

# Product Information

## Fluo-3, AM Ester

Catalog Number	Unit Size
50013	10 x 100 ug
50016	20 x 50 ug
50014	1 mg
50015	1 mL, 1 mM in anhydrous DMSO

### Storage and Handling

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

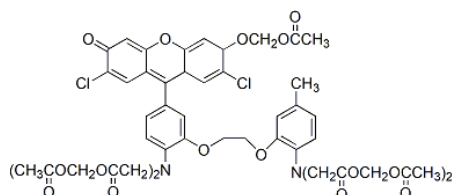
We recommend using anhydrous DMSO for making stock solutions from AM ester solids. Both DMSO and AM ester should be warmed to room temperature before mixing. Dissolution can be kinetically slow, so allow sufficient time for the AM ester to dissolve. The DMSO stock solution can be stored tightly sealed 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>51</sub>H<sub>50</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>23</sub>

MW: 1129.9

CAS: 121714-22-5



### Properties

Color & Form:

50013, 50014, 50016: Orange red solid

50015: Orange red solution

Solubility: Soluble in DMSO

Abs/Em (after hydrolysis): 506/526 nm (low or high [Ca<sup>2+</sup>])

Extinction Coefficient: 86,000 M<sup>-1</sup>cm<sup>-1</sup> (506 nm)

K<sub>d</sub> (Ca<sup>2+</sup>) (after hydrolysis): 450 nM

### Product Description

Fluo-3 is a widely used long wavelength fluorescent calcium indicator developed by professor Roger Tsien and colleagues (1,2). The indicator absorbs at 506 nm and thus can be efficiently excited by the 488 argon-ion laser. Fluo-3 is essentially non-fluorescent without Ca<sup>2+</sup> present, but the fluorescence at 526 nm increases at least 40 times on Ca<sup>2+</sup> binding. Unlike Fura-2 and Indo-1, neither the excitation nor the emission maximum of Fluo-3 shifts significantly before and after Ca<sup>2+</sup> binding. As a result, the ratiometric technique for measuring [Ca<sup>2+</sup>] is not applicable to Fluo-3. Also, because Fluo-3 binds Ca<sup>2+</sup> more weakly than Fura-2 and Indo-1, it is more useful for measuring high transient Ca<sup>2+</sup> concentration during Ca<sup>2+</sup> spikes.

Fluo-3 AM ester is a membrane-permeant form of Fluo-3 that can be loaded into cells by incubation. Fluo-3 AM itself does not respond to calcium. However, once inside cells it is hydrolyzed to Fluo-3 free acid by cytoplasmic esterases.

### 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. 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 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® and dye AM ester and mix well for a final concentration of 1-5 uM.

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.

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.
5. Add the 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.

6. 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<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 Fluo-3 was reported to be 450 nM in cell-free media. However, the K<sub>d</sub> is usually affected by a number of factors in cells including pH, proteins concentrations, ionic strength, temperature and viscosity. Thus, calibration of the K<sub>d</sub> 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 (59001), an ionophore that is commonly used for intracellular calibration of calcium indicators, Calcium Calibration Buffer Kit (59100) 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), 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

- 1) J. Biol. Chem. 264, 8171(1989); 2) J. Biol. Chem. 264, 8179(1989); 3) Meth. Enzymol. 192, 38 (1990). 4) A Practical Guide to the Study of Calcium in Living Cells, Volume 40. San Diego: Academic Press, 1994.

## Calcium Indicator Dyes

Cat. No.	Product	Unit Size
50013	Fluo-3, AM Ester	10 x 100 ug
50016	Fluo-3, AM Ester	20 x 50 ug
50014	Fluo-3, AM Ester	1 mg
50015	Fluo-3, AM Ester, 1 mM in Anhydrous DMSO	1 mL
50010	Fluo-3, Pentaammonium Salt	1 mg
50011	Fluo-3, Pentapotassium Salt	1 mg
50012	Fluo-3, Pentasodium Salt	1 mg
50018	Fluo-4, AM Ester	10 x 50 ug
50019	Fluo-4, Pentapotassium Salt	0.5 mg
50040	Indo-1, Pentaammonium Salt	1 mg
50041	Indo-1, Pentapotassium Salt	1 mg
50042	Indo-1, Pentasodium Salt	1 mg
50043	Indo-1, AM Ester	10 x 100 ug
50043-1	Indo-1, AM Ester	20 x 50 ug
50044	Indo-1, AM Ester	1 mg
50029	Fura-2 AM Ester, 1 mM in Anhydrous DMSO	1 mL
50033	Fura-2 AM Ester	10 x 100 ug
50033-1	Fura-2 AM Ester	20 x 50 ug
50034	Fura-2 AM Ester	1 mg
50035	Furaptra (Mag-Fura-2), Tetrapotassium Salt	1 mg
50036	Furaptra (Mag-Fura-2), Tetrasodium Salt	1 mg
50037	Furaptra (Mag-Fura-2), AM Ester	10 x 100 ug
50039	Furaptra (Mag-Fura-2), AM Ester	20 x 50 ug
50038	Furaptra (Mag-Fura-2), AM Ester	1 mg
50020	Rhod-2, Triammonium Salt	1 mg
50021	Rhod-2, Tripotassium Salt	1 mg
50022	Rhod-2, Trisodium Salt	1 mg
50023	Rhod-2, AM Ester	10 x 100 ug
50024	Rhod-2, AM Ester	1 mg
50025	Rhod-590, AM Ester	10 x 50 ug
50026	Rhod-590, Tripotassium Salt	500 ug

## BAPTA Chelators

Cat. No.	Product
50000	BAPTA, AM Ester
50001	BAPTA, Tetraesium 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

Cat. No.	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

Please visit our website at [www.biotium.com](http://www.biotium.com) for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, fluorescent CF® dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.

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