

Product Information

Dihydrorhodamine 123

Product List

Catalog no.	Product	MW	Unit size
10055	Dihydrorhodamine 123	346	10 mg
10056	Dihydrorhodamine 123, dihydrochloride salt	419	10 mg
10056-1			20 x 500 ug

Molecular Information:

Product	MW	Formula	Solubility
Dihydrorhodamine 123	346	C ₂₁ H ₁₈ N ₂ O ₃	Soluble in DMF (>10 mg/mL)
Dihydrorhodamine 123, dihydrochloride salt	419	C ₂₁ H ₂₀ Cl ₂ N ₂ O ₃	

CAS number: 109244-58-8

Color and Form: Pinkish solid

Absorption/Emission: 505/534 nm (end product)

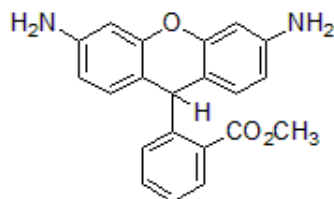


Figure 1. Dihydrorhodamine 123

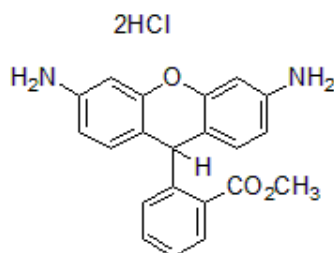


Figure 2. Dihydrorhodamine 123, dihydrochloride salt

Storage and Handling

Store desiccated at -20°C under nitrogen or argon, and protect from light. Product is stable for at least 12 months from date of receipt when stored as recommended.

We recommend preparing a 10 mM dye stock solution in DMF on the day of use. The stock solution may also be stored with desiccant and protected from light at -20°C, for at least 1 month.

Note: DMSO is not recommended for making stock solutions, because it may cause oxidation of the compound.

Product Description

Dihydrorhodamine 123 is the reduced form of rhodamine 123 (70010), which is a commonly used fluorescent mitochondrial dye. Dihydrorhodamine 123 itself is nonfluorescent, but it readily enters cells and is oxidized by oxidative species or by cellular redox systems to the fluorescent rhodamine 123 that accumulates in mitochondrial membranes (1). Dihydrorhodamine 123 is useful for detecting reactive oxygen species (ROS) including superoxide (in the presence of peroxidase or cytochrome c (2,3) and peroxynitrite (4,5)). Dihydrorhodamine 123 dihydrochloride (10056) is a more stable and water soluble form of dihydrorhodamine 123.

References

1) J. Immunol. Meth. 178, 89(1995); 2) Eur. J. Biochem. 217, 973(1993); 3) Arc. Biochem. Biophys. 302, 348(1993); 4) Biochemistry 34, 3544(1995); 5) Free Rad. Biol Med. 16, 149(1994).

Experimental Protocols

The following protocol is provided as an example. Cellular treatments, dye concentration, and/or staining time may require optimization for different cell types or applications.

We recommend including the following positive and negative controls:

- Untreated cells
- Positive control cells treated with a ROS inducing agent validated for your cell type. We have used 100 uM menadione in cell culture medium for 1 hour at 37°C to induce ROS in HeLa cells.
- Cells treated with antioxidant agent validated for your cell type to suppress ROS. We have used 5 mM N-acetylcysteine (NAC) in cell culture medium for 1 hour at 37°C to suppress menadione-induced ROS in HeLa cells.

Staining procedure

1. Optional: Treat cells with inducers or inhibitors of reactive oxygen species.

Note: If treating cells with oxidizing agents, rinse cells three times with buffer or culture medium to remove the compounds before staining to avoid direct oxidation of the dye by the compound.
2. Dilute dihydrorhodamine dye stock solution in complete cell culture medium at a final concentration of 5 uM.
3. Remove medium from the cells and replace with medium containing dye.
4. Incubate for 30 minutes at 37°C.
5. Detect staining in the FITC channel by fluorescence microscopy or flow cytometry. Washing is not required, but can be performed to reduce background for microscopy.

Related Products

Catalog number	Product
70010	Rhodamine 123
10057	Dihydroethidium (Hydroethidium)
10058	H ₂ DCFDA (2'7'-Dichlorodihydrofluorescein diacetate)
00301	2,3-Diaminonaphthalene
00300	DAA
70054... 70075	MitoView™ Mitochondrial Dyes
30001	JC-1 Mitochondrial Membrane Potential Detection Kit
70005	TMRE, 2 mM in DMSO
70016	TMRE
70017	TMRM
30100	Griess Reagent Kit
00239	Nitric Oxide Generation Kit
00249	NOS Inhibitor Kit

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