TMRE

Cat. No.: HY-D0985A CAS No.: 115532-52-0 Molecular Formula: $\mathsf{C}_{26}\mathsf{H}_{27}\mathsf{CIN}_2\mathsf{O}_7$

Molecular Weight: 514.95

Target: Fluorescent Dye

Pathway: Others

4°C, sealed storage, away from moisture and light Storage:

* In solvent: -80°C, 1 year; -20°C, 6 months (sealed storage, away from moisture and

light)

Product Data Sheet

SOLVENT & SOLUBILITY

In Vitro

DMSO: 27.78 mg/mL (53.95 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent Mass Concentration	1 mg	5 mg	10 mg
	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
- 1.1Preparation 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 $4 \ mathbb{m}$ for 3-5 minutes and then discard the supernatant. Wash twice with PBS, 5 minutes each time. The cell density is $1 \times 10^6 / mL$.
- b.Add 1 mL of working solution, and then incubate at room temperature for 5-30 minutes.
- c.Centrifuge at 400 g at 4\pi 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 $100 \, \mu L$ of working solution, gently shake it to completely cover the cells, and then incubate at room temperature for 30- $60 \, \text{minutes}$.
- 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].

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CUSTOMER VALIDATION

- Adv Sci (Weinh). 2023 Dec 25:e2303388.
- Autophagy. 2023 Feb 13.
- Autophagy. 2021 Dec;17(12):4341-4362.
- Biomater Res. 2023 Jul 1;27(1):63.
- Cancer Lett. 2021 Dec 1;522:171-183.

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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]. Bantseev 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.

 $\label{lem:caution:Product} \textbf{Caution: Product has not been fully validated for medical applications. For research use only.}$

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