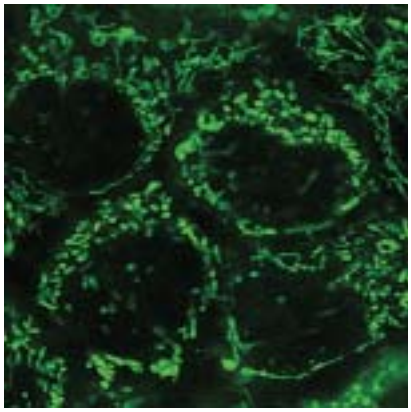
Cell Biology study



Cell Biology is one of the major growth area of Life Sciences in the post-genomic area. Cell Biology study is using largely chromogenic and fluorescent probes , beside cell culture (section E1-E29), genomics (chapter D), and immunodetections (chapter A).

This chapter is dedicated to cell biology probes, allowing a cell view at the sub-cellular and molecular level. Such cell biology probes recognize specific molecules by affinity (membrane receptor, cytoplasmic enzymes...), and generally their own properties are changed (color, fluorescence). Detection is performed by various techniques, from microplate assays to cytometry or microscopy. A large place is dedicated to fluorescent probes that present several advantages in term of sensitivity, automation, and multiparametric analysis. They are valuable tools for localization (imaging), quantification, and dynamics study (non-invasive methods).

Cell biology probes cover following areas :

- Detection of ions (Ca^{2+}, Mg^{2+}, Cl, K, Na) that are messengers (flux processes) and cofactors (enzymes) : section E32-E64.
- Other intracellular biochemical processes (pH (E65), Redox (E92), Oxydation (E100-E107)caspases activity, glutaminases (E108),...)
- Membrane probes (amphiphilic probes (E70), lipids (E75))
- Other intracellular biochemical processes (pH (E65), Redox (E92), Oxydation (E100-E107)caspases activity, glutaminases (E108),...)
- Cell and organtelles tracing (E96)
- DNA/RNA study (E119)
- Mitochondria (E129) and neural cells (E136) studies

It is impossible to present here an extensive list of probes for cell biology studies. Other are available on our searchable tools http ://www.interchim.com/interchim/customers/default.cfm or on inquire. Assay kits using these probes for diverse important cell biology applications are presented in starting page E145.

Calcium Indicators/Studies

Technical tip

Interchim and Berthold collaboration supports further your works. Many of our fluorescence and luminescence reagents and kits were validated with

BERTHOLD TECHNOLOGIES

imaging systems.

NightOWL LB981

Imaging system equiped with slow scan CCD camera for : in-vivo as well as for in-vitro imaging

- Whole animals
- Plants
- Blots
- Cell culture dishes
- Gels
- Microplates
- Arrays.

See also microplate absorbance, fluorescence and luminescence microplate readers.
See also new LED light sources (FRAEN) to convert optical microscopes to fluorescence microscopes

Calcium is an important intracellular messenger ion, responsible for the activation and deactivation of numerous biological events in cells, including neurosynaptic transmission, secretion of hormones, muscle contraction... To study the role of calcium in these events, one needs to quantitatively monitor its concentration. The most widely used method of Ca^{2+} monitoring is the use of fluorescent Ca^{2+} indicators, a technique pioneered by professor Roger Tsien and colleagues (1989). These indicators measure Ca^{2+} concentration via their fluorescent spectral changes upon Ca^{2+} binding.

With our FluoProbes® we aim to provide new Ca^{2+} indicators with improved performances as leakage resistance (PE3 dyes), higher signals (Fluo-4) or new applications (FF dyes for transient Ca^{2+} high concentrations). But you will also find popular fluorescent calcium indicators at very high purity and competitive prices for cell biology studies, and screening assays. Fura-2, Indo-1, Fluo-3 and Rhod-2 are available in both membrane-impermeant salt forms and membrane-permeant AM ester forms. For each Ca^{2+} indicator, we have several salt forms available.
All three **salt forms** ($NH4^+$, Na^+ and K^+) have the same Ca^{2+} response for a given indicator. However, you may want to select a salt form compatible with your biological system. The salt forms of the indicators are water-soluble and can be loaded into cells via microinjection or scrape loading.
The **AM esters** of the indicators are membrane-permeant and thus can be loaded into cells 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 Ca^{2+}. However, once they have entered the cells, they are rapidly hydrolyzed by intracellular esterases into the parent Ca^{2+} indicators, thus becoming sensitive to Ca^{2+}.

The section ends with luminescent dyes coelenterazines, that can be used for fine-tuned Ca^{2+} measurements, and accessory reagents (ionophores and channel blockers, caged compounds, chelators and loading/chelating agents).

Selection guide

The primary features to consider for Ca^{2+} dyes selection are their Ca^{2+} dissociation constant (Kd), then their Ca^{2+} response range, excitation/emission wavelengths, spectral shift, and relative fluorescent quantum yields. Therefore, you should select a Ca^{2+} indicator that best suits your need in consideration of your biological system, instrument settings and any other fluorescent probes that you may use at the same time. The Kd values give an estimation of the detectable Ca^{2+} concentration range, usually 0.1Kd to 10Kd. However, one should be cautious in using these in vitro determined Kd values, because the Kd values in cells can vary considerably due to differences in ionic strength, pH, viscosity and Ca^{2+} buffering by intracellular lipids and proteins (Petr M.J. and Wurster R.D. Cell Calcium, 21, 233(1997)).

Standard calcium indicators
Most popular indicators are **Fura-2**, and **Indo-1**, both being ratiometric. The shift in excitation wavelength eliminates variable effects such as cell loading, photobleaching, detector sensitivity, cell thickness, and optical path. **Bapta** and **Quin-2** were the first usable calcium indicators and they are still popular for specific applications.
In the visible region, **Fluo-3** has become a standard for Ca^{2+} measurement, thanks to its huge dynamic range upon calcium binding (100-200 fold increase of fluorescence). Excitation efficiency can be doubled with improved **Fluo-4** derivative.

Leakage resistant calcium indicators
Most of available indicators for cytosolic measurement of Ca^+, including popular and sensitive Fura-2, Indo-1 and Fluo-3, tend to leak out of cells and accumulate in organelles. This leads to incomplete Ca^{2+} cell loading, undesired background and so prevaricate datas notably in particular cell types. New leakage resistant Ca^{2+} indicators include **IndoPE3** and **FuraPE3**, **Kojo** and **Lojo**.

