Rhod-2 AM (Bromide)

Dyes and Stains

A cell-permeable calcium dye that exhibits fluorescence upon increasing Ca2+ concentrations

Catalog #100-0992

1 mg

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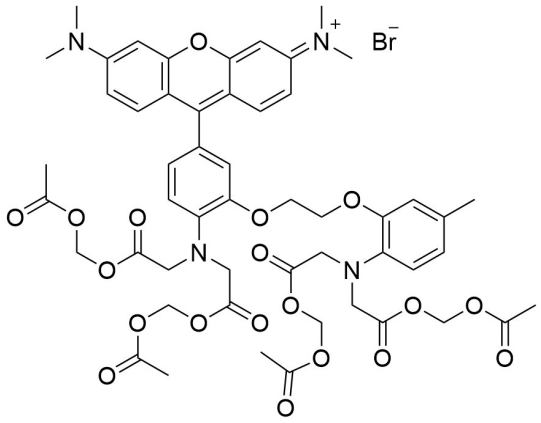
Product Description

Rhod-2 AM (Bromide) is a cell-permeable acetoxymethyl (AM) ester dye which fluoresces in the presence of Ca2+. Cellular uptake is facilitated by the AM group and is removed by cytoplasmic esterases, resulting in intracellular accumulation of a cell-impermeable Ca2+ indicator, Rhod-2 (Smith et al.). Increasing Ca2+ concentrations significantly enhance Rhod-2 AM fluorescence, which makes the long wavelength of this dye valuable for applications involving cells with high levels of autofluorescence. Increased intracellular calcium concentrations are a critical indicator leading to the activation of apoptotic pathways (Mukherjee et al.). Use Rhod-2 AM (Bromide) dye to measure mitochondrial calcium influx by detection using flow cytometry, fluorescence microscopy, and microplate readers.

CAS Number:	145037-81-6
Chemical Formula:	$C_{52}H_{59}N_4O_{19}$ • Br
Molecular Weight:	1124.0 g/mol
Excitation Wavelength:	553 nm
Emission Wavelength:	577 nm
Cutoff:	570 nm
Structure:	



Properties

Storage:	Store at -20°C.
Shelf Life:	Stable until expiry date (EXP) on label. Protect product from prolonged exposure to light.
Format:	Red solid

Directions for Use

Please read the entire protocol before proceeding. The following protocol is for staining adherent cells in a black-wall/clear-bottom plate.

Preparation of Rhod-2 AM (Bromide) Stock and Working Solution

1. To prepare a stock solution, dissolve Rhod-2 AM (Bromide) in dimethyl sulfoxide (DMSO) at 2 - 5 mM.

   NOTE: If not used immediately, aliquot and store at -20°C. Do not exceed the expiry date as indicated on the label. Avoid repeated freeze-thaw cycles.
2. To prepare the Rhod-2 AM (Bromide) working solution, dilute the stock solution (prepared in step 2) to 2 - 20 µM with Hanks' Balanced Salt Solution with 20 mM HEPES (HHBS) or a buffer of choice. Use the working solution immediately; do not store.

   NOTE: Although 4 - 5 µM is the recommended final concentration for most cell lines, titrate the dye for optimal performance.
3. (OPTIONAL): Additional reagents may be added to the working solution to improve performance as follows:
   - To increase the solubility of Rhod-2 AM (Bromide), add 0.04% Pluronic® F-127 (AAT Bioquest Catalog #20053).
   - To reduce leakage of de-esterified indicators in cells that contain organic anion transporters, add ReadiUse™ probenecid (AAT Bioquest) at a final concentration of 0.5 - 1.0 mM.

Staining Samples

1. Plate cells in a black-wall/clear-bottom plate with culture medium and allow cells to adhere overnight.
2. Add the Rhod-2 AM (Bromide) working solution directly to the culture medium at the desired concentration.

   NOTE: If serum in the culture medium interferes with your stimulant of choice, replace the culture medium with HHBS before adding the working solution.
3. Incubate at 37°C and 5% CO_2 for at least 30 minutes.

   NOTE: The optimal incubation time should be determined for different cell lines.
4. Remove the Rhod-2 AM (Bromide) working solution and wash the cells with HHBS or a buffer of choice.

   NOTE: For cells that contain organic anion transporters, use buffer containing 1 mM ReadiUse™ probenecid.

Fluorescence Detection

Add a stimulant of choice and monitor the fluorescence intensity as follows:

- Fluorescence microscope: TRITC or appropriate filter set
- Fluorescence microplate reader containing a programmable liquid-handling system, such as a functional drug-screening system (FDSS), FLIPR®, or FlexStation®: Ex/Em = 540/590 nm (cutoff = 570 nm)

References

1. Mukherjee SB et al. (2002) Increase in cytosolic Ca2+ levels through the activation of non-selective cation channels induced by oxidative stress causes mitochondrial depolarization leading to apoptosis-like death in Leishmania donovani promastigotes. J Biol Chem 277(27): 24717–27.
2. Smith NA et al. (2018) Fluorescent Ca2+ indicators directly inhibit the Na,K-ATPase and disrupt cellular functions. Sci Signal 11(515): eaal2039.