FT-78932

Fluo-3, Ca^{2+} indicators

Product Information

Product name Cat.number	MW ($g \cdot mol^{-1}$)	$\lambda_{exc}\backslash\lambda_{em}$ max. Free Ca^{2+} (nm)	$\lambda_{exc}\backslash\lambda_{em}$ max. High Ca^{2+} (nm)	mol. abs. ($M^{-1}cm^{-1}$)	Kd (nM)	Soluble in
Fluo-3, AM ester FP-78932A	1129.8	503 / weak	505 / 526 [(a)]			DMSO
Fluo-3, AM ester FPgrade FP-R1245A	1129.8	503 / weak	505 / 526 [(a)]			DMSO
Fluo-3, AM ester 1 mM in dry DMSO FP-M2036A	1129.8	503 / weak	505 / 526 [(a)]			DMSO
Fluo-3, $5NH_4$ salt FP-37020A	854.7	503 / weak	505 / 526	100 000	390 [(c)]	Water >pH6
Fluo-3, K salt FP-036691	960.0	503 / weak	505 / 526	100 000	390	Water >pH6
Fluo-3, Na salt FP-I3021A	879.4	503 / weak	505 / 526	100 000	390	Water >pH6

(a) after hydrolysis
(b) $\lambda_{exc}\backslash\lambda_{em}$ (EtOac,no Mg2+) = 366nm / 475nm
(c) Reported values vary from 325 to 465 nm. K_d325nm determined at 22°C in 100 mM KCl, 10 mM, MOPS, pH 7.2, 0 to 10 mM CaEGTA.

Storage: **Indicator salts** can be stored desicated and protected from light at room temperature, +4°C or –20°C >1 year.
AM esters can be stored desicated and protected from light at –20°C > 6 months.

Technical and Scientific Information

- **Fluo-3**

Fluo-3 and their derivatives all exhibit large fluorescence intensity increases on binding Ca^{2+}. Unlike indicators fura-2 and indo-1, there is no spectral shift. They are non-fluorescent until hydrolyzed intracellularly or in the presence of Ca^{2+}. There lower binding affinity allows measurement of higher peaks of Ca^{2+} transients than with Fura 2.
Fluo-3 has an absorption spectrum compatible with excitation at 488 nm by argon-ion laser sources, and a >100 fold fluorescence intensity increase in response to Ca^{2+} binding. These properties are taken to good account in microscopy and Flow cytometry.
Fluo-3 has a fluorescence quantum yield (QY) of 0.15 (Value determined at 22°C in 100 mM KCl, 10 mM, MOPS, pH 7.2 containing 39.8 µM free Ca^{2+}).

Applications:

- Microscopy imaging for the study of the spatial dynamics of many elementary processes in Ca^{2+} signaling.

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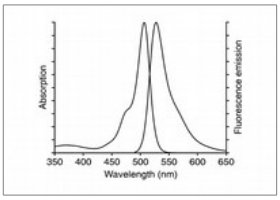
- Flow cytometry analysis for experiments involving photoactivation of "caged" chelators, second messengers and neurotransmitters
- Cell-based assays, especially for testing xenobiotics effect on intracellular Ca^{2+}, up to microplates assays for pharmaceutical High Thoughput Screening.

Spectra of Fluo-3



Absorption and fluorescence emission spectra of Ca^{2+}-saturated Fluo-3 (AM hydrolysed) in pH 7.2 buffer.

Available forms of Ca^{2+} indicators

These calcium indicators are available as Acetoxymethyl ester (AM ester) and salts form.
AM ester are membrane-permeant and thus increases greatly cell loading that can be performed by simple incubation of the cells or tissue preparation in a buffer containing the AM ester. Pluronic® F-127, a mild non-ionic detergent, can facilitate AM esters loading. The AM esters themselves do not bind to Ca2+. However, once they have entered the cells, they are rapidly hydrolyzed by intracellular esterases into the parent Ca2+ compounds, thus becoming fully fluorescent upon binding to Ca2+.
Salts form are membrane-impermeant, but can be loaded into cells via microinjection or scrape loading.

Instruction for use

Handling and Storage

Indicator salts : stock solutions of the salts may be prepared in distilled water or aqueous buffers (pH>6) and stored frozen (<20°C) and protected from light; these solutions should be stable for at least six months. Indicator salt are orange red solid and soluble in DMSO and water (pH >6)
AM esters should be reconstituted in anhydrous dimethylsulfoxide (DMSO) then used as soon as possible thereafter (within a week) to avoid hydrolysis with subsequent loss of cell loading capacity. DMSO stock solutions of AM esters should be frozen and desiccated and protected from light.

Possible AM ester degradation may be assayed as follows:
- prepare a 1µM solution in calcium-free buffer.
- measure the fluorescence intensity (excitation at 485 nm, emission at 520 nm).
- add 5≥ µM Ca^{2+} for Fluo-3 and Fluo-4
- check fluorescence again.

Both intensity readings should be very low. Significantly increased fluorescence upon calcium addition (i.e., in the second reading) indicates partial hydrolysis of the AM ester.

- **Fluo-3**

Guidelines for use – cell loading

The following protocol was designed for Fluo-3. It may be optimized depending on type of Ca2+ indicator, type and number of loaded cells, time, temperature are some other experimental factors.
The water-soluble salt forms of Ca^{2+} indicators may be loaded into cells via microinjection, addition to patch pipette solutions, scrape loading, or using pinocytotic cell-loading reagent.

