

Product Information

Fluo-4 AM Ester

Catalog Number: 50018

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 Fluo-4 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: C₅₁H₅₀F₂N₂O₂₃

CAS number: 273221-67-3

Molecular Weight: 1097

Color and Form: Orange-red solid

Solubility: Soluble in DMSO

Absorption/Emission: 494 nm/506 nm
(after hydrolysis, with Ca²⁺)

K_d for Ca²⁺ in Buffer: ~335 nM (after hydrolysis)

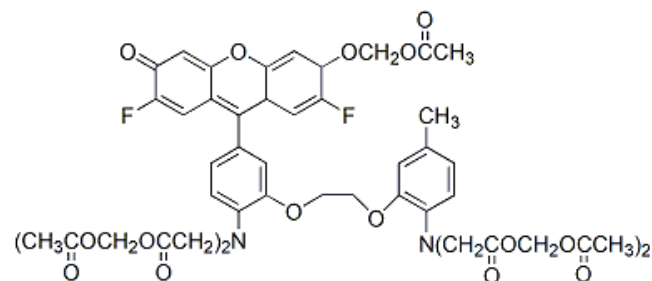


Figure 1. Structure of Fluo-4 AM Ester.

Product Description

Fluo-4 is an analog of Fluo-3, the widely used long wavelength fluorescent calcium indicator developed by Roger Tsien and colleagues (1-3). The two chlorine substituents of Fluo-3 are replaced by fluorines, which results in increased excitation at 488 nm that gives higher fluorescence signal. Fluo-4 is essentially nonfluorescent without Ca²⁺ present, but the fluorescence increases at least 100 times upon Ca²⁺ binding with little shift in wavelength. Additionally, because Fluo-4 binds Ca²⁺ more weakly than Fura-2 and Indo-1, it is more useful for measuring high transient Ca²⁺ concentration during Ca²⁺ spikes.

Fluo-4 AM Ester is a membrane-permeant form of Fluo-4 that can be loaded into cells via incubation. Fluo-4 AM Ester itself does not respond to Ca²⁺, but it is readily hydrolyzed to Fluo-4 by endogenous esterases once the dye is inside the cells. Fluo-4 AM Ester is often used to measure intracellular calcium in high-throughput drug screening.

General Protocol for Cell Loading

The following is an example protocol for loading cells with AM esters of calcium indicator dyes (4). 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 MgSO₄,
1.25 mM CaCl₂, 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:

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

where 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 Fluo-4 was reported to be 335 nM 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 to see our full selection of ion indicator dyes and related reagents.

References

- Cell Calcium 27, 97 (2000);
- J Biol Chem 264, 8171 (1989);
- J Biol Chem 264, 8179 (1989);
- 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 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 ₂ 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
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
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
50025	Rhod-590 AM Ester
50026	Rhod-590, Tripotassium 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