

## Fluo-4 AM Ester

Membrane-permeant form of Fluo-4 calcium indicator.



### Product attributes

CAS number	273221-67-3
Cell permeability	Membrane permeant
Indicator type	Non-ratiometric
Excitation/Emission	494/516 nm (after hydrolysis)

## Product Description

Membrane-permeant AM ester form of Fluo-4 that can enter into cells via incubation. Because of the relatively low water solubility of the AM ester, the mild detergent [Pluronic® F-127 \(cat# 59004\)](#), while optional, is recommended to facilitate cell loading. Fluo-4 AM ester itself does not bind  $\text{Ca}^{2+}$ , but it is readily hydrolyzed to Fluo-4 by endogenous esterases once inside cells. Fluo-4 AM ester is often used to measure intracellular calcium in high throughput drug screening.

Fluo-4 is an analog of Fluo-3 with the two chlorine substituents replaced by fluorines, which results in increased fluorescence excitation at 488 nm that gives higher fluorescence signal. Fluo-4 has its absorption maximum at 494 nm, thus making it excitable by the argon-ion laser. Fluo-4 is essentially nonfluorescent without  $\text{Ca}^{2+}$  present, but the fluorescence increases at least 100 times on  $\text{Ca}^{2+}$  binding. Also, because Fluo-4 binds  $\text{Ca}^{2+}$  more weakly (higher  $K_d$ ) than do fura-2 and indo-1, it is more useful for measuring high transient  $\text{Ca}^{2+}$  concentration during  $\text{Ca}^{2+}$  spikes.

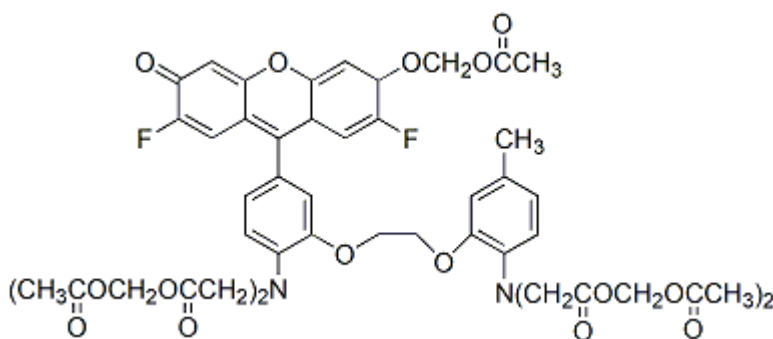
Fluo-4 AM ester can be used to detect intracellular free calcium by fluorescence microscopy, flow cytometry, or fluorescence microplate reader.

BAPTA-based ion indicators like Fluo-4 have been shown to be fixable in situ by [EDC/EDAC \(cat# 59002\)](#). The fixation of indicator dyes is useful for downstream immunofluorescence and IHC studies ([Cell Calcium 1997, 21\(3\), 175](#)).

As the indicator does not covalently bind to cellular components, it may be actively effluxed from the cell by organic anion transporters. The rate of efflux increases with temperature, and may vary between cell types, resulting in variable retention times of a few minutes to hours. Experiments using indicators in cells usually are performed within one or two hours of loading, but it may be possible to re-load cells with indicator if needed. The organic anion transporter inhibitor [Probenecid \(#50027\)](#) can be used to slow the rate of indicator efflux from cells.

We also offer Fluo-4 as a membrane-impermeant pentapotassium salt version, [cat# 50019](#).

- Orange red solid soluble in DMSO
- Store  $-20^{\circ}\text{C}$  and protect from light, especially when in solution
- $\lambda_{\text{Ex}}/\lambda_{\text{Em}}$  (with  $\text{Ca}^{2+}$ ): 494/516 nm (after hydrolysis)
- $K_d$  for  $\text{Ca}^{2+}$  in buffer:  $\sim 335$  nM (after hydrolysis)
- $\text{C}_{51}\text{H}_{50}\text{F}_2\text{N}_2\text{O}_{23}$
- MW: 1096.95
- [273221-67-3]



Pluronic is a registered trademark of BASF.

## References

1. Cell Calcium 27, 97, (2021), [DOI: 10.1054/ceca.1999.0095](https://doi.org/10.1054/ceca.1999.0095)
2. Methods Cell Biol, 99, 113, (2021), [DOI: 10.1016/B978-0-12-374841-6.00005-0](https://doi.org/10.1016/B978-0-12-374841-6.00005-0)