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

Catalog number: 21060
Unit size: 1 mg

Component	Storage	Amount
Rhod-2, AM *CAS#: 145037-81-6*	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. 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 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 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

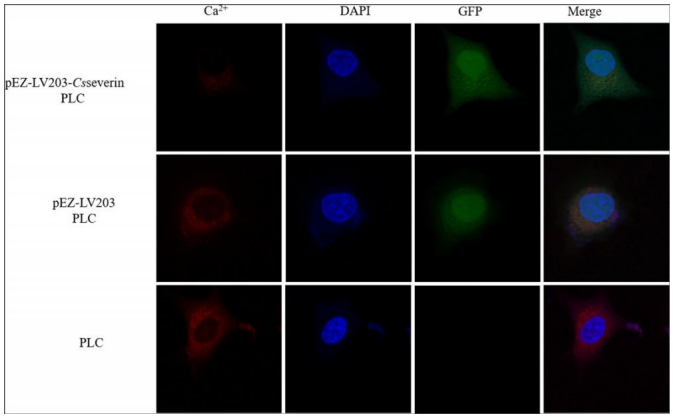

Figure 1. The intracellular distribution of free Ca^{2+} was analyzed by laser scanning confocal microscopy.
Intracellular free Ca^{2+} was detected by red fluorescent probe dihydrorhod-2 AM (Rhod-2 AM). The nuclei were stained with DAPI (blue). The pEZ-LV203 vector harboring the eGFP reporter gene produced green fluorescent protein. Source: **Csseverin inhibits apoptosis through mitochondria-mediated pathways triggered by Ca^{2+} dyshomeostasis in hepatocarcinoma PLC cells** by Shi M et al., *PLOS,* Nov. 2017.

DISCLAIMER

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