

# Product Information

## Rhod-2, AM Ester

Catalog no.	Product	Size
50023	Rhod-2, AM Ester	10 x 100 ug
50024	Rhod-2, AM Ester	1 mg

### Storage and Handling

Store desiccated at -20°C, protected from light, especially when in solution. Product is stable for at least 12 months from date of receipt when stored as recommended.

We recommend using anhydrous DMSO (catalog no. 90082) for making stock solutions from AM Ester solids. Both DMSO and AM Ester should be warmed to room temperature before mixing. Dissolution can be kinetically slow, so allow sufficient time for the AM ester to dissolve. The DMSO stock solution can be stored tightly sealed at -20°C for at least 6 months and is stable to freeze/thaw cycles as long as it is protected from moisture. Warm the stock solution to room temperature each time before opening the vial to avoid condensation, which may hydrolyze the AM ester during storage.

**Molecular Information:** C<sub>52</sub>H<sub>59</sub>BrN<sub>4</sub>O<sub>19</sub>

**CAS number:** 145037-81-6

**Molecular Weight:** 1124

**Color and form:** Red solid

**Solubility:** Soluble in DMSO

**Absorption/Emission:** 556/576 nm (low or high [Ca<sup>2+</sup>]) (after hydrolysis)

**K<sub>d</sub> (Ca<sup>2+</sup>) (after hydrolysis):** 1 uM

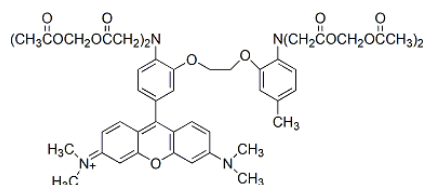


Figure 1. Rhod-2, Acetoxymethyl Ester

### Product Description

Rhod-2, AM Ester is the membrane-permeant form of rhod-2 calcium indicator and can therefore be loaded into cells via incubation. Because of the relatively low water solubility of the AM ester, Pluronic® F-127 (see related products), a mild detergent, is often used as a dispersing agent to facilitate the loading. Rhod-2, AM Ester itself is non-fluorescent and does not bind calcium, but it is readily hydrolyzed to rhod-2 by endogenous esterases once the compound enters cells. Rhod-2 is similar to fluo-3 in that the excitation and emission spectra do not undergo a shift and the sensor is essentially nonfluorescent before Ca<sup>2+</sup> binding but becomes more fluorescent with increasing Ca<sup>2+</sup> concentration. However, the absorption (556 nm) and emission (576 nm) maxima of rhod-2 are longer than those of fluo-3. The longer absorption and emission wavelengths of rhod-2 may make it useful for some applications where autofluorescence is a problem, or where another fluorescent dye of shorter wavelengths is used at the same time. The fluorescent enhancement for rhod-2 from low [Ca<sup>2+</sup>] to high [Ca<sup>2+</sup>] is smaller than that for fluo-3, and also rhod-2 is somewhat less fluorescent than fluo-3 in general.

Biotium offers A-23187 (see related products), an ionophore that is commonly used for intracellular calibration of calcium indicators, Calcium Calibration Buffer Kit (see related products) for preparing a range of calibration buffers with defined calcium concentrations, and BAPTA Ca<sup>2+</sup> chelators in soluble and cell-permeant forms. We also offer EDC (also known as EDAC, see related products), which can be used to fix calcium indicators in cells, if histochemical studies will be performed following physiological experiments. Visit [www.biotium.com](http://www.biotium.com) to see our full selection of ion indicator dyes and related reagents.

### References

1) Meyer, T., et al. *Biochemistry* 29, 32 (1990); 2) Minta, A., et al. *J. Biol. Chem.* 264, 8171 (1984); 3) *A Practical Guide to the Study of Calcium in Living Cells*, Volume 40. San Diego: Academic Press, 1994.

### Experimental Protocols

#### Materials required but not provided

- Anhydrous DMSO (catalog no. 90082)
- Krebs-Ringer-HEPES-glucose buffer (KRH-glc): 136 mM NaCl, 10 mM HEPES, 4.7 mM KCl, 1.25 mM MgSO<sub>4</sub>, 1.25 mM CaCl<sub>2</sub>, 25 mM glucose, pH 7.4.

