saline Solubility: 0.2 mg/mL (0.63 mM); Suspended solution; Need ultrasonic</li> <li>Add each solvent one by one: 10% DMSO &gt;&gt; 90% (20% SBE-β-CD in saline) Solubility: ≥ 0.2 mg/mL (0.63 mM); Clear solution</li> </ol>																								

### BIOLOGICAL ACTIVITY

Description	Nile red (Nile blue oxazine) is a lipophilic stain. Nile red has environment-sensitive fluorescence. Nile red is intensely fluorescent in a lipid-rich environment while it has minimal fluorescence in aqueous media. Nile red is an excellent vital stain for the detection of intracellular lipid droplets by fluorescence microscopy and flow cytometry. Nile red stains intracellular lipid droplets red. The fluorescence wavelength is 559/635 nm <sup>[1]</sup> .
In Vitro	<ol style="list-style-type: none"> <li>Preparation of Phalloidin-TRITC working solution           <ol style="list-style-type: none"> <li>Preparation of the stock solution Dissolve Phalloidin-TRITC in Methanol to obtain 10 mM of stock solution. Note: It is recommended to store the stock solution at -20°C or -80°C away from light and avoid repetitive freeze-thaw cycles.</li> <li>Preparation of Phalloidin-TRITC working solution</li> </ol> </li> </ol>

Dilute the stock solution in serum-free cell culture medium to obtain 1-10  $\mu\text{M}$  of working solution.  
Note: Please adjust the concentration of Phalloidin-TRITC working solution according to the actual situation.

2. Cell staining

2.1 Suspension cells (6-well plate)

- Centrifuge at 1000 g at 4 $\text{^\circ}$ C for 3-5 minutes and then discard the supernatant. Wash twice with PBS, 5 minutes each time.
- Add 1 mL of working solution, and then incubate at room temperature for 30-60 minutes.
- Centrifuge at 400 g at 4 $\text{^\circ}$ C for 3-4 minutes and then discard the supernatant.
- Wash twice with PBS, 5 minutes each time.
- Resuspend cells with serum-free cell culture medium or PBS. Observation by fluorescence microscopy or flow cytometry.

2.2 Adherent cells

- Culture adherent cells on sterile coverslips.
- Remove the coverslip from the medium and aspirate excess medium.
- Add 100  $\mu\text{L}$  of working solution, gently shake it to completely cover the cells, and then incubate at room temperature for 30-60 minutes.
- Wash twice with medium, 5 minutes each time. Observation by fluorescence microscopy.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

#### In Vivo

When Nile red-stained *Caenorhabditis elegans* is viewed for green fluorescence, discrete lipid bodies can be observed throughout the intestine and other tissues either in clusters or evenly dispersed, depending on the animal's genotype or experimental treatment<sup>[3]</sup>.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## CUSTOMER VALIDATION

- Nat Neurosci. 2023 Apr;26(4):542-554.
- Nano Today. 47 (2022) 101675
- Nat Commun. 2023 Aug 28;14(1):5242.
- Nat Commun. 2022 Oct 5;13(1):5871.
- Nat Commun. 2020 Jan 13;11(1):240.

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## REFERENCES

- [1]. Greenspan P, et al. Nile red: a selective fluorescent stain for intracellular lipid droplets. J Cell Biol. 1985 Mar;100(3):965-73.
- [2]. Gibrán S Alemán-Nava, et al. How to use Nile Red, a selective fluorescent stain for microalgal neutral lipids. J Microbiol Methods. 2016 Sep;128:74-79.
- [3]. Wilber Escorcía, et al. Quantification of Lipid Abundance and Evaluation of Lipid Distribution in *Caenorhabditis elegans* by Nile Red and Oil Red O Staining. J Vis Exp. 2018 Mar 5;(133):57352.
- [4]. Elizabeth C Pino, et al. Biochemical and high throughput microscopic assessment of fat mass in *Caenorhabditis elegans*. J Vis Exp. 2013 Mar 30;(73):50180.

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

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