High concentration indicators
To measure high Ca^{2+} concentrations, new modified forms of Fluo-3, Fura-2 and Indo-1 are now preferred to Furaptra. Furaptra that was used previously need to do corrections because of its magnesium affinity, especially in mitochondria (Golovina 1997). Now our Fluo-3FF, Fura-2FF, and Indo-1FF offer several advantages (London 1996), while eliciting fluorescence properties (abs./em., photostability, and QY), similar to Fluo-3, Fura-2 and Indo-1. Other benefits are :

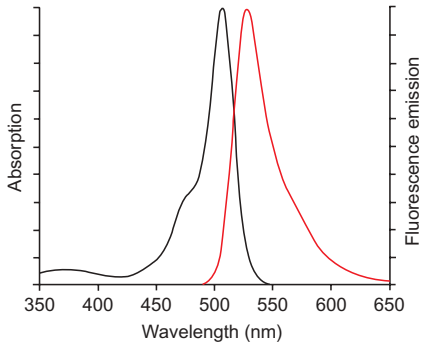
- Reduced buffering of intracellular calcium.
- Suitable for shorter lived transients (reduced perturbation).

Near membrane calcium detection
Measuring Ca^{2+} in transient cell (as in myocytes) is hampered by rapid Ca^{2+} concentration changes near plasma membrane. The **FFP18** dye has been shown to be the best one made for that topic, thanks to its association with the membrane. The indo version **FIP18** gets similar success. Now, the AM esters are available to perform non invasive experiments!

Fluo-3

Fluo-3 has its absorption maximum at 506 nm, thus making it excitable by the 488 argon-ion laser. Unlike Fura-2 and Indo-1, neither the excitation nor the emission maximum of the sensor shifts significantly before and after Ca^{2+} binding. As a result, the ratiometric measurement technique is not applicable to Fluo-3. Fluo-3 is essentially non-fluorescent without Ca^{2+}, but the fluorescence increases at least 40 times upon Ca^{2+} binding. Also, because Fluo-3 binds Ca^{2+} more weakly than do Fura-2 and Indo-1 (higher Kd : 390 nM), it is more useful for measuring high transient Ca^{2+} concentration during Ca^{2+} spikes. A major application of fluo-3 AM is drug high throughput screening.
Fluo-3 is available as different salts (water soluble, membrane-impermeant but can be loaded into cells via microinjection or scrape loading), and as AcetoxyMethyl ester (membrane permeant).

Literature : Minta, A., et al. J. Biol. Chem. 264, 8171(1989) ; Kao, J.P.Y., et al. J. Biol. Chem. 264, 8179(1989) ; Schroeder et al. J. Biomol. Screening 1(2), 75(1996). Absorption and fluorescence emission spectra of Ca^{2+}-saturated fluo-3 in pH 7.2 buffer



Absorption and fluorescence emission spectra of Ca^{2+} saturated Fluo-3.

abs em. Fluo-3, AM ester

$C_{51}H_{50}Cl_2N_2O_{23}$ MW : 1129.87
Soluble in DMSO
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (after hydrolysis) : see Fluo-3 FP-37020
Membrane-permeant Fluorescence of AM ester derivative is very weak, until its AM group is cleaved by intracellular esterase to yield the highly fluorescent Fluo-3 compound (FP-37020A).

Description	Cat.#	Qty
Fluo-3, AM ester	**FP-78932A**	1 mg
	FP-78932C	20 x 50 µg

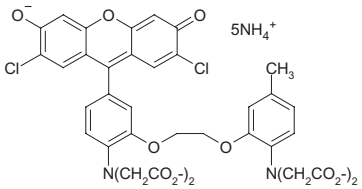
abs em. Fluo-3, AM ester 1mM in DMSO

$C_{51}H_{50}Cl_2N_2O_{23}$ MW : 1129.87
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (after hydrolysis) : see Fluo-3 FP-37020A
Convenient packaging of Fluo-3-AM (FP-78932A), widely used for HTS.

Description	Cat.#	Qty
Fluo-3, AM ester 1mM in DMSO	**FP-M2036A**	1 ml

abs em. Fluo-3, pentaammonium salt

$C_{36}H_{45}Cl_2N_7O_{13}$ MW : 854.8
Soluble in buffer pH > 6
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (low $[Ca^{2+}]$) : 503 nm/negligible
$\lambda_{exc.}/\lambda_{em.}$ (high $[Ca^{2+}]$) : 505 nm/526 nm ; EC (506 nm) : 100 000 $M^{-1}cm^{-1}$
K_d : 390 nM
Membrane-impermeant but can be loaded into cells via microinjection or scrape loading.



Description	Cat.#	Qty
Fluo-3, pentaammonium salt	**FP-37020A**	1 mg

abs em. Fluo-3, K salt

$C_{36}H_{25}Cl_2K_5N_7O_{13}$ MW : 960.02
Soluble in buffer pH > 6
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (after hydrolysis) : see Fluo-3 (FP-37020A)

Description	Cat.#	Qty
Fluo-3, K salt	**FP-03669A**	1 mg

abs em. Fluo-3, Na salt

$C_{36}H_{25}Cl_2N_2Na_5O_{13}$ MW : 879.46
Soluble in buffer pH > 6
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (after hydrolysis) : see Fluo-3 (FP-37020A)

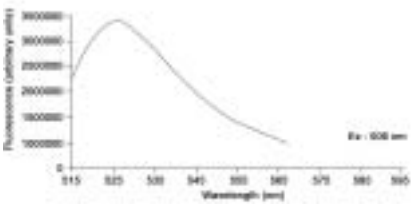
Description	Cat.#	Qty
Fluo-3, Na salt	**FP-I3021A**	1 mg

Related products :

- Fluo-4 for a brighter, more photostable dye, FP-72971.
- Kojo™ for reduced leakage, FP-AY6291
- Fluo-3FF for measurement of high Calcium concentrations, FP-AM626.
- MagFluo-3 for Ca^{2+}/Mg^{2+} measurements, FP-BB423.

Technical tip

Leakage
A major problem with Fluo-3 and Fluo-4 dyes is leakage from cells. Sulfinpyrazole and probenecid are being used to prevent it. Now our new Kojo™ or Lojo™ dyes offer a preferable solution !
Other leakage resistant Ca^{2+} indicators are Fura PE3 (FP-AM603) et Indo PE3 (FP-AM602).



