

# Product Information

## Rhod-590 AM Ester

**Catalog Number:** 50025

**Unit Size:** 10 x 50 ug

### Storage and Handling

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

We recommend using anhydrous DMSO (Cat. No. 90082) to make stock solutions from AM ester solids. Both DMSO and Rhod-590 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 and protected from light 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>64</sub>H<sub>75</sub>N<sub>4</sub>O<sub>19</sub>

**Molecular Weight:** 1204

**Color and Form:** Purple solid

**Solubility:** Soluble in DMSO

**Absorption/Emission (after hydrolysis):**

590 nm/616 nm (low [Ca<sup>2+</sup>])

595 nm/626 nm (high [Ca<sup>2+</sup>])

**K<sub>d</sub> for Ca<sup>2+</sup> in Buffer:** 0.61 uM

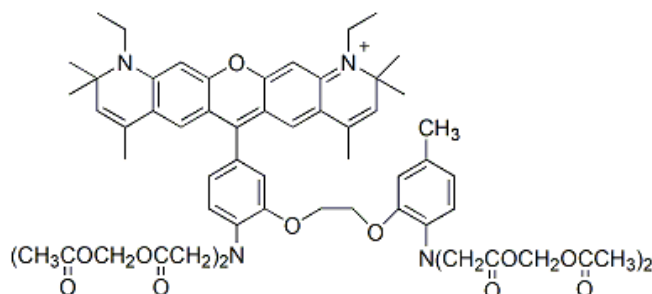


Figure 1. Structure of Rhod-590 AM Ester.

## Product Description

Rhod-590 is a fluorescent calcium indicator that has absorption and emission maxima that are longer than those of Fluo-3. The longer absorption and emission wavelengths of Rhod-590 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-590 from low [Ca<sup>2+</sup>] to high [Ca<sup>2+</sup>] was smaller than that for Fluo-3, and also in general, Rhod-590 is somewhat less fluorescent than Fluo-3.

Rhod-590 AM Ester is a membrane-permeant form of Rhod-590 that can be loaded into cells via incubation. Rhod-590 AM Ester itself does not respond to Ca<sup>2+</sup>, but it is readily hydrolyzed to Rhod-590 by endogenous esterases once the dye is inside the cells.

## General Protocol for Cell Loading

The following is an example protocol for loading cells with AM esters of calcium indicator dyes (1). You may need to optimize the buffer system or concentration of calcium indicator dye for your experimental system. Because of the relatively low water solubility of the AM ester, the mild detergent Pluronic® F-127 (see Related Products), while optional, is recommended to facilitate cell loading.

## Materials required but not provided

- DMSO, Anhydrous (Cat. No. 90082).
- Bovine Serum Albumin (Cat. No. 22014)
- Krebs-Ringer-HEPES-glucose buffer (KRH-glc):  
136 mM NaCl, 10 mM HEPES, 7.4 mM KCl, 1.25 mM MgSO<sub>4</sub>,  
1.25 mM CaCl<sub>2</sub>, 25 mM glucose, pH 7.4

## Protocol

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

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

4. Wash cells twice with KRH-glc + 0.5% BSA.

- Add the AM ester solution from step 3 to cells and incubate for 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.

- 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:

$$[Ca^{2+}] = K_d [(F - F_{min}) / (F_{max} - F)]$$

F is the fluorescence of the indicator at experimental calcium concentration,  $F_{min}$  is the fluorescence in the absence of calcium, and  $F_{max}$  is the fluorescence of the indicator at saturated calcium concentration.

The  $K_d$  for Rhod-590 was reported to be 0.61  $\mu$ M in cell-free media. However, the  $K_d$  is usually affected by a number of factors in cells, including pH, protein concentrations, ionic strength, temperature, and viscosity. Thus, calibration of the  $K_d$  is necessary for accurate measurement of intracellular calcium concentrations. For literature with detailed information on loading, calibrating, and imaging indicator dyes, please see References.

Biotium offers A-23187 (Cat. No. 59001), an ionophore that is commonly used for intracellular calibration of calcium indicators, Calcium Calibration Buffer Kit (Cat. No. 59100) for preparing a range of calibration buffers with defined calcium concentrations, and BAPTA  $Ca^{2+}$  chelators in soluble and cell-permeant forms. We also offer EDC (also known as EDAC), which can be used to fix calcium indicators in cells if histochemical studies will be performed following physiological experiments. See Related Products, or visit [www.biotium.com](http://www.biotium.com) to see our full selection of ion indicator dyes and related reagents.

### References

- A Practical Guide to the Study of Calcium in Living Cells, Volume 40, San Diego: Academic Press (1994).

Please visit our website at [www.biotium.com](http://www.biotium.com) for information on our life science research products, including fluorescent calcium and other ion indicators, ion chelators, fluorescent pH indicators, cell membrane and organelle dyes, and fluorescent CF® Dye antibody conjugates and reactive dyes.

Pluronic F-127 is a registered trademark of BASF.

Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use.

### Related Products

| Cat. No.     | Product                                    |
|--------------|--------------------------------------------|
| 90082        | DMSO, Anhydrous                            |
| 22014        | Bovine Serum Albumin 30% Solution          |
| 59000        | Pluronic® F-127                            |
| 59004        | Pluronic® F-127, 20% Solution in DMSO      |
| 59005        | Pluronic® F-127, 10% in H <sub>2</sub> O   |
| 59100        | Calcium Calibration Buffer Kit             |
| 59001        | A-23187, Free Acid                         |
| 59006        | 4-Bromo A-23187, Free Acid                 |
| 59007        | Ionomycin, Calcium Salt                    |
| 59002        | EDC (EDAC)                                 |
| 50027        | Probenecid, Sodium Salt, Water Soluble     |
| 50026        | Rhod-590, Tripotassium Salt                |
| 50013..50016 | 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                |
| 50043        | Indo-1, AM Ester                           |
| 50040        | Indo-1, Pentaammonium Salt                 |
| 50041        | Indo-1, Pentapotassium Salt                |
| 50042        | Indo-1, Pentasodium Salt                   |
| 50033, 50034 | Fura-2, AM Ester                           |
| 50029        | Fura-2, AM Ester, 1 mM Solution            |
| 50030        | Fura-2, Pentaammonium Salt                 |
| 50031        | Fura-2, Pentapotassium Salt                |
| 50032        | Fura-2, Pentasodium Salt                   |
| 50037-50039  | Furaptra (Mag-Fura-2), AM Ester            |
| 50035        | Furaptra (Mag-Fura-2), Tetrapotassium Salt |
| 50036        | Furaptra (Mag-Fura-2), Tetrasodium Salt    |
| 50023, 50024 | Rhod-2, AM Ester                           |
| 50020        | Rhod-2, Triammonium Salt                   |
| 50021        | Rhod-2, Tripotassium Salt                  |
| 50022        | Rhod-2, Trisodium Salt                     |
| 50000        | BAPTA, AM Ester                            |
| 50001        | BAPTA, Tetracesium Salt                    |
| 50002        | BAPTA, Tetrapotassium Salt                 |
| 50003        | BAPTA, Tetrasodium Salt                    |
| 50004        | 5',5'-Dibromo BAPTA, Tetrapotassium Salt   |
| 50005        | 5',5'-Difluoro BAPTA, AM Ester             |
| 50006        | 5',5'-Difluoro BAPTA, Tetrapotassium Salt  |
| 50007        | 5',5'-Dimethyl BAPTA, AM Ester             |
| 50008        | 5',5'-Dimethyl BAPTA, Tetrapotassium Salt  |