FT-66158

Rhod-2

Product Information

Product name cat.number	MW ($g \cdot mol^{-1}$)	$\lambda_{exc}/\lambda_{em}$ max. Low Ca^{2+} (nm)	$\lambda_{exc}/\lambda_{em}$ max. High Ca^{2+} (nm)	mol. abs. ($M^{-1}cm^{-1}$)	Kd (nM)	Soluble In
Rhod-2, AM ester bromide **FP-661582**, 1mg FP-661584, 1mg Pure grade FP-661585, 50mg Pure grade FP-661584, 20x50µg FP-HH6180, 1 ml, 1mM DMSO, Pure grade	1123.97	548 nm / negligible	552 / 578	low Ca^{2+}] 91 000 high[Ca^{2+}] 96 000 [		DMSO
					570	DMSO, water pH>6
Rhod-2, K salt **FP-M2038A**, 1mg	869.08					DMSO
Rhod-2, NH_4 salt **FP-66159A**, 1 mg	805.08					Buffer pH>6
Rhod-2, Na salt **FP-AM709A**, 1 mg	820.8					DMSO Buffer pH>6

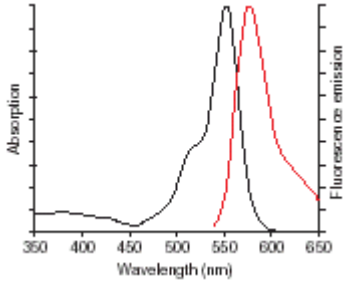
Storage: **Indicator salts** can be stored desicated and protected from light at room temperature, +4°C or –20°C >1 year.
AM esters can be stored desicated and protected from light at –20°C > 6 months.

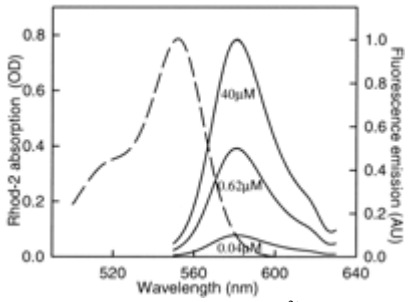
Introduction

Rhod-2 is non fluorescent before Ca2+ binding but becomes fluorescent with increasing Ca2+ concentration. The fluorescent enhancement for rhod-2 from low [Ca2+] to high [Ca2+] is smaller than that for fluo-3, and also in general rhod-2 is somewhat less fluorescent than fluo-3. However, the wavelengths of absorption (556 nm) and emission (576 nm) maxima of Rhod-2, are longer than those of Fluo-3. This may make it useful for some applications where sample autofluorescence is a problem, where sample contains photoreceptors, when photoactivatable chelators or another fluorescent dye of shorter wavelength is used at the same time. Its Kd of 570 nM places Rhod-2 above Fluo-3 and Fura-2, thus for higher Ca2+ concentrations studies.

Rhod-2 salt forms are membrane-impermeant but can be loaded into cells via microinjection or scrape loading.

Membrane-permeant form and thus can be loaded into cells via incubation. Because of the relatively low water solubility of the AM ester, Pluronic F-127, a mild detergent, is often used as a dispersing agent to facilitate the loading. Rhod-2/AM itself does not bind Ca^{2+}, but it is readily hydrolyzed to rhod-2 by endogenous esterases once the dye is inside the cells.





Absorption (*dashed curve*) and Ca^{2+}-dependent fluorescence (*solid curves*) spectra of Rhod-2 (10 µM) in solutions with various Ca^{2+} concentrations (0.04-40 µM). (Congwu Du, *Biophys. J.*, Jan 2001; 80: 549)

FT-66158

Directions for use

Handling and Storage

For loading, a 1-10 mM stock solution is prepared using dry (anhydrous) dimethylsulfoxide and kept at -20ºC. In order to prevent the deterioration of the AM esters that occurs with repeated thawing and freezing, aliquots should be prepared. Loading is usually performed in a serum-free culture medium at a concentration ranging from 1-10 µM. Pluronic may be added to the loading medium if there is difficulty in loading. (Not all cells require Pluronic.)

The cells can be incubated at 20ºC (or 37ºC), and the time of incubation may range from 30 to 60 minutes. They should be washed two to three times with fresh serum-free culture medium for clean results. Note that incubation conditions may vary from cell to cell and should be optimized for each cell type. Protocols abound in the literature for a variety of cells.

Guidelines for use – calcium activity in skeletal muscle ()

1. Isolate single muscle fibres
2. Load the mitochondria by incubating fibres with 5µM Rhod-3 AM for 2h. Then wash for a further 2h.
3. After loading, fibres were mounted in the perfusion channel of a muscle bath where they could be stimulated electrically to produce a tetanus.
4. Dyes were excited with 568 nm light and the emitted light collected through a 585 nm long-pass filter.

References

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Related Products

- Rhod-4 AM, CQ6061
- Fluo-8 NW, CJ2560
- Fluo-8 AM, CP7501
- Fluo-8L AM, CP7551
- Fluo-8H AM, CP7531
- Fura-2 AM, FP-42776A

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