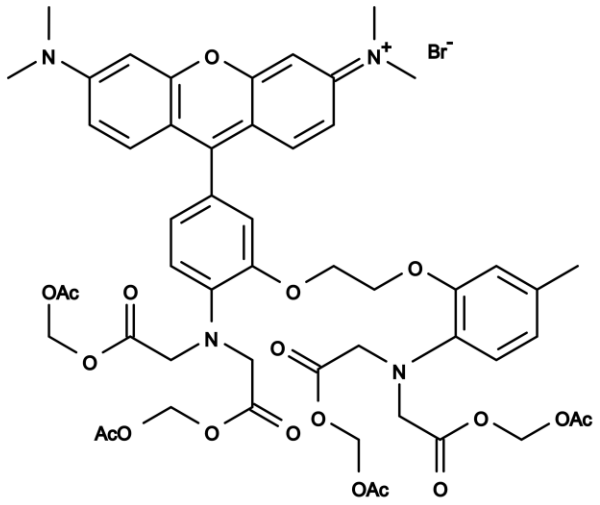
Rhod-2 AM, fluorescent Ca indicator

Cat. No. CTG-CV0004 Store the kit at -15 to -25°C

Product Description

Rhod-2 AM has the longest fluorescent emission signal of the commonly used calcium indicators. It contains a rhodamine-like fluorophore, making it suitable for use with argon and krypton laser excitation sources. Rhod-2 signal intensity one of the strongest of all the calcium probes. Rhod 2 is especially suitable probe for intracellular calcium monitoring by confocal laser-scanning microscopy and flow cytometry. The dissociation constant of Rhod 2 with calcium is among the highest of all the fluorescent calcium probes, providing a wider range for monitoring calcium concentration. Rhod-2 AM is an acetoxymethyl ester derivative of Rhod-2 that can be easily loaded into cells by passive incubation. Wavelength Maxima: Excitation 549nm, Emission 578nm.



Specifications

Purity	> 93%
CAS Number	145037-81-6
Excitation	552nm
Emission	581nm
Chemical name	9-[4-[Bis[2-[(acetyloxy)methoxy]-2-oxoethyl]amino]-3-[2-[2-[bis[2-[(acetyloxy) methoxy]-2-oxoethyl] amino]-5-methylphenoxy]ethoxy] phenyl]-3,6-bis (dimethylamino) xanthylium bromide
Molecular weight	1123.96
Molecular formula	$C_{52}H_{59}BrN_4O_{19}$
PubChemidentifier	25229581
Solubility	Soluble in DMSO to 1 mg/ml

Kit Components

50ug

Kit storage/stability

Store at -20°C. It is important to note that this product is reported to be light sensitive. Store In the Dark. Store under desiccating conditions. Handling Wherever possible, you should prepare and use solutions on the same day. However, if you need to make up stock solutions in advance, we recommend that you store the solution as aliquots in tightly sealed vials at -20°C. Generally, these will be useable for up to one month.

Usage
Celltechgen provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please request the Safety Data Sheet (SDS) provided for the product.

Experimental Protocol

Introduction
The cell-permeant AM ester derivative of rhod-2 (R-1244, R-1245) has a net positive charge. This property promotes its sequestration into mitochondria in some cells, most likely via membrane potential-driven uptake. By reducing rhod-2 AM to the colorless, nonfluorescent dihydrorhod-2 AM, the discrimination between cytosolic and mitochondrially localized dye can be further improved.

The AM ester of dihydrorhod-2 exhibits Ca2+-dependent fluorescence only after it is oxidized and its AM esters are cleaved to yield the rhod-2 indicator, processes that occur rapidly in the mitochondrial environment. Reduction of rhod-2 AM (R-1244, R-1245) to dihydrorhod-2 AM can be readily accomplished using the protocol given below.

Protocol
1. Dissolve 50 µg of rhod-2 AM (one vial of the set of 20 supplied as product R-1245) in 100 µL of anhydrous dimethylsulfoxide (DMSO).
2. Add a small excess of sodium borohydride ($NaBH_4$) either as a solid or as a methanol solution. The smallest amount of solid $NaBH_4$ that can be practicably transferred will provide a sufficient excess.
3. Incubate for 10 minutes or until the reaction mixture appears colorless, whichever is sooner.
4. Use the reaction solution in DMSO (about 0.4 mM dihydrorhod-2 AM) directly for cell loading according to usual AM ester loading protocols (see our product information sheets Rhod-2 and X-rhod-1 Calcium Indicators (MP1244) or Acetoxymethyl (AM) Esters (G002)).
5. Dihydrorhod-2 AM will spontaneously and quite rapidly revert to the oxidized form, marked by reappearance of color in the stock solution. Therefore experiments using dihydro rhod-2 AM should be carried out as soon as possible after preparation.

References
1. Cell 82, 415 (1995).