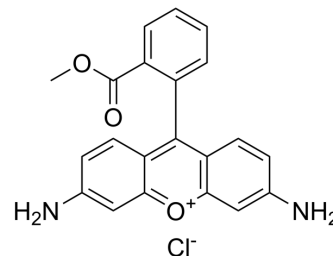


Rhodamine 123

Cat. No.:	HY-D0816
CAS No.:	62669-70-9
Molecular Formula:	C ₂₁ H ₁₇ ClN ₂ O ₃
Molecular Weight:	380.82
Target:	Fluorescent Dye
Pathway:	Others
Storage:	4°C, sealed storage, away from moisture and light * In solvent : -80°C, 2 years; -20°C, 1 year (sealed storage, away from moisture and light)



SOLVENT & SOLUBILITY

In Vitro

DMSO : 62.5 mg/mL (164.12 mM; Need ultrasonic)

	Solvent Concentration	Mass	1 mg	5 mg	10 mg
Preparing Stock Solutions	1 mM		2.6259 mL	13.1296 mL	26.2591 mL
	5 mM		0.5252 mL	2.6259 mL	5.2518 mL
	10 mM		0.2626 mL	1.3130 mL	2.6259 mL

Please refer to the solubility information to select the appropriate solvent.

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

- Preparation of Rhodamine 123 working solution
 - Preparation of the stock solution
Dissolve 1 mg Rhodamine 123 in 525 µL DMSO to obtain 5 mM of stock solution.
 - Preparation of Rhodamine 123 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 Rhodamine 123 working solution according to the actual situation.
- Cell staining
 - Suspension cells (6-well plate)
 - 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 1×10⁶/mL.
 - 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 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

Kinase Assay ^[1]

Measurements are made at room temperature with continuous stirring of the mitochondrial suspension using spectrophotometer equipped with a magnetic stirrer with fluorescent cation R123 as probe. Excitation and emission wavelengths are 503 nm and 527 nm, respectively. The incubation medium is the respiration buffer. R123 and sodium pyruvate are added to final concentrations of 50 nM and 10 mM, respectively. Isolated mitochondria maintain a steady membrane potential ($\pm 5\%$) throughout the duration of the recording^[1].

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

CUSTOMER VALIDATION

- Phytomedicine. 2020 Nov;78:153329.
- Life Sci. 2024 Feb 14;122505.
- The Journal of Nutritional Biochemistry. 2020 Sep;83:108404.
- J Ethnopharmacol. 2023 May 9, 116566.
- Foods. 2023 Dec 20, 13(1), 23.

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REFERENCES

- [1]. Emaus, R. K., Grunwald, R., & Lemasters, J. J. (1986). Rhodamine 123 as a probe of transmembrane potential in isolated rat-liver mitochondria: spectral and metabolic properties. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 850(3), 436–448.
- [2]. M. Huang, et al. Mitochondrial Inner Membrane Electrophysiology Assessed by Rhodamine-123 Transport and Fluorescence. *Ann Biomed Eng.* 2007 Jul; 35(7): 1276–1285.

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

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