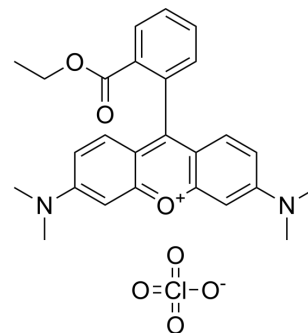


TMRE

Cat. No.:	HY-D0985A
CAS No.:	115532-52-0
Molecular Formula:	C ₂₆ H ₂₇ ClN ₂ O ₇
Molecular Weight:	514.95
Target:	Fluorescent Dye
Pathway:	Others
Storage:	4°C, sealed storage, away from moisture and light * In solvent : -80°C, 1 year; -20°C, 6 months (sealed storage, away from moisture and light)



SOLVENT & SOLUBILITY

In Vitro	DMSO : 27.78 mg/mL (53.95 mM; Need ultrasonic)				
	Preparing Stock Solutions	Solvent	1 mg	5 mg	10 mg
		Concentration			
		Mass			
	1 mM	1.9419 mL	9.7097 mL	19.4194 mL	
	5 mM	0.3884 mL	1.9419 mL	3.8839 mL	
	10 mM	0.1942 mL	0.9710 mL	1.9419 mL	
Please refer to the solubility information to select the appropriate solvent.					
In Vivo	1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline Solubility: ≥ 2.08 mg/mL (4.04 mM); Clear solution 2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.08 mg/mL (4.04 mM); Clear solution				

BIOLOGICAL ACTIVITY

Description	Rhodamine dyes are membrane-permeable cationic fluorescent probes that specifically recognize mitochondrial membrane potentials, thereby attaching to mitochondria and producing bright fluorescence, and at certain concentrations, rhodamine dyes have low toxicity to cells, so they are commonly used to detect mitochondria in animal cells, plant cells, and microorganisms ^[1] .
In Vitro	1. Preparation of TMRE working solution 1.1 Preparation of the stock solution Dissolve 1 mg TMRE in DMSO to obtain 5 mM of stock solution. 1.2 Preparation of TMRE working solution Dilute the stock solution in serum-free cell culture medium or PBS to obtain 1-20 μM of working solution. Note: Please adjust the concentration of TMRE working solution according to the actual situation.

2. Cell staining

2.1 Suspension cells (6-well plate)

a. Centrifuge at 1000 g at 4°C for 3-5 minutes and then discard the supernatant. Wash twice with PBS, 5 minutes each time. The cell density is 1×10^6 /mL.

b. Add 1 mL of working solution, and then incubate at room temperature for 5-30 minutes.

c. Centrifuge at 400 g at 4°C for 3-4 minutes and then discard the supernatant.

d. Wash twice with PBS, 5 minutes each time.

e. Resuspend cells with serum-free cell culture medium or PBS. Observation by fluorescence microscopy or flow cytometry.

2.2 Adherent cells

a. Culture adherent cells on sterile coverslips.

b. Remove the coverslip from the medium and aspirate excess medium.

c. Add 100 μ L of working solution, gently shake it to completely cover the cells, and then incubate at room temperature for 30-60 minutes.

d. Wash twice with medium, 5 minutes each time. Observation by fluorescence microscopy or flow cytometry.

Note: If detection by flow cytometry, cells need to be resuspended before staining.

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

PROTOCOL

Cell Assay ^[1]

The entire experiment should be performed at room temperature because temperature will directly impact mitochondrial transmembrane potential and TMRE staining. Cells should never be placed, centrifuged, incubated, or washed at 4°C or have ice-cold buffers or media added. Treat the cells with a cytotoxic stimulus. Harvest cells and resuspend at 5×10^5 cells/mL in culture medium containing 150 nM TMRE. Incubate for 5 min at room temperature in the dark. Add Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP) (5 μ M final concentration) to an aliquot of untreated cells and incubate for 5 min at room temperature in the dark. Turn on the appropriate laser on the flow cytometer. Set up a histogram plot to detect TMRE using log scale^[1].

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

CUSTOMER VALIDATION

- Nat Aging. 2024 May 9.
- Adv Sci (Weinh). 2023 Dec 25:e2303388.
- Autophagy. 2023 Feb 13.
- Autophagy. 2021 Dec;17(12):4341-4362.
- Mol Ther. 2024 Apr 23:S1525-0016(24)00253-3.

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REFERENCES

[1]. Crowley LC, et al. Measuring Mitochondrial Transmembrane Potential by TMRE Staining. Cold Spring Harb Protoc. 2016 Dec 1;2016(12):pdb.prot087361.

[2]. Crowley LC, et al. Measuring Mitochondrial Transmembrane Potential by TMRE Staining. Cold Spring Harb Protoc. 2016 Dec 1;2016(12):pdb.prot087361.

[3]. Bantsev V, et al. Confocal laser scanning microscopy imaging of dynamic TMRE movement in the mitochondria of epithelial and superficial cortical fiber cells of bovine lenses. Mol Vis. 2005 Jul 14;11:518-23.

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

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