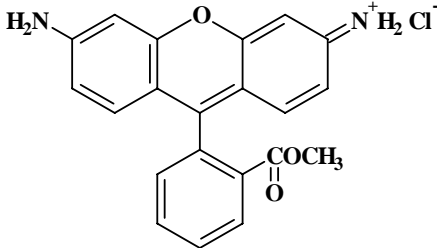


## Instruction Manual

Catalog Number	PK-CA707-70010
Description	Rhodamine 123 (Rh123) is a popular green fluorescent mitochondrial dye that stains mitochondria in living cells in a membrane potential-dependent fashion. This membrane permeable dye localizes in mitochondria of viable cells to emit a yellowish-green fluorescence. Rh123 is utilized for staining a wide variety of cells, including plant cells and bacteria. Since there is a correlation between the amount of ATP in a cell and the fluorescence intensity of Rh123, this compound is applied for the detection of intracellular ATP. Rh123 is also used in cancer research and in flow cytometry studies involving mitochondrial membrane potential.
Quantity	50 mg
Excitation / Emission Maxima	$\lambda_{ex}/\lambda_{em}$ (in MeOH) = 505/534 nm Extinction coefficient = 97,000 (505 nm, in MeOH)
Molecular Structure	 <p>The chemical structure of Rhodamine 123 consists of a central xanthene ring system. It features a primary amine group (-NH<sub>2</sub>) at the 6-position and a trimethylammonium group (-N<sup>+</sup>(CH<sub>3</sub>)<sub>3</sub>) at the 10-position. At the 4-position, there is a phenyl ring substituted with an acetyl group (-COCH<sub>3</sub>).</p>
Molecular Weight / Molecular Formula	381 Da; C <sub>21</sub> H <sub>17</sub> ClN <sub>2</sub> O <sub>3</sub>
Purity	>96% (as determined by HPLC)
Appearance / Formulation / Solubility	Orange-red solid; soluble in MeOH and DMF.
Storage & Stability	Store desiccated at 4°C. Protect from light.
Applications	<p>General Staining Procedure:</p> <ol style="list-style-type: none"> <li>1. Dissolve 0.4 mg Rh123 in 1 ml DMSO to prepare 1 mM Rh123-DMSO solution.</li> <li>2. Prepare cells with a glass slide. The cell number will be 5×10<sup>4</sup> to 5×10<sup>5</sup> cells per ml.</li> <li>3. Incubate the slide and wash cells with PBS or Hank's medium.</li> <li>4. Dilute the 1 mM Rh123 solution with culture medium to prepare 1-20 μM Rh123 buffer solution.</li> <li>5. Add the Rh123 buffer solution to the glass slide and incubate at 37 °C for 30 minutes to 1 hour.<sup>a)</sup></li> <li>6. Remove the Rh123 buffer solution and wash cells with culture medium.<sup>b)</sup></li> <li>7. Observe the cells using a fluorescence microscope with a fluorescein filter.</li> </ol> <p>a) Incubate the Rh123 buffer solution at 37 °C prior to adding to cells. b) For fixing after washing cells, add 10% formarin buffer and incubate for 15-20 min, and then wash with PBS.</p>
References	<p>References:</p> <ol style="list-style-type: none"> <li>1) Millot, J.M., et al. Cytometry 17, 50(1994)</li> <li>2) Bernal, S.D., et al Science 218, 1117(1982)</li> <li>3) Johnson, L.V., et al. J. Cell Biol. 88, 526(1981)</li> </ol>
Caution	Potentially harmful. Avoid prolonged or repeated exposure. Avoid getting in eyes, on skin, or on clothing. Wash thoroughly after handling. If eye or skin contact occurs, wash affected areas with plenty of water for 15 minutes and seek medical advice. In case of inhaling or swallowing, move individual to fresh air and seek medical advice immediately.

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