Rhod-5N, AM

Catalog number: 21070
Unit size: 1 mg

Component	Storage	Amount (Cat No. 21070)
Rhod-5N, AM	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)

OVERVIEW

Calcium measurement is critical for numerous biological investigations. Fluorescent probes that show spectral responses upon binding Ca2+ have enabled researchers to investigate changes in intracellular free Ca2+ concentrations by using fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers. Rhod-5N has a lower binding affinity for Ca2+ (Kd = ~320 μM) than any other BAPTA-based indicator and is suitable for Ca2+ measurements from 10 μM to 1 mM. Like the parent Rhod-2 indicator, Rhod-5N is essentially nonfluorescent in the absence of divalent cations and exhibits strong fluorescence enhancement with no spectral shift upon binding Ca2+. Rhod-5N AM is cell-permeable version of Rhod-5N.

KEY PARAMETERS

Fluorescence microscope

Emission	TRITC filter set
Excitation	TRITC filter set
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Cutoff	570
Emission	590
Excitation	540
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Bottom read mode/Programmable liquid handling

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Rhod-5N AM Stock Solution

1. Prepare a 2 to 5 mM stock solution of Rhod-5N AM in high-quality, anhydrous DMSO.

PREPARATION OF WORKING SOLUTION

Rhod-5N AM Working Solution

1. On the day of the experiment, either dissolve Rhod-5N AM in DMSO or thaw an aliquot of the indicator stock solution to room temperature.

2. Prepare a 2 to 20 μM Rhod-5N AM working solution in a buffer of your choice (e.g., Hanks and Hepes buffer) with 0.04% Pluronic® F-127. For most cell lines, Rhod-5N AM at a final concentration of 4-5 μM is recommended. The exact concentration of indicators required for cell loading must be determined empirically.

   Note: The nonionic detergent Pluronic® F-127 is sometimes used to increase the aqueous solubility of Rhod-5N AM. A variety of Pluronic® F-127 solutions can be purchased from AAT Bioquest.

   Note: If your cells contain organic anion-transporters, probenecid (1-2 mM) may be added to the dye working solution (final in well concentration will be 0.5-1 mM) to reduce leakage of the de-esterified indicators. A variety of ReadiUse™ Probenecid products, including water-soluble, sodium salt, and stabilized solutions, can be purchased from AAT Bioquest.

SAMPLE EXPERIMENTAL PROTOCOL

Following is our recommended protocol for loading AM esters into live cells. This protocol only provides a guideline and should be modified according to your specific needs.

1. Prepare cells in growth medium overnight.

2. On the next day, add 1X Rhod-5N AM working solution to your cell plate.

   Note: If your compound(s) interfere with the serum, replace the growth medium with fresh HHBS buffer before dye-loading.

3. Incubate the dye-loaded plate in a cell incubator at 37 °C for 30 to 60 minutes.

   Note: Incubating the dye for longer than 1 hour can improve signal intensities in certain cell lines.

4. Replace the dye working solution with HHBS or buffer of your choice (containing an anion transporter inhibitor, such as 1 mM probenecid, if applicable) to remove any excess probes.
5. Add the stimulant as desired and simultaneously measure fluorescence using either a fluorescence microscope equipped with a TRITC filter set or a fluorescence plate reader containing a programmable liquid handling system such as an FDSS, FLIPR, or FlexStation, at Ex/Em = 540/590 nm cutoff 570 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

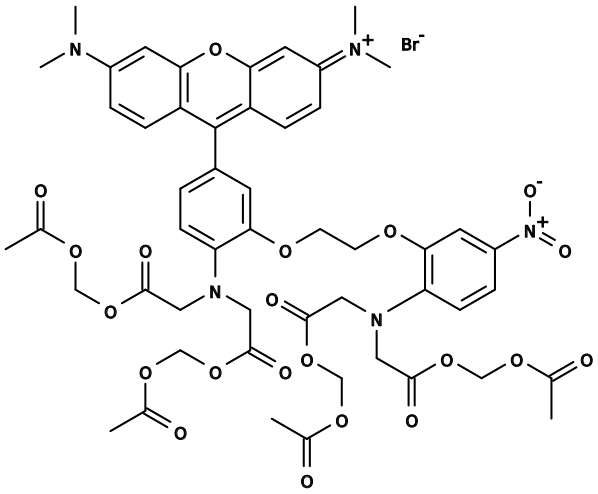

Figure 1. Chemical structure for Rhod-5N, AM

DISCLAIMER

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