Emission spectrum of Ca^{2+} saturated Kojo.

Several new dyes derived from Fluo 3 were developed Fluo-3, PE3 and Kojo™ for low retention. Further characterization are in progress.

Kojo™

Kojo™ is a new calcium indicator replacing Fluo-3 to solve leakage concerns, in cells. There is no need to modify your instrumentation settings, but thanks to leakage resistance sensitivity is slightly lower, or less indicator be used, most importantly longer experiment can be analyzed.

abs em. **Kojo™, AM**
Soluble in DMSO
Store at -20°C and protect from light
MW : 1260
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see Kojo™ (FP-AY630) ; EC : 26 000 $M^{-1}cm^{-1}$
Membrane-permeant

Description	Cat.#	Qty
Kojo™, AM	**FP-AY6291**	500 µg

abs em. **Kojo™, K salt**
Soluble in water
MW : 1056
$\lambda_{exc.}/\lambda_{em.}$: 506/525 nm ; EC : 80 000 $M^{-1}cm^{-1}$
K_d : 500 nM
Membrane-impermeant

Description	Cat.#	Qty
Kojo™, K salt	**FP-AY6301**	1 mg

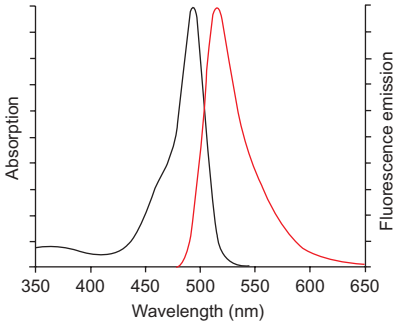
abs em. **Fluo-3-PE3**
$\lambda_{exc.}/\lambda_{em.}$: see fluo-3 (FP-37020A)

This Fluo-3 derivate shows similar fluorescent properties to Fluo-3 but it is designed to improve retention compared to Fluo-4. Kd is slightly higher. It is well retained in cells for over 15 hours it seems essentially in mitochondria.

Description	Cat.#	Qty
Fluo-3-PE3	**FP-BC0221**	1 mg

Fluo-4

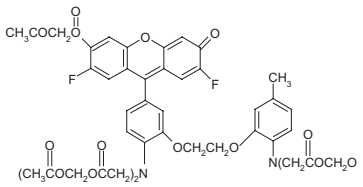
Fluo-4 is an analog of Fluo-3 with the two chlorine substituents replaced by fluorines. This increases fluorescence excitation at 488 nm and consequently about twice signal levels for confocal microscopy, flow cytometry and especially microplate screening applications. K_d (345 nM) is slightly lower than the Fluo-3 one.



abs em. Fluo-4, AM ester

$C_{51}H_{50}F_2N_2O_{23}$ MW : 1096.96
Soluble in DMSO
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see Fluo-4 (FP-M2020A)
Membrane-permeant. Fluorescence of AM ester derivative is very weak, until its AM group is cleaved by intracellular esterase to yield the highly fluorescent Fluo-4 compound.

Description	Cat.#	Qty
Fluo-4, AM ester	**FP-729712**	10 x 50 µg
	FP-729713	500 µg
	FP-729714	5 x 1 mg



abs em. Fluo-4, AM, 1mM in DMSO

Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see Fluo-4 (FP-M2020A)

Description	Cat.#	Qty
Fluo-4, AM 1mM in DMSO	**FP-M2021A**	500 µl

abs em. Fluo-4, K salt

Soluble in buffer with pH > 5
MW : 927.09
$\lambda_{exc.}/\lambda_{em.}$ (low [Ca^{2+}]) : 491 nm/negligible
$\lambda_{exc.}/\lambda_{em.}$ (high [Ca^{2+}]) : 494 nm/516 nm ; EC : 88 000 M^{-1} cm^{-1}
K_d : 345 nM
Membrane-impermeant but can be loaded into cells via microinjection or scrape loading.

Description	Cat.#	Qty
Fluo-4, K salt	**FP-M2020A**	500 µg

abs em. Fluo-4 terButyl

Used to prepare Fluo-4 conjugates (peptide, dextran...).
terButyl group should be removed by trifluoroaccetic treatment.

Description	Cat.#	Qty
Fluo-4 terButyl	**FP-BC2031**	inquire

Related Products :

- Fluo-Lojo™ for brighter dye with reduced leakage (FP-AY6311).
- Fluo-4FF for high Ca^{2+} concentrations detection (FP-F9928).
- Mag-Fluo-4 for Ca^{2+}/Mg^{2+} measurements (FP-M2025).

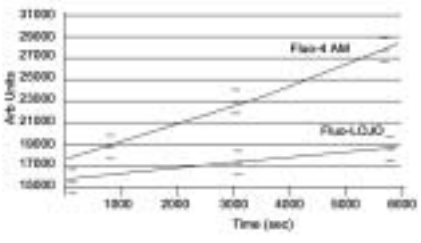
Lojo™

Several new dyes are under development ; KJM, Fluo-535 for longer-wavelength, Fluo-3, PE3 and Lojo™ for low retention. Further characterization, are in progress. Please inquire if you have special interest in them.

abs em. Lojo™, AM

Soluble in DMSO
Store at -20°C and protect from light
MW : 1277
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see Lojo™ K salt (FP-AY6321) ; EC : 26 000 $M^{-1}cm^{-1}$
Membrane-permeant

Description	Cat.#	Qty
Lojo™, AM	**FP-AY6311**	500 µg

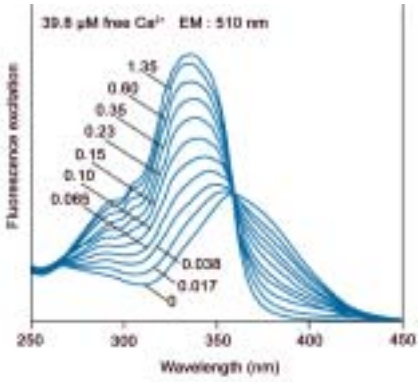


Leakage comparaison between Fluo-4 and Lojo™ on cortical neurons (both at 2 µM).

abs em. Lojo™, K salt

Soluble in water
Store at -20°C and protect from light
MW : 1023
$\lambda_{exc.}/\lambda_{em.}$: 491/515 nm ; EC : 80 000 $M^{-1}cm^{-1}$
K_d : 440 nM
Membrane-impermeant

Description	Cat.#	Qty
Lojo™, K salt	**FP-AY6321**	1 mg



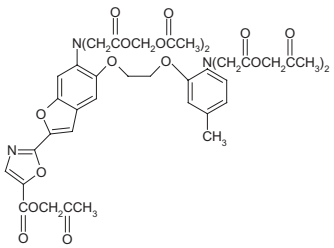
Absorption spectra of fura-2 at different concentrations Ca^{2+}.