#### Protocol for cell loading

The following is an example protocol for loading cells with AM esters of calcium indicator dyes (3). You may need to optimize the buffer system or concentration of calcium indicator dye for your experimental system. The use of Pluronic® F-127 (see Related Products), a non-ionic detergent that facilitates AM ester solubilization, is optional.

1. Prepare a 1-5 mM stock solution of the rhod-2, AM ester using anhydrous DMSO.
2. (Optional): Mix 1 uL 20% Pluronic® F-127 in DMSO with 1 uL rhod-2, AM ester stock solution.
3. Add 1 mL KRH-glc buffer containing 0.5% bovine serum albumin (BSA) to the tube containing Pluronic® and rhod-2, AM ester dye and mix well for a final concentration of 1-5 uM.

**Note:** The final concentration of the dye should be as low as possible in order to minimize background fluorescence and nonspecific staining.

3. Wash cells twice with KRH-glc + 0.5% BSA.
4. Add the rhod-2, AM ester solution from step 3 to cells and incubate 30 minutes, protected from light.

**Note:** Incubating cells at 37°C promotes dye compartmentalization in organelles, particularly mitochondria. For measuring cytoplasmic calcium it is recommended to incubate cells at room temperature to reduce dye compartmentalization.

5. Rinse cells several times with KRH-glc + 0.5% BSA.

#### Considerations for measuring cellular calcium concentration

Calcium concentration and fluorescence are related according to the equation:

$$[Ca^{2+}] = K_d \left[ \frac{(F - F_{min})}{(F_{max} - F)} \right]$$

where F is the fluorescence of the indicator at experimental calcium concentration, F<sub>min</sub> is the fluorescence in the absence of calcium and F<sub>max</sub> is the fluorescence of the indicator at saturated calcium concentration.

The K<sub>d</sub> for calcium indicators in cells may be affected by a number of factors including pH, protein concentration, ionic strength, temperature, and viscosity. Thus, calibration of the K<sub>d</sub> is necessary for accurate measurement of intracellular calcium concentrations.

### Calcium Indicator Dyes

Catalog number	Product
50013	Fluo-3, AM Ester
50015	Fluo-3, AM Ester, 1 mM in Anhydrous DMSO
50010	Fluo-3, Pentaammonium Salt
50011	Fluo-3, Pentapotassium Salt
50012	Fluo-3, Pentasodium Salt
50018	Fluo-4, AM Ester
50019	Fluo-4, Pentapotassium Salt
50040	Indo-1, Pentaammonium Salt
50041	Indo-1, Pentapotassium Salt
50042	Indo-1, Pentasodium Salt
50043	Indo-1, AM Ester
50029	Fura-2 AM Ester, 1 mM in Anhydrous DMSO
50033	Fura-2 AM Ester
50035	Furaptra (Mag-Fura-2), Tetrapotassium Salt
50036	Furaptra (Mag-Fura-2), Tetrasodium Salt
50037	Furaptra (Mag-Fura-2), AM Ester
50020	Rhod-2, Triammonium Salt
50021	Rhod-2, Tripotassium Salt
50022	Rhod-2, Trisodium Salt
50023	Rhod-2, AM Ester
50025	Rhod-590, AM Ester
50026	Rhod-590, Tripotassium Salt

### BAPTA Chelators

Catalog number	Product
50000	BAPTA, AM Ester
50001	BAPTA, Tetracesium Salt
50005	5,5'-Difluoro BAPTA, AM Ester
50007	5',5'-Dimethyl BAPTA, AM Ester
50004	5',5'-Dibromo BAPTA, Tetrapotassium Salt
50009	5-Methyl-5'-nitro BAPTA, Tetrapotassium Salt
50017	5-Mononitro BAPTA, Tetrapotassium Salt

### Related Products

Catalog number	Product
90082	DMSO, Anhydrous
59000	Pluronic® F-127
59005	Pluronic® F-127, 10% in dH <sub>2</sub> O
59004	Pluronic® F-127, 20% Solution in DMSO
59100	Calcium Calibration Buffer Kit
59001	A-23187, Free Acid
59006	4-Bromo A-23187, Free Acid
59007	Ionomycin, Calcium Salt
59002	EDC (EDAC)
41024-4L	Water, Ultrapure Molecular Biology Grade
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative

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