Rhod-4™, AM

Catalog number: 21120, 21121, 21122, 21123
Unit size: 1 mg, 5x50 ug, 10x50 ug, 20x50 ug

Component	Storage	Amount (Cat No. 21120)	Amount (Cat No. 21121)	Amount (Cat No. 21122)	Amount (Cat No. 21123)
Rhod-4™, AM	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)	5x50 ug	10x50 ug	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. Rhod-2 is most commonly used among the red fluorescent calcium indicators. However, Rhod-2 AM is only moderately fluorescent in live cells upon esterase hydrolysis, and has very small cellular calcium responses. Rhod-4™ has been developed to improve Rhod-2 cell loading and calcium response while maintaining the spectral wavelength of Rhod-2. In CHO and HEK cells Rhod-4™ AM has cellular calcium response that is 10 times more sensitive than Rhod-2 AM. AAT Bioquest offers versatile packing sizes of Quest Rhod-4 to meet your special needs, e.g., 1 mg; 10x50 µg; 20x50 µg; HTS packages with no additional packaging charge.

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-4™ AM Stock Solution
Prepare a 2 to 5 mM stock solution of Rhod-4™ AM in high-quality, anhydrous DMSO.

PREPARATION OF WORKING SOLUTION

Rhod-4™ AM Working Solution
On the day of the experiment, either dissolve Rhod-4™ 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-4™ 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-4™ 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-4™ 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

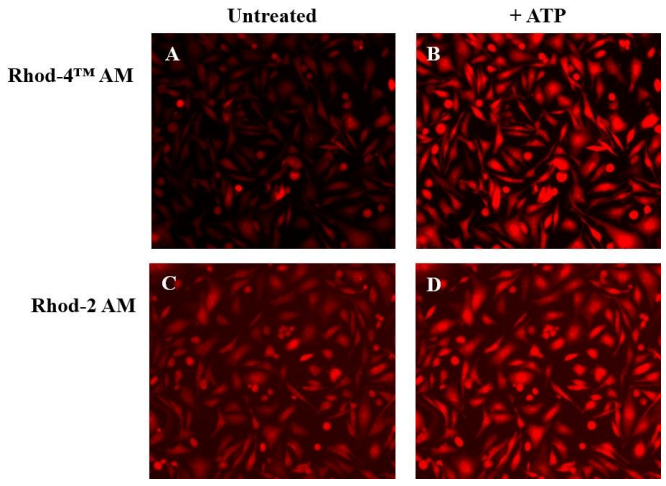

Figure 1. ATP-stimulated calcium responses of endogenous P2Y receptors were measured in CHO-K1 cells with Rhod-4™ AM (Cat# 21120) and Rhod-2 AM (Cat# 21064). CHO-K1 cells were seeded overnight at 50,000 cells/100 µL/well in a Costar 96-well black wall/clear bottom plate. The growth medium was removed, and the cells were incubated with 100 µL of dye loading solution using Rhod-4™

AM (4 μM, A and B) or Rhod-2 AM (4 μM, C and D) for 1 hour in a 37 °C, 5% CO2 incubator. The staining solution was replaced with 200 μL HHBS, then the cells were imaged before (A and C) and after (B and D) ATP treatment with a fluorescence microscope (Olympus IX71) using TRITC channel.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.