Fura-2

Fura-2 is a widely used UV-excitable fluorescent calcium indicator. Upon calcium binding, the fluorescent excitation maximum undergoes a blue shift from 363 nm (Ca^{2+}-free) to 335 nm (Ca^{2+}-saturated), while the fluorescence emission maximum is relatively unchanged at ~510 nm. The indicator is typically excited at 340 nm and 380 nm respectively and the ratio of the fluorescent intensities corresponding to the two excitations is used for calculating the intracellular concentrations. Measurement of calcium concentration using this ratiometric method avoids interference due to uneven dye distribution and photobleaching (Bright, 1989). Fura-2 has been used in many cellular systems and applications, and is preferred to Indo-1 for microscopy ratio-imaging.
Fura-2 shows also high affinities for other divalent cations such as Zn^{2+} and Mn^{2+}.

Fura-2 indicator is now available as **"FuraPE3"** derivate which is leakage resistant !

Literature :
Bright G.R. et al, in Fluorescence Microscopy of Living Cells in Culture, Part B, (Methods in Cell Biology Vol. 30), Academic Press (1989) p. 157
Bals S., et al. Cell Calcium 11, 385 (1990)
Gorczynska E. and Handelsman, D.J. J. Biol. Chem. 266, 23739 (1991)
Grynkiewicz G., et al. J. Biol. Chem. 260, 3340(1985)
Jaconi M.E. et al. J. Cell Biol. 110, 1555(1990)
Ward C.A. and Moffat, M.P. J. Mol. Cell. Cardiol. 24, 937(1992)



abs em. **Fura-2, AM ester**

$C_{44}H_{47}N_3O_{24}$ MW : 1001.87
Soluble in DMSO
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$: (hydrolyzed) : see Fura-2 (FP-42777)
Membrane-permeant
Fluorescence of AM ester derivative is very weak, until its AM group is cleaved by intracellular esterase to yield the highly fluorescent Fura-2 compound.

Description	Cat.#	Qty
Fura-2, AM ester	**FP-42776A**	1 mg
	FP-42776B	10 x 100 µg
	FP-42776C	20 x 50 µg
	FP-85312A	1 ml at 1mM in dry DMSO

abs em. **Fura-2, K salt**

$C_{29}H_{22}K_5N_3O_{14}$ MW : 832.02
Soluble in buffer pH > 6
$\lambda_{exc.}/\lambda_{em.}$: (low $[Ca^{2+}]$) : 363/512 nm ; EC : 27000 $cm^{-1}M^{-1}$
$\lambda_{exc.}/\lambda_{em.}$: (high $[Ca^{2+}]$) : 335/505 nm ; EC : 35000 $cm^{-1}M^{-1}$
$K_d(Ca^{2+})$: 145 nM (>250nM in presence of Mg^{2+} 1mM)
Membrane-impermeant but can be loaded into cells via microinjection or scrape loading

Description	Cat.#	Qty
Fura-2, K salt	**FP-42777A**	1 mg

abs em. **Fura-2, NH_4 salt**

$C_{29}H_{37}N_8O_{14}$ MW : 721.66
Soluble in buffer pH > 6
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$: see Fura-2 (FP-42777A)

Description	Cat.#	Qty
Fura-2, NH_4 salt	**FP-AK166A**	1 mg

abs em. **Fura-2, Na salt**

$C_{29}H_{22}N_3Na_5O_{14}$ MW : 751.46
Soluble in buffer pH > 6
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$: see Fura-2 (FP-42777A)

Description	Cat.#	Qty
Fura-2, Na salt	**FP-31493A**	1 mg

Related Products :

- Fura-2FF for high Ca^{2+} concentrations measurement (FP-AM629).
- FuraPE3 for leakage resistance (FP-AM603).
- Furaptra - AM ester (Mag-Fura-2 - AM) for Ca/Mg measurements (FP-35374)

Fura-PE3

Fura-PE3 elicits similar properties as Fura-2, but is retained in cells longer than Fura-2.

References :
Ristocetin-mediated interaction of human von Willebrand factor with platelet glycoprotein Ib evokes a transient calcium signal: observations with Fura-PE3; Milner et al. ; J Lab Clin Med 1998 Jan 131:1 49-62.
New fluorescent calcium indicators designed for cytosolic retention or measuring calcium near membranes ; Vorndran C et al.; Biophys J 1995 Nov 69:5 2112-24.

abs em. Fura-PE3, AM ester

$C_{55}H_{63}N_5O_{29}$ MW : 1258.13
Soluble in DMSO
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$: (hydrolyzed) : see Fura-PE3 salt (FP-AM604)
Cell-membrane permeant : can be loaded into cells by simple incubation.
Fluorescence of this AM ester derivative is very weak, but becomes that of Fura-PE3 salt after hydrolysis of AM group by intracellular esterases binding of divalent cations to the anionic indicator.

Description	Cat.#	Qty
Fura-PE3, AM ester	**FP-AM603A**	500 µg
	FP-AM603B	10 x 50 µg

abs em. Fura-PE3, K salt

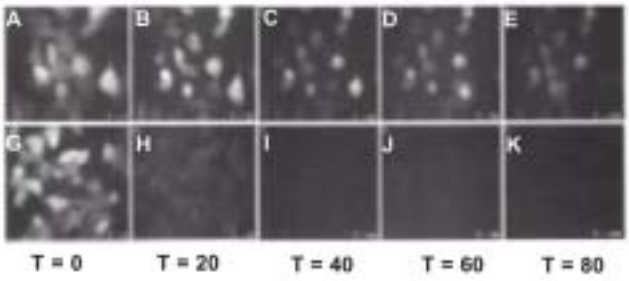
$C_{37}H_{33}N_5O_{17}K_6$ MW : 1054.31
Soluble in water pH > 6
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$: (low $[Ca^{2+}]$) : 364/502 nm
$\lambda_{exc.}/\lambda_{em.}$: (high $[Ca^{2+}]$) : 335/495 nm ; EC : 33 000 $M^{-1}cm^{-1}$
K_d(pH 7.2) : 250 nM
Membrane-impermeant but can be loaded into cells via microinjection or scrape loading.

