

Rhod-2, AM *UltraPure Grade* *CAS#: 145037-81-6*

 Catalog number: 21062, 21063, 21064
 Unit size: 1 mg, 50 mg, 20x50 ug

Component	Storage	Amount (Cat No. 21062)	Amount (Cat No. 21063)	Amount (Cat No. 21064)
Rhod-2, AM	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)	1 vial (50 mg)	20x50 ug

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. The long-wavelength Rhod-2 Ca²⁺ indicators are valuable alternatives to Fluo-3 for experiments in cells and tissues that have high levels of autofluorescence. Rhod-2 AM is cell-permeable version of Rhod-2.

KEY PARAMETERS
Fluorescence microscope

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

Fluorescence microplate reader

Excitation	540
Emission	590
Cutoff	570
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-2 AM *UltraPure Grade* Stock Solution

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

PREPARATION OF WORKING SOLUTION
Rhod-2 AM *UltraPure Grade* Working Solution

On the day of the experiment, either dissolve Rhod-2 AM in DMSO or thaw an aliquot of the indicator stock solution to room temperature. Prepare a dye working solution of 2 to 20 μM in a buffer of your choice (e.g., Hanks and Hepes buffer) with 0.04% Pluronic® F-127. For most cell lines, Rhod-2 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-2 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 solution, 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-2 AM working solution into 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.

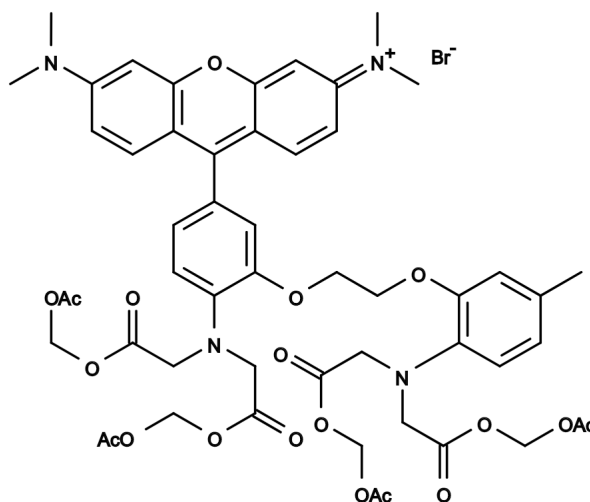
EXAMPLE DATA ANALYSIS AND FIGURES


Figure 1. Chemical structure for Rhod-2, AM *UltraPure Grade* *CAS#: 145037-81-6*

DISCLAIMER

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