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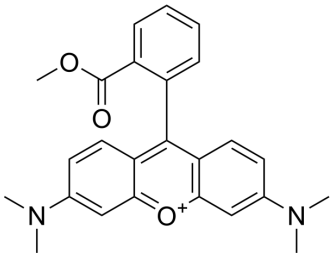
TMRM

Cat. No.:	HY-D0984
CAS No.:	115532-49-5
Molecular Formula:	$C_{25}H_{25}N_2O_3$
Molecular Weight:	401.48
Target:	Fluorescent Dye
Pathway:	Others
Storage:	Please store the product under the recommended conditions in the Certificate of Analysis.



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 TMRM working solution 1.1Preparation of the stock solution Dissolve 1 mg TMRM in 525 µL DMSO to obtain 5 mM of stock solution. 1.2Preparation of TMRM 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 TMRM working solution according to the actual situation. 2.Cell staining 2.1 Suspension cells (6-well plate) a.Centrifuge at 1000 g at 4□ 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□ 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 µL 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]	Cultures are exposed to Millipore-filtered solutions (0.22 µm) containing TMRM and/or drugs for 1 hr at 37°C (except the

experiment involving different durations of exposure to TMRM). After treatment, solutions are removed and growth media reapplied under sterile conditions, and cultures are post-incubated for 18 hours at 37°C (except for the experiment involving analysis at different time points after exposure). Cells are then stained with 2 mg/mL bisbenzimide for 20 min at room temperature. Coverslips are subsequently washed in saline and imaged using 2P microscopy. Apoptotic cells are identified as brightly fluorescent nuclei under UV excitation indicating DNA fragmentation. Cell survivability is calculated as the percentage of live, unstained cells (±SD) in five microscopic fields per treatment[1].

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

CUSTOMER VALIDATION

- Adv Mater. 2023 Mar 29;e2211609.
- Adv Sci (Weinh). 2023 Jan 15;e2203869.
- Autophagy. 2021 Nov;17(11):3592-3606.
- J Thromb Haemost. 2021 Aug 19.
- Int J Mol Sci. 2023 Jul 17, 24(14), 11554.

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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]. Chowdhury SR, et al. Simultaneous evaluation of substrate-dependent oxygen consumption rates and mitochondrial membrane potential by TMRM and safranin in cortical mitochondria. Biosci Rep. 2015 Dec 8;36(1):e00286.

[3]. Monteith A, et al. Imaging of mitochondrial and non-mitochondrial responses in cultured rat hippocampal neurons exposed to micromolar concentrations of TMRM. PLoS One. 2013;8(3):e58059.

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