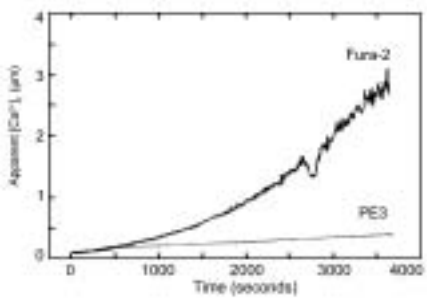
Description	Cat.#	Qty
Fura-PE3, K salt	**FP-AM604A**	500 µg

Technical tip

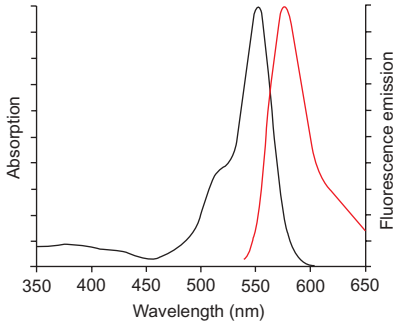
A visual comparison of Fura-2 (G-L) and PE3 (A-F) leakage in BPV cells, showing clearly the lower leakage of Fura-PE3.

BPV cells, adhered to coverslips, were loaded with fura-PE3(AM) (A-E) or fura-2(AM) (G-K).
Images (same microscope field) were recorded at 360 nm excitation at 20-min intervals beginning immediately after cells were washed. Camera gain and intensifier voltages were set based on the brightness of cells at the first time point and maintained constant thereafter.
Between the recording of images, excitation light was blocked by a shutter. Figure is adapted from Biophysical Journal ; C. Vondran et al.; Department of Zoology, University of Texas at Austin vol. 69 page 2121,1995.





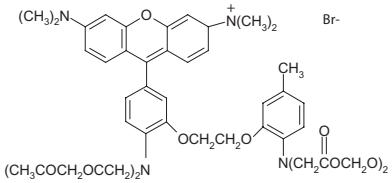
Ca^{2+} indicator leakage detected by dye titration in cell medium.



Rhod-2

Rhod-2 is non fluorescent before Ca^{2+} binding but becomes fluorescent with increasing Ca^{2+} concentration. The fluorescent enhancement for Rhod-2 from low [Ca^{2+}] to high [Ca^{2+}] is smaller than that for fluo-3, and also in general rhod-2 is somewhat less fluorescent than fluo-3. However, the wavelengths of absorption (556 nm) and emission (576 nm) maxima of Rhod-2, are longer than those of Fluo-3. This may make it useful for some applications where sample autofluorescence is a problem, where sample contains photoreceptors, when photoactivatable chelators or another fluorescent dye of shorter wavelength is used at the same time. Its K_d of 570 nM places Rhod-2 above Fluo-3 and Fura-2, thus for higher Ca^{2+} concentrations studies.

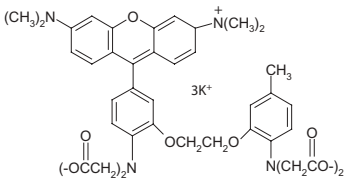
Reference :
Minta, A., et al. J. Biol. Chem. 264, 8171(1989); Kao, J.P.Y., et al. J. Biol. Chem. 264, 8179(1989).



abs em. **Rhod-2, AM ester bromide**

$C_{52}H_{59}BrN_4O_{19}$ MW : 1123.97
Soluble in DMSO
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see Rhod-2 salt (FP-M2038A)
A cell membrane-permeant Ca^{2+} ratiomeric dye for the quantitative assessment of intracellular free calcium concentration. Can be loaded into cells by simple incubation, once inside cells, hydrolysis of AM group gives the highly fluorescent Rhod-2 salt (FP-M2038A).

Description	Cat.#	Qty
Rhod-2, AM ester bromide	**FP-661582**	1 mg
	FP-661584	20 x 50 µg



abs em. **Rhod-2, K salt**

$C_{40}H_{39}K_3N_4O_{11}$ MW : 869.08
Soluble in buffer pH > 6
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (low [Ca^{2+}]) : 548 nm/negligible ; EC : 91 000 $cm^{-1}M^{-1}$
$\lambda_{exc.}/\lambda_{em.}$ (high [Ca^{2+}]) : 552 /578 nm ; EC : 96 000 $cm^{-1}M^{-1}$
K_d : 570 nM
Cell impermeant

Description	Cat.#	Qty
Rhod-2, K salt	**FP-M2038A**	1 mg

abs em. **Rhod-2, AM ester chloride**

$C_{52}H_{59}ClN_4O_{19}$ MW : 1079.52
Soluble in DMSO
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see Rhod-2 salt (FP-M2038A)
Cell permeant

Description	Cat.#	Qty
Rhod-2, AM ester chloride	**FP-T3282A**	1 mg

abs em. **Rhod-2, NH_4 salt**

$C_{40}H_{51}N_7O_{11}$ MW : 805.08
Soluble in buffer pH > 6
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see Rhod-2 salt (FP-M2038A)
Cell impermeant

Description	Cat.#	Qty
Rhod-2, NH_4 salt	**FP-66159A**	1 mg

abs em. **Rhod-2, Na salt**

$C_{40}H_{39}N_4Na_3O_{11}$ MW : 820.75
Soluble in DMSO or buffer pH > 6
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see Rhod-2 salt (FP-M2038A)
Cell impermeant

Description	Cat.#	Qty
Rhod-2, Na salt	**FP-AM709A**	1 mg

Related Products :
Rhod-2FF for of high Ca^{2+} concentrations measurement (FP-BB413).

X-Rhod dyes.

