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Fluo-4 AM, green fluorescent calcium indicator

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

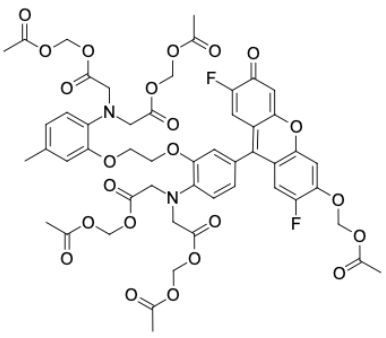
Fluo-4 AM is a cell-permeable Ca^{2+}-indicator that is metabolized by intracellular esterase, leading to a bright green fluorescent signal upon Ca^{2+}-binding (excitation/emission λ at 494/506 nm). Fluo-4 AM is used for visualization and measurement of intracellular Ca^{2+}. It is well suited for fluorometric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

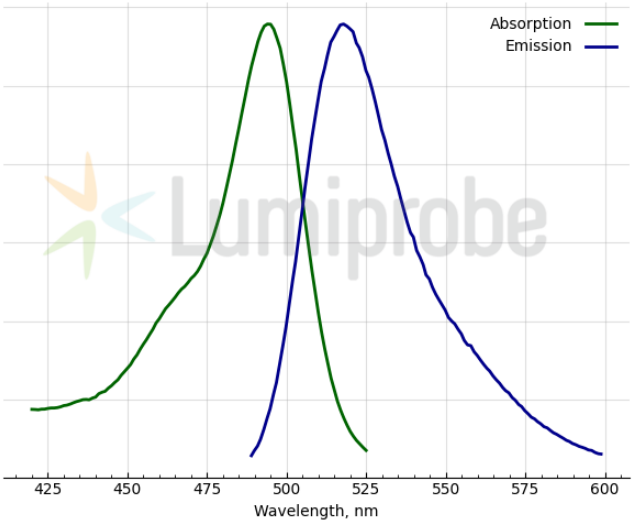
As Fluo-4 AM does not covalently bind to cellular components, it may be actively effluxed from the cell by organic anion transporters. *In vivo* cell imaging with Fluo-4 AM is usually performed within one or two hours after loading, but the dye can be re-loaded to cells if it is needed. Fluo-4 AM can 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 µM 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 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:	$C_{51}H_{50}F_2N_2O_{23}$
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}H and HPLC-MS (95+%)
Storage conditions:	24 months after receival at -20°C in the dark. Transportation: at room temperature for up to 3 weeks. Desiccate.

Spectral properties

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