

SUMMARY

shipped at room temperature; store at -20 °C

CAS Number: 121714-22-5

$C_{51}H_{50}Cl_2N_2O_{23}$, **Molecular Weight:** 1129.85

Unit: 1 mg

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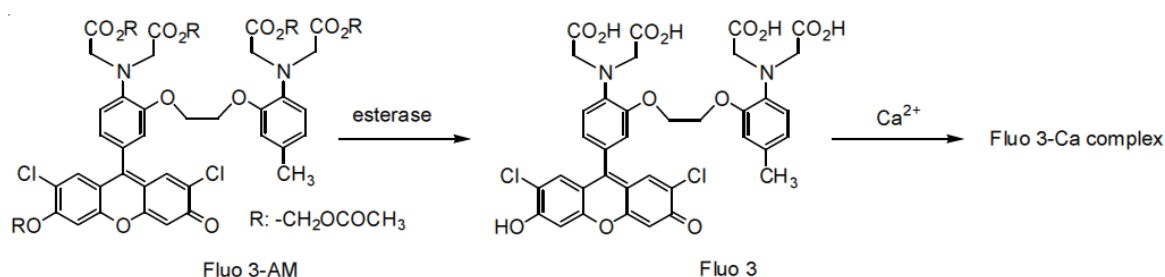
Chemical Name: 1-[2-Amino-5-(2,7-dichloro-6-hydroxy-3-oxo-9-xanthenyl)phenoxy]-2-(2-amino-5-methylphenoxy)ethane-N,N,N',N'-tetraacetic acid, pentaacetoxymethyl ester.

Appearance: red, solid

Fluorescent spectrum: pass test

Solubility: 2 mg/ml acetonitrile, 0.9 mg/ml DMSO

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Hydrolysis of the AM ester

Properties:

Fluo-3 is a long wavelength calcium probe. It is practically non-fluorescent in its free ligand form, but its fluorescence increases 60 - 80 times when it forms complexes with calcium. Thus, it has been widely used with confocal laser fluorescent microscopy because the microscope has an argon laser. The long wavelength of the fluorescent signal is also convenient for minimizing photodamage to sample cells. Fluo-3 is also useful for caged calcium and others that are cleaved by the photo-irradiation in the UV region. Fluo-3 AM is an acetoxymethyl ester derivative of Fluo-3 that can be easily loaded into cells by incubation.

General Protocol (for human T cells)*

* Cell staining conditions differ by cell types, so it is necessary to optimize the conditions for each experiment.

Reagents:

- 2 mM Fluo-3 AM/DMSO (1 mg Fluo-3 AM in 442 ml DMSO)
- Pluronic F127
- Hanks•balanced salt solution (HBSS)
- HEPES buffer saline (10 mM HEPES, 1 mM Na₂HPO₄, 137 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 0.5 mM MgCl₂, 5 mM glucose, 0.1% BSA, pH 7.4)

Procedure:

1. Add 16.5 mg Pluronic F127 to Fluo-3 AM/DMSO solution. Pluronic F127 prevents aggregation of Fluo-3 AM in HBSS and helps uptake with cells.
2. Dilute the Fluo-3 AM solution with HBSS to prepare 4 mM Fluo-3 AM working solution (not storable!).
3. Add the Fluo-3 AM working solution to the cells, and incubate at 37 °C for 20 minutes.
4. Add 5 volumes of HBSS containing 1% fetal calf serum, and continue the incubation for another 40 minutes.
5. Wash the cells 3 times with HEPES buffer saline. Then resuspend the cells to prepare 1 x 10⁵ cells/ml solution using HEPES buffer saline.
6. Incubate at 37 °C for 10 minutes. Then use the cells for fluorescent calcium ion detection.
7. Monitor the fluorescence at 528 nm (excitation: 490 - 500 nm).

Order Information, Shipping and Storage

Order#	Product	Quantity
MFP-F241	Fluo-3 AM	1 mg
shipped at room temperature; store at -20 °C		

Contact and Support

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