Tel 33 (0)4 70 03 88 55 ♦ Hot line 33 (0)4 70 03 73 06 ♦ Fax 33 (0)4 70 03 82 60

abs em. Rhod-2 chloride

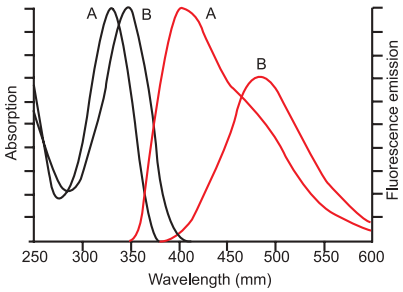
$C_{40}H_{43}ClN_4O_{11}$ MW : 791.26
Soluble in buffer pH > 6
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see Rhod-2 salt (FP-M2038A)
Cell impermeant

Description	Cat.#	Qty
Rhod-2 chloride	**FP-T3281A**	1 mg

Indo-1

Similar to Fura-2, **Indo-1** is also an UV-excitable fluorescent Ca^{2+} sensor. However, compared to Fura-2, the fluorescent emission maximum undergoes a large blue shift from 482 nm to 398 nm upon Ca^{2+} binding. Thus, Ca^{2+} concentration can be determined by measuring the fluorescence intensities at two wavelengths, usually 480 nm and 410 nm. As with Fura-2, this ratiometric measurements technique avoids problems associated with uneven dye distribution, cell or tissue thickness and photobleaching. Indo-1 has been widely used, and preferred to Fura-2, for ratiometry using a flow cytometer that can be used to measure fluorescent signals at dual wavelengths.
Indo-1 indicator is now available as **"IndoPE3"** derivate which is leakage resistant !

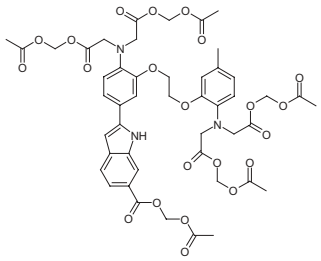
References :
Grynkiewicz, G., et al. J. Biol. Chem. 260, 3340(1985)
Circulation Res. 69, 46(1991); Stefenelli, et al. Am. Heart J. 120, 590(1990) ; Wahl, M. et al. Cell Calcium 11, 487(1990).



Absorption and fluorescence emission spectra ($\lambda_{exc.}$: 338 nm) of Indo-1 or pH 7.2 buffer.
A : hight (Ca^{2+}) ; B : no Ca^{2+}

abs em. Indo-1, AM

$C_{47}H_{51}N_3O_{22}$ MW : 1009.94
Soluble in DMSO
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolysed) : see Indo-1, salt (FP-42774A)
A cell permeable Ca^{2+} ratiometric dye for the quantitative assessment of intracellular free calcium concentration.
- Can be loaded into cells by simple incubation.
- Once inside cells, hydrolysis of AM group gives the highly fluorescent IndoPE3 product (FP-AM601).



Description	Cat.#	Qty
Indo-1, AM	**FP-427755**	1 mg
	FP-42775A	20 x 50 µg

abs em. Indo-1, K salt

$C_{32}H_{26}K_5N_3O_{12}$ MW : 840.06
Soluble in buffer pH > 6
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (low $[Ca^{2+}]$) : 346/475 nm ; EC : 33 000 $cm^{-1}M^{-1}$
$\lambda_{exc.}/\lambda_{em.}$ (high $[Ca^{2+}]$) : 330/401 nm ; EC : 33 000 $cm^{-1}M^{-1}$
K_d : 230 nM
Membrane-impermeant but can be loaded into cells via microinjection electroporation, or scrape loading.

Description	Cat.#	Qty
Indo-1, K salt	**FP-42774A**	1 mg

abs em. Indo-1, Na salt

$C_{32}H_{26}N_3Na_5O_{12}$ MW : 759.53
Soluble in buffer pH > 6
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see Indo-1, salt (FP-42774A)

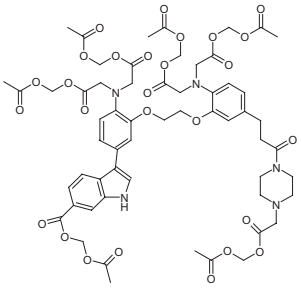
Description	Cat.#	Qty
Indo-1, Na salt	**FP-31603A**	1 mg

abs em. Indo-1, NH_4 salt

$C_{32}H_{46}N_8O_{12}$ MW : 734.77
Soluble in buffer pH > 6
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see Indo-1, salt (FP-42774A)

Description	Cat.#	Qty
ndo-1, NH_4 salt	**FP-AM293A**	1 mg

Related Products :
indo-1FF for of high Calcium concentrations measurement (FP-AM626).



Indo-PE3

Indo-PE3 elicits similar properties as Fura-2, but is retained in cells longer than Indo-1.

abs em. **Indo PE3, AM**

$C_{58}H_{67}N_5O_{27}$ MW : 1266.20
Soluble in DMSO
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see IndoPE3, salt (FP-AM601A)

A cell permeable leakage resistant Ca^{2+} ratiometric dye

- Can be loaded into cells by simple incubation.
- Once inside cells, hydrolysis of AM group gives the highly fluorescent IndoPE3 product (FP-AM601A).

Description	Cat.#	Qty
Indo PE3, AM	**FP-AM602A**	500 µg
	FP-AM602B	10 x 50 µg

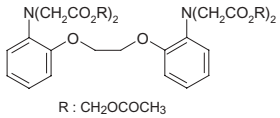
abs em. **Indo PE3, K salt**

$C_{40}H_{37}N_5O_{15}K_6$ MW : 1062.38
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (low $[Ca^{2+}]$) : 350/475 nm ; EC : 33 000 $M^{-1}cm^{-1}$
$\lambda_{exc.}/\lambda_{em.}$ (high $[Ca^{2+}]$) : 350/408 nm
K_d : 260 nM

Description	Cat.#	Qty
Indo PE3, K salt	**FP-AM601A**	500 µg

Bapta

Bapta is highly selective for Ca^{2+} over Mg^{2+} : better than EDTA and EGTA, and with lower pH sensitivity ; it releases Ca^{2+} ions about 50–400 times faster than EGTA. Absorption spectrum of BAPTA is Ca^{2+}-dependent. BAPTA becomes weakly fluorescent in aqueous solutions in presence of Ca^{2+} ($\lambda_{em.}$: 363 nm, QY = 0.03).
These features make Bapta useful to control the level of both intracellular and extracellular Ca^{2+} in presence of Mg^{2+} concentrations that interfere with other dyes, and as chelating agent for the preparation of buffers for Ca^{2+}/Mg^{2+} measurements.

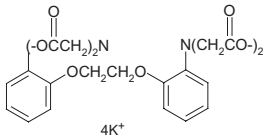


R : CH_2OCOCH_3

abs em. **Bapta-AM**

$C_{34}H_{40}N_2O_{18}$ MW : 764.69
Soluble in DMSO
Store at -20°C and protect from light
$\lambda_{exc.}$ (free) : 287nm ; EC : 5 900 $M^{-1}cm^{-1}$
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see Bapta salt (FP-453551)
Ratiometric Ca^{2+} indicator with a 105 fold greater affinity for Ca^{2+} than Mg^{2+}.
Membrane permeant.
Upon AM hydrolysis, yields the fluorescent Bapta salt.
After complexation with Ca^{2+} the absorption maximum shifts from 254 to 279 at pH > 6.

