Rhod-FF, AM

Catalog number: 21077, 21078
Unit size: 1 mg, 10x50 ug

Component	Storage	Amount (Cat No. 21077)	Amount (Cat No. 21078)
Rhod-FF, AM	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)	10x50 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-FF AM is cell-permeable, and generates Rhod-FF upon esterase hydrolysis in cells. Rhod-FF has a lower binding affinity for Ca2+ and is suitable for Ca2+ measurements from 10 to 200 uM. Like the parent Rhod-2 indicator, Rhod-FF is essentially nonfluorescent in the absence of divalent cations and exhibits strong fluorescence enhancement with no spectral shift upon binding Ca2+.

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

PREPARATION OF WORKING SOLUTION

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

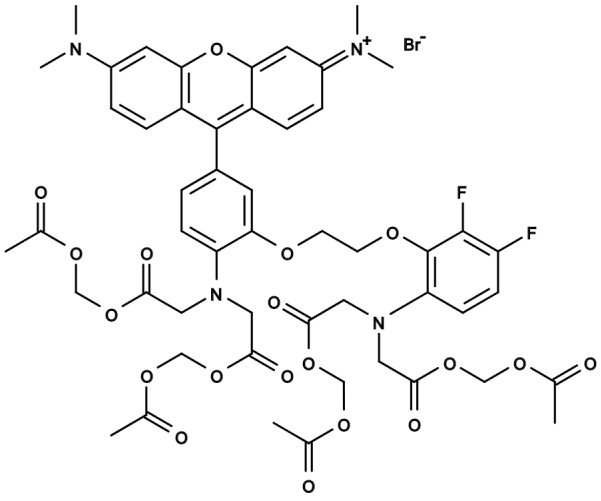

Figure 1. Chemical structure for Rhod-FF, AM

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.