The AM analogs indicators could be loaded directly:
1. Prepare a 1-5mM stock solution in DMSO.

Note: It is often more convenient and effective to add the non-ionic detergent Pluronic® F-127 to get further a better dissolution of AM indicator: mix the AM ester stock solution in DMSO with an equal volume of 20% (w/v) Pluronic® F-127 in DMSO before dilution in the loading medium, making the final Pluronic® F-127 concentration about 0.02%.

2. Prepare a 1–5 µM working solution in the appropriate buffered physiological medium.

Note: Avoid amine-containing buffers such as Tris.

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Note: The organic anion-transport inhibitors probenecid (1–2.5 mM) or sulfinpyrazone (0.1–0.25 mM) may be added to the cell medium to further reduce leakage of the de-esterified indicator. Stock solutions of sulfinpyrazone and probenecid being quite alkaline; readjust the pH of media after addition.

3. Incubated cells with the AM ester by mixing equal volumes for 15–60 minutes at +20/+37°C.

Notes: One can load adherent cells without lifting
Exact loading concentration, time and temperature will need to be determined empirically; in general it is desirable to use the minimum dye concentration required to yield fluorescence signals with adequate signal to noise. Subcellular compartmentalization, an inherent problem with the AM ester loading technique, is usually lessened by lowering the incubation temperature.

4. De-esterification : remove the AM ester solution by washing in indicator-free medium and incubate cells 30 minutes to allow complete de-esterification of intracellular AM esters.

Note: eventual indicator leakage may be quenched if needed by addition of anti-fluorescein antibody

5. Tetracarboxylate form of the indicator can be measured with known Ca^{2+} concentrations.

Calcul of calcium concentration:
Calcium concentration and fluorescence are related according to the equation :

$$[Ca^{2+}]=K_d\,[(F-F_{min})/(F_{max}-F)]$$

F : fluorescence of the indicator at experimental calcium concentration
F_{min} : fluorescence in the absence of calcium
F_{max} : fluorescence of the indicator at saturated calcium concentration.
K_d : vary according to a number of factors in cells including pH, proteins concentrations, ionic strength, temperature and viscosity. **Calibration of the K_d** should be necessary for accurate measurement of intracellular calcium concentrations. See our kit « Calcium Calibration kit », FP-21527A.

Other technical information:

* Calcium-binding and spectroscopic properties of fluorescent indicators can vary quite markedly with several parameters, as cellular environment.

- For example, Fluo-3 fluorescence in the nucleoplasm has been found to be twice that in the cytoplasm. In situ response calibrations of intracellular indicators typically yield Kd values significantly higher than *in vitro* determinations.
- Heavy metal cations (e.g., Mn^{2+}, Zn^{2+}, Pb^{2+}) also interfere with several indicators, sometimes with higher affinity than Ca^{2+}. Perturbations to calcium measurements caused by presence of these ions can be controlled using the heavy metal-selective chelator TPEN.
- In situ calibrations may thus be required. They are performed by exposing loaded cells to controlled Ca^{2+} buffers in the presence of ionophores such as A-23187, 4-bromo A-23187 or ionomycin. An alternative method is to saturate the intracellular indicator with Mn^{2+} by adding 2 mM Mn^{2+} to the extracellular medium in the presence of ionophore.
- Variations in the intracellular indicator concentration between cells or in different cell compartments affect quantitative Ca^{2+} measurements. Co-loading cells with a pair of spectrally contrasted indicators (typically Fluo-3) can give an appreciation of such variations, provided indicators are equally consistent.
- Variations of Ca^{2+} with in time might also be questioned, but this is often in fact the goal of the assay!

Guidelines for use – screening assays (HTS)

All above guidelines are suitable for 84- and 384-wells microplate assays used at great scale by pharmaceutical screenings, with fluorimeter such as Molecular Devices FLIPR™ (Fluorometric Imaging Plate Reader).

Protocol may be modified for maximum results according the cell type.

Related products

- TPEN: FP-44736A
- Calcium Calibration Buffer Kit: 21527
- Pluronic® acid F-127: FP-37361A
- Ionomycin: FP-53989A
- A23187 free acid, FP-28362A
- 4-Bromo A23187 free acid, FP-37222A
- Fluo-8 AM, 4 times brighter than Fluo-3 CP7502
- Fluo-8 NW, CJ2550
- Rhod-4 AM, 4 times brighter than Rhod-2 CQ6062

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Ordering information

Catalog size quantites and prices may be found at http://www.interchim.com
Please inquire for higher quantities (avaibility, shipment conditions).

Fluo-3, AM ester	FP-78932A	1 mg
Fluo-3, AM ester	FP-78932B	10x100 µg
Fluo-3, AM ester	FP-78932C	20x50 µg
Fluo-3, AM ester FluoroPure grade	FP-R1245A	20x50 µg
Fluo-3, AM ester FluoroPure grade	FP-R1245B	5 mg
Fluo-3, AM ester	FP-M2036A	1 ml 1mM in dry DMSO
Fluo-3, pentaammonium salt	FP-37020A	1 mg
Fluo-3, K salt	FP-03669A	1 mg
Fluo-3, Na salt	FP-I3021A	1 mg

For any information, please ask : Fluoprobes / Interchim; Hotline : +33(0)4 70 03 73 06

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