Description	Cat.#	Qty
Bapta-AM	**FP-486103**	25 mg



abs em. **Bapta, K salt**

$C_{22}H_{20}N_2O_{10}K_4$ MW : 628.88
Soluble in pH > 6
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (low $[Ca^{2+}]$) : 254 nm/low ; EC : 5 000 $M^{-1}cm^{-1}$
$\lambda_{exc.}/\lambda_{em.}$ (high $[Ca^{2+}]$) : 279 nm/363 nm
QY : 0.03
K_d : 160 nM
Membrane-impermeant but can be loaded into cells via microinjection electroporation, or scrape loading.

Description	Cat.#	Qty
Bapta, K salt	**FP-453551**	1 g

Related Products :

- BAPTA high calcium indicators.
- BAPTA based chelators.

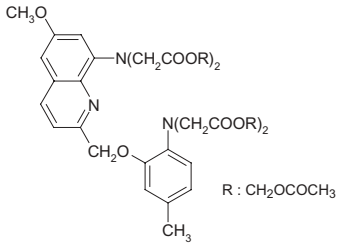
Quin-2

Quin-2 was largely used until Fura-2/Fluo-3 were available, that shows higher absorption and quantum yield. It is still used in some specific applications, as buffering intracellular Ca^{2+} transients and depleting cytosolic free Ca^{2+}, but also to prevent crocidolite-induced DNA strand breakage in human white blood cells (Brown 2004).

Reference :
D. M. Brown et al.; Calcium and ROS-mediated activation of transcription factors and TNF- cytokine gene expression in macrophages exposed to ultrafine particles; Am J Physiol Lung Cell Mol Physiol, Feb 2004; 286: 344 - 353.

Quin-2, AM

$C_{38}H_{43}N_3O_{18}$ MW : 829.78
Soluble in DMSO, acetonitrile, acetone.
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see Quin-2 salt (FP-AY6581)
Cell-permeant AM ester.



Description	Cat.#	Qty
Quin-2, AM	**FP-405126**	25 mg

Quin-2, K salt

$C_{26}H_{23}K_4N_3O_{10}$ MW : 693.89
Soluble at pH > 6
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (low $[Ca^{2+}]$) : 353/495 nm ; EC : 4 000 $M^{-1}cm^{-1}$
$\lambda_{exc.}/\lambda_{em.}$ (high $[Ca^{2+}]$) : 333/495 nm ; EC : 3 900 $M^{-1}cm^{-1}$
K_d : 60 nM
Membrane-impermeant but can be loaded into cells via microinjection electroporation, or scrape loading.

Description	Cat.#	Qty
Quin-2, K salt	**FP-AY6581**	25 mg

Quin-2 free acid

$C_{26}H_{27}N_3O_{10}$ MW : 541.52
Store at 4°C and protect from light
Cell-impermeant free acid.

Description	Cat.#	Qty
Quin-2 free acid	**FP-R1371A**	5 mg

Ca^{2+} indicators conjugates (dextran)
The preparation of indicators conjugated to dextran was designed to remedy to the dye leakage from cytoplasm to organelles (mitochondria) or to extracellular medium. Loading of these conjugates however needs invasive techniques. FluoProbes® strongly recommends our new Ca^{2+} calcium indicators as described above with classic dyes.

High Calcium Indicators

High Ca^{2+} concentrations, present in some organelles (mitochondria, vacuoles) and in excitable cells (fibroblast i.e.), were hardly detected : standard dyes Fluo-3, Fluo-4 and Rhod-2 have too high affinity for Ca^{2+}, so usually Furaptra was preferred. However, measurements needed corrections because of its magnesium affinity, especially in mitochondria (Golovina 1997).
Now, new modified forms of the standard dyes are available, eliciting similar fluorescence properties ($\lambda_{exc.}/\lambda_{em.}$ photostability, and QY), but offering several advantages (London 1996). Other benefits are :

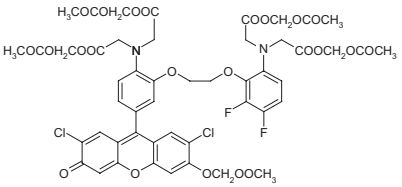
- Reduced buffering of intracellular calcium
- Suitability for shorter-lived transients (reduced perturbations)

References :
Science; Vera A. Golovina and Mordecai P. Blaustein 1997; Baltimore Md. vol. 275, page 1646

Fluo-3FF

Fluo-3FF is a modified Fluo-3 eliciting similar fluorescence properties but with higher K_d (41 µM). Thus dedicated to higher Ca^{2+} concentrations. Its also insensitive to magnesium.

References :
Cseresnyes Z., et al. 1997. Neuron 19, 403
David G., et al. 1997. J. Physiol. (London) 504, 83.



abs em. **Fluo-3FF, AM**

$C_{49}H_{46}Cl_2F_2N_2O_{23}$ MW : 1139.81
Soluble in DMSO
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see Fluo-3FF salt (FP-AM631A)
Cell-permeable, acetoxymethyl ester (AM) derivative of Fluo-3FF. Intracellular hydrolysis of AM group gives the highly fluorescent Fluo-3FF (FP-AM631A), with a very large fluorescent response upon Ca^{2+} binding.

Description	Cat.#	Qty
Fluo-3FF, AM	**FP-AM626A**	500 µg
	FP-AM626B	10 x 50 µg

abs em. **Fluo-3FF, K salt**

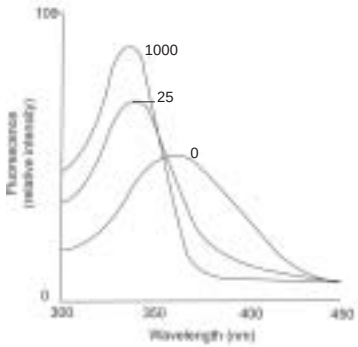
$C_{35}H_{21}Cl_2F_2K_5N_2O_{13}$ MW : 981.97
Soluble in Water
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$: 506/526 nm
K_d : 41 µM
A non-ratiometric dye which has a very large fluorescent response upon Ca^{2+} binding. Membrane-impermeant but can be loaded into cells via microinjection, electroporation, or scrape loading.

