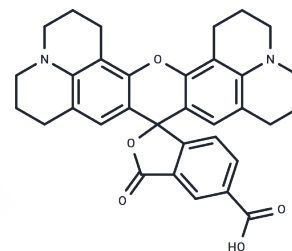


5-ROX

Chemical Properties

CAS No. :	216699-35-3
Formula:	C33H30N2O5
Molecular Weight:	534.6
Appearance:	no data available
Storage:	keep away from direct sunlight Powder: -20°C for 3 years In solvent: -80°C for 1 year



Biological Description

Description	5-ROX (5-Carboxy-X-rhodamine), a rhodamine dye, exhibits strong fluorescence in aqueous buffer with an excitation wavelength (λ_{excit}) of 580 nm ($\epsilon = 3.6 \times 10^4 / (\text{M} \cdot \text{cm})$) and an emission wavelength (λ_{emit}) of 604 nm ($\phi = 0.94$).
Targets(IC50)	Others
In vitro	<p>I. Nucleic acid or protein labeling</p> <p>1. Material preparation:</p> <ol style="list-style-type: none"> 1) 5-ROX dye (usually in the form of NHS ester or succinimidyl ester). 2) Nucleic acid or protein sample. 3) Buffer (such as 0.1 M NaHCO_3, pH 8.5). 4) Organic solvent (such as DMSO or DMF, used to dissolve the dye). <p>2. Labeling steps:</p> <ol style="list-style-type: none"> 1) Dissolve the dye: Dissolve 5-ROX dye in anhydrous DMSO or DMF, usually at a concentration of 10 mM. <p>Note: The dye solution should be kept away from light and used as soon as possible.</p> <ol style="list-style-type: none"> 2) Prepare the labeling reaction: Add 5-ROX solution to the nucleic acid or protein sample, usually at a molar ratio of 10:1 (dye: target molecule); add an appropriate amount of buffer (such as 0.1 M NaHCO_3, pH 8.5) to the reaction system to ensure that the reaction environment is suitable for the formation of amide bonds. 3) Incubation: Incubate at room temperature for 1-2 hours (protein labeling) or 2-4 hours (nucleic acid labeling), avoiding strong light exposure. 4) Purification: Use ultrafiltration centrifuge tubes, gel filtration columns or HPLC to purify unbound dye molecules. 5) Storage: Dissolve the labeled product in an appropriate buffer (such as TE buffer) and store at -20°C away from light. <p>II. Real-time PCR (qPCR) application</p> <p>1. Material preparation:</p> <ol style="list-style-type: none"> 1) 5-ROX fluorescent dye. 2) Real-time PCR reaction mixture (including template, primers, probe and polymerase). 3) Appropriate fluorescence detection equipment (such as qPCR instrument with orange-red light channel). <p>2. Usage steps:</p> <ol style="list-style-type: none"> 1) Add 5-ROX: Add an appropriate amount of 5-ROX according to the equipment requirements (usually a final concentration of 50-100 nM per 50 μL reaction system).

- 2) Mix well: Gently mix the reaction solution to avoid bubbles.
- 3) Run PCR: Detect fluorescence signals within the wavelength range supported by the device (excitation ~580 nm, emission ~600 nm).
- Notes:
- 1) Avoid light: 5-ROX is light-sensitive and should be kept away from light as much as possible during operation and storage. It is recommended to store it at 4°C for short term and -20°C for long term.
- 2) Solvent selection: Use anhydrous DMSO or DMF to ensure the stability of the dye.
- 3) Optimize labeling efficiency: The pH and dye concentration of the sample should be adjusted according to the characteristics of the target molecule to optimize the labeling efficiency.
- 4) Safety: Avoid skin contact and inhalation, and wear gloves and goggles during operation.

Solubility Information

Solubility	DMSO: 120 mg/mL (224.47 mM), Sonication is recommended. (< 1 mg/mL refers to the product slightly soluble or insoluble)
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Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	1.8706 mL	9.3528 mL	18.7056 mL
5 mM	0.3741 mL	1.8706 mL	3.7411 mL
10 mM	0.1871 mL	0.9353 mL	1.8706 mL
50 mM	0.0374 mL	0.1871 mL	0.3741 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Reference

Kricka LJ. Clinical and biochemical applications of luciferases and luciferins. Anal Biochem. 1988 Nov 15;175(1):14-21.

Kenneth J. Livak, Thomas D. Schmittgen. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method, Methods, Volume 25, Issue 4, 2001, Pages 402-408, ISSN 1046-2023,

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