Fluo-4, Ca^{2+} indicators

Product Information

Product name Cat.number	**MW** (g·mol⁻¹)	CAS	**$\lambda_{exc}\backslash\lambda_{em}$ max. High Ca^{2+}** (nm)	**mol. abs.** ($M^{-1}cm^{-1}$)	**Kd**	**Soluble in**
Fluo-4 AM FP-729718, 5 x 50 µg FP-729712, 10 x 50 µg FP-729716, 1 mg FP-729714, 5 mg	1096.95	273221-67-3	494 / 516	88 000	345 nM	DMSO
Fluo-4, K salt FP-M20201, 10 x 50 µg FP-M20202, 1 mg	927.08		494 / 516	88 000	345 nM	Water >pH6
Fluo-4FF AM FP-F9928A, 10 x 50 µg	1118.92		494 / 516	75 000	9.7 µM	DMSO
Fluo-4FF, K salt FP-R1264A, 500 µg	949.07		494 / 516	75 000	9.7 µM	Water >pH6

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

Calcium measurement is critical for numerous biological investigations. Fluorescent probes that show spectral responses upon binding calcium have enabled researchers to investigate changes in intracellular free calcium concentrations by using fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers. Fluo-3 and Fluo-4 are most commonly used among the visible light-excitable calcium indicators. 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 and consequently higher fluorescence signal levels. Cells may be loaded with the AM ester forms of these calcium indicators by adding the dissolved indicator directly to dishes containing cultured cells.

Applications:

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

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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-4**

Guidelines for use – cell loading

AM esters are the non-polar esters that readily cross live cell membranes, and rapidly hydrolyzed by cellular esterases inside live cells. AM esters are widely used for loading a variety of polar fluorescent probes into live cell non-invasively. However, cautions must be excised when AM esters are used since they are susceptible to hydrolysis, particularly in solution. They should be reconstituted in high-quality, anhydrous dimethylsulfoxide (DMSO). DMSO stock solutions should be stored desiccated at -20 °C and protected from light. Under these conditions, AM esters should be stable for several months.

The AM analogs indicators could be loaded directly:

1. Prepare a 2-5mM stock solution in high-quality, anhydrous 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. On the day of the experiment, either dissolve calcium indicators solid in DMSO or thaw an aliquot of the indicator stock solutions to room temperature. Prepare a 2-20 µM working solution in the appropriate buffered physiological medium (such as Hanks and Hepes buffer) with 0.04% *Pluronic® F-127*. For most cell lines we recommend the final concentration of calcium indicators be 4-5 uM. The exact concentration of indicators required for cell loading must be determined empirically. To avoid any artifacts caused by overloading and potential dye toxicity, it is recommended to use the minimal probe concentration that can yield sufficient signal strength. .

Note: Avoid amine-containing buffers such as Tris.
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

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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 (HHBS or buffer of your choice) 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 :

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

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.

- 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 fluorometric 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

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