

## Fluo-4 AM, green fluorescent calcium indicator

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

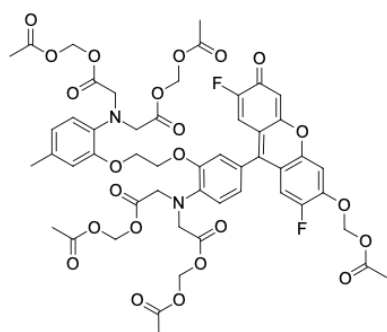
Fluo-4 AM is a cell-permeable  $\text{Ca}^{2+}$ -indicator that is metabolized by intracellular esterase, leading to a bright green fluorescent signal upon  $\text{Ca}^{2+}$ -binding (excitation/emission  $\lambda$  at 494/506 nm). Fluo-4 AM is used for visualization and measurement of intracellular  $\text{Ca}^{2+}$ . 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  $\text{Ca}^{2+}$ -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  $\text{Ca}^{2+}$  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.

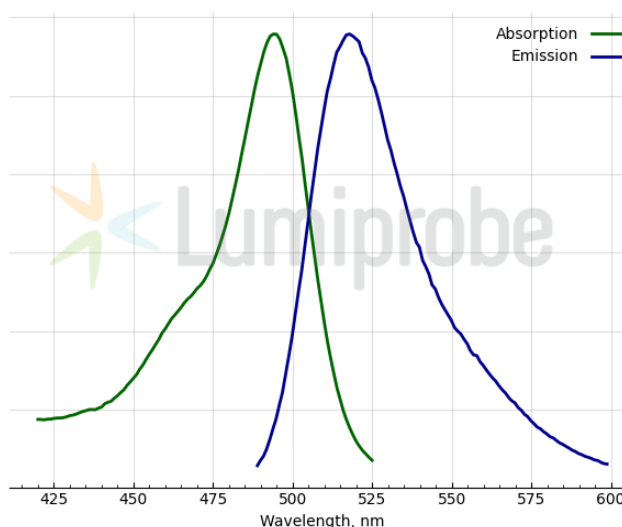
As Fluo-4 AM does not covalently bind to cellular components, it may be actively effluxed from the cell by organic anion transporters. *In vivo* cell imaging with Fluo-4 AM is usually performed within one or two hours after loading, but the dye can be re-loaded to cells if it is needed. Fluo-4 AM can also be fixed *in situ* by [EDC/EDAC](#) for downstream immunofluorescence studies.

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  $\mu\text{M}$  and incubation at 37 °C for 15-60 min as a start point of your protocol.

[1] Gee K.R. et al. Chemical and physiological characterization of fluo-4  $\text{Ca}^{2+}$ -indicator dyes. *Cell Calcium*. 2000. 27(2). 97-106.



**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:  $\text{C}_{31}\text{H}_{39}\text{F}_2\text{N}_2\text{O}_{23}$   
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  $^1\text{H}$  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.

### Spectral properties

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