

Fluo-4 AM, green fluorescent calcium indicator

<http://www.lumiprobe.com/p/fluo-4-am>

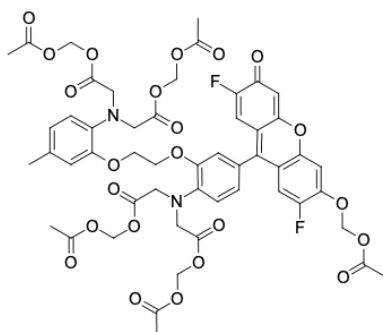
Fluo-4 AM is a cell-permeable Ca^{2+} -indicator that is metabolized by intracellular esterase, leading to a bright green fluorescent signal upon Ca^{2+} -binding (excitation/emission λ at 494/506 nm). Fluo-4 AM is used for visualization and measurement of intracellular Ca^{2+} . It is well suited for fluorometric and imaging applications such as microscopy, flow cytometry, spectrofluorometry, and fluorometric high-throughput microplate screening assays ^[1].

Fluo-4 AM is similar in structure and spectral properties to the widely used Ca^{2+} -indicator, Fluo-3, but it has certain advantages over Fluo-3, such as brighter fluorescence emission, high rate of cell permeation, and a K_d for Ca^{2+} in buffer of 345 nM. Because of its higher fluorescence emission intensity, Fluo-4 AM can be used at lower intracellular concentrations, making its use less toxic for live cells.

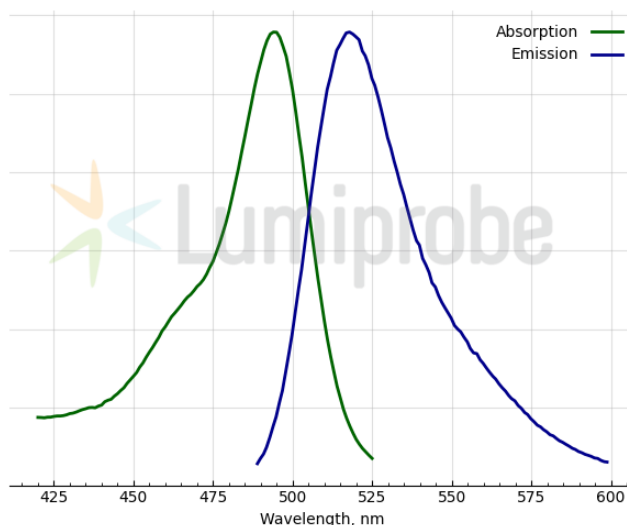
As Fluo-4 AM does not covalently bind to cellular components, it may be actively effluxed from the cell by organic anion transporters. *In vivo* cell imaging with Fluo-4 AM is usually performed within one or two hours after loading, but the dye can be re-loaded to cells if it is needed. To determine the cellular localization of the dye, Fluo-4 AM can be fixed *in situ* with [EDC/EDAC](#) and counterstained immunochemically using anti-BAPTA antibodies ^[2].

Fluo-4 AM has low solubility in the water. It is recommended to prepare 1 mM stock solution in [labeling grade DMSO](#) prior to cell loading. Use the final concentration of 1-5 μM and incubation at 37 °C for 15-60 min as a start point of your protocol.

^[1] Cell Calcium. 2000. 27(2). 97-106. ^[2] Cell Calcium. 1997. 21(3). 175-183.



Structure of Fluo-4 AM



Absorption and emission spectra of Fluo-4 AM (calcium-bound form)

General properties

Appearance: orange-red powder
Molecular weight: 1096.95
CAS number: 273221-67-3
Molecular formula: $\text{C}_{31}\text{H}_{39}\text{F}_2\text{N}_3\text{O}_{23}$
IUPAC name: N-[4-[6-((Acetyloxy)methoxy)-2,7-difluoro-3-oxo-3H-xanthen-9-yl]-2-[2-[2-bis[2-((acetyloxy)methoxy]-2-oxoethyl)amino]-5-methylphenoxy]ethoxy]phenyl]-N-[2-((acetyloxy)methoxy)-2-oxoethyl]glycine (acetyloxy)methyl ester
Solubility: good in DMSO
Quality control: NMR ¹H and HPLC-MS (95+%)
Storage conditions: 24 months after receipt at -20°C in the dark. Transportation: at room temperature for up to 3 weeks. Desiccate.
Legal statement: This Product is offered and sold for research purposes only. It has not been tested for safety and efficacy in food, drug, medical device, cosmetic, commercial or any other use. Supply does not express or imply authorization to use for any other purpose, including, without limitation, *in vitro* diagnostic purposes, in the manufacture of food or pharmaceutical products, in medical devices or in cosmetic products.

Spectral properties

Excitation/absorption 494
maximum, nm:
Emission maximum, 518
nm: