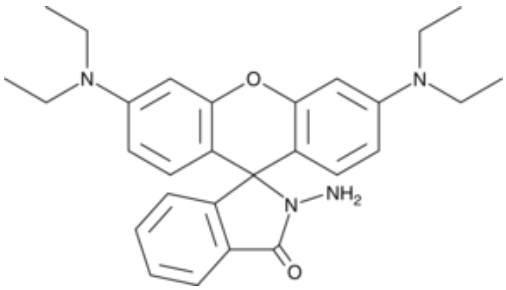
Product Data Sheet

Product Name: Rhodamine B hydrazide
Cat. No.: GC44832

Chemical Properties

Cas. No.	74317-53-6		
SMILES	CCN(CC)C1=CC2=C(C=C1)C3(C(C=CC=C4)=C4C(N3N)=O)C5=CC=C(N(CC)CC)C=C5O2		
Formula	$C_{28}H_{32}N_4O_2$	M.Wt	456.6
Solubility	DMF: 30 mg/ml,DMF:PBS(pH7.2) (1:2): 0.33 mg/ml,DMSO: 20 mg/ml,Ethanol: 0.1 mg/ml	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		

Structure



Protocol

This plan only provides a guide, please modify it to meet your specific needs.

1. Prepare staining solution

(1) Prepare stock solution: Dissolve Rhodamine B hydrazide in DMSO and prepare a stock solution with a concentration of 5-10mM.

Note: Unused stock solution should be aliquoted and stored in the dark at -20°C or -80°C to avoid repeated freezing and thawing.

Caution: Product has not been fully validated for medical applications. For research use only.
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(2) Prepare working solution: Dilute the stock solution with a suitable buffer (such as serum-free medium or PBS) and prepare a working solution with a concentration of 5-10μM.

Note: Please adjust the concentration of the working fluid according to the actual situation and prepare it now.

2. Cell suspension staining

(1) Suspension cells: Centrifuge at 1000 *g* for 3-5 minutes at 4°C, discard the supernatant, and wash twice with PBS for 5 minutes each time.

(2) Adherent cells: Wash twice with PBS, add trypsin to digest the cells, and centrifuge at 1000 *g* for 3-5 minutes after digestion is completed.

(3) Add Rhodamine B hydrazide working solution to resuspend the cells, and incubate at 37°C in the dark for 2 hours. The optimal incubation time for different cells is different, please explore by yourself according to the specific experimental needs.

(4) After the incubation, centrifuge at 1000 *g* for 5 minutes, remove the supernatant, add PBS and wash 2-3 times, 5 minutes each time.

(5) Resuspend cells in pre-warmed serum-free cell culture medium or PBS and observe by fluorescence microscope or flow cytometry.

3. Cell adhesion staining

(1) Culture adherent cells on sterile coverslips.

(2) Remove the coverslip from the culture medium, suck out the excess culture medium, and place the coverslip in a humid environment.

(3) Add 100μL of dye working solution from one corner of the coverslip and shake gently to evenly cover all cells with the dye.

(4) Incubate at 37°C in the dark for 2 hours. The optimal incubation time for different cells is different, please explore by yourself according to the specific experimental needs.

(5) After the incubation, discard the dye working solution and use pre-warmed culture solution to

wash the coverslip 2 to 3 times.

4. Detect intracellular nitric oxide production

(1) Incubate cells with 5μM Rhodamine B hydrazide (2.5%, v/v DMSO) for 4 hours at 37°C in the dark;

(2) Discard the dye solution and wash twice with PBS;

(3) Incubate the cells with DMEM containing 375μM SNAP (S-nitroso-N-acetyl-D,L-penicillamine, a NO releasing agent) for 2 hours at 37°C in the dark;

(4) Wash twice with PBS, then soak the sample in PBS.

5. Microscope detection: The maximum excitation wavelength and emission wavelength of Rhodamine B hydrazide are 546/610nm respectively.

Precautions:

1) Fluorescent dyes all have quenching problems. Please avoid light as much as possible to slow down fluorescence quenching.

2) For your safety and health, please wear a lab coat and disposable gloves.

References:

[1]. Chi-Ming Wu,et,al. Sensitivity evaluation of rhodamine B hydrazide towards nitric oxide and its application for macrophage cells imaging. 2011 Dec 5;708(1-2):141-8. doi: 10.1016/j.aca.2011.10.005. Epub 2011 Oct 8.

Background

Rhodamine B hydrazide is a water-soluble fluorescent probe with excitation/emission spectra of 510/578 nm, respectively. It has been used for the detection of copper, peroxynitrite, nitric oxide, hydrogen peroxide, glucose, diacetyl, and hemoglobin.