Description	Cat.#	Qty
Fluo-3FF, K salt	**FP-AM631A**	500 µg

Fura-2FF

Fura-2FF is a modified form of Fura-2 eliciting similar properties but with higher Kd and insensitivity to Mg^{2+}. This dedicates it to higher Ca^{2+} concentrations, and makes it superior to Furaptra (shows the real Ca^{2+} concentration).

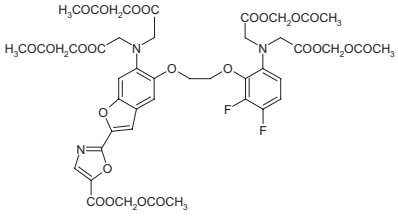
References :
- Spatially and functionally distinct Ca^{2+} stores in sarcoplasmic and endoplasmic reticulum; Vera. A Golovina, and Blaustein M.P. (1997) ; Science 275: 1643-1648.
- Cytosolic-free Calcium Increases to Greater Than 100 Micromolar in ATP-depleted Proximal Tubules; Weinberg J.M., et al. (1997); J. Clin. Investigations 100:713-722.
- Intracellular Ca^{2+} Thresholds That Determine Survival or Death of Energy-Deprived Cells; Dong Z., et al. (1998); Amer. J. Pathology 152:231-240.
- Minimal Requirements for Calcium Oscillations Driven by the IP3 Receptor; Hajnoczy G. and Thomas A. (1997) ; EMBO Journal 16:3533-3545.



Fluorescence excitation spectra of fura-2 FF as a function of added Ca^{2+} (micromoles). (Emission is set to 510 nm).

abs em. Fura-2FF, AM

$C_{43}H_{45}F_2N_3O_{24}$ MW : 1025.84
Soluble in DMSO
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see Fura-2FF salt (FP-AM627A)
Membrane-permeant, ratiometric high Ca^{2+} dye.
Intracelullar hydrolysis of AM group, gives the highly fluorescent Fura-2FF (FP-AM627A), with a very large fluorescent response upon Ca^{2+} binding.



Description	Cat.#	Qty
Fura-2FF, AM	**FP-AM629A**	500 µg
	FP-AM629B	10 x 50 µg

abs em. Fura-2FF, K salt

$C_{28}H_{18}F_2K_5N_3O_{14}$ MW : 853.97
Soluble in water
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$: 360/505 nm ; EC (360 nm) : 33 000 $M^{-1}cm^{-1}$
K_d : 35 µM
Membrane-impermeant but can be loaded into cells via microinjection electroporation, or scrape loading.

Description	Cat.#	Qty
Fura-2FF, K salt	**FP-AM627A**	500 µg

abs em. Bis-Fura-2, K salt

$C_{34}H_{20}K_6N_4O_{18}$ MW : 1007.17
Soluble in water pH > 6
Store at 4°C
$\lambda_{exc.}/\lambda_{em.}$ (free) : 366/511nm ; EC : 56 000 $M^{-1}cm^{-1}$
$\lambda_{exc.}/\lambda_{em.}$ (high Ca^{2+}) : 338/504nm ; EC : 68 000 $M^{-1}cm^{-1}$
K_d: 370 nm
Several other Fura derivatives (i.e. fluorinated in other positions) are available on request, when lower affinity for Ca^{2+} is wished (K_d ranging from 5 to 700nM). Please inquire.

Description	Cat.#	Qty
Bis-Fura-2, K salt	**FP-M1436A**	1 mg

Fluo-4FF/ Fluo-5F/ Fluo-5N/ Mag Fluo-4

Fluo-5F, Fluo-4FF, Fluo-5N and Mag-Fluo-4 are modified Fluo-4 eliciting similar fluorescent properties but with higher K_d (ranging from 2 to 90 µM), they are thus dedicated to higher Ca^{2+} concentrations (rods, neurones, sarcoplasmic reticulum/ myocytes). Fluo-4FF is the most widely used, with intermediate K_d of ca 10 µM, and insensitivity to magnesium.
All acetoxymethyl ester (AM) derivatives are almost non-fluorescent, and membrane-permeant thus easily loaded in cells. After intracellular hydrolysis of the AM group, they give the highly fluorescent salt form, with a very large fluorescent response upon Ca^{2+} binding with no spectral shift.

abs em. Fluo-4FF, AM

$C_{50}H_{46}F_4N_2O_{23}$ MW : 1118.92
Soluble in DMSO
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$: 456 nm (weak) ; EC : 25 000 $M^{-1}cm^{-1}$
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see Fluo-4FF salt (FP-R1264A)

Description	Cat.#	Qty
Fluo-4FF, AM	**FP-F9928A**	500 µg

abs em. Fluo-4FF, K salt

$C_{35}H_{21}F_4K_5N_2O_{13}$ MW : 949.07
Soluble in Water
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (water) : 491/weak ; EC : 72 000 $M^{-1}cm^{-1}$
$\lambda_{exc.}/\lambda_{em.}$ (Ca^{2+}) : 494/516 nm ; EC : 75 000 $M^{-1}cm^{-1}$
K_d : 9.7 µM

Description	Cat.#	Qty
Fluo-4FF, K salt	**FP-R1264A**	500 µg

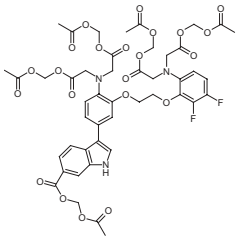
abs em. mag-Fluo-4

$K_d(Ca^{2+})$: 22 µM
See product FP-M2025 page E58.

Indo-1FF

Indo1-FF is a modified Indo-1, eliciting similar properties but with higher K_d (33 µM), thus it is dedicated to higher Ca^{2+} concentrations, and with Mg^{2+} insensitivity. It is also used in flow cytometry.

References :
-Ca^{2+}-induced exocytosis in individual human neutrophils : high and low affinity granule populations and submaximal responses ; Nuesse O., (1998) ; EMBO Journal 17:1279-1288.
-Use of Indo-1FF for measurements of rapid micromolar cytoplasmic free Ca^{2+} increments in a single smooth muscle cell ; Ganitkevich V.Y. (1998) ; Cell Calcium 28:313-322.



abs em. Indo-1FF, AM

$C_{46}H_{47}F_2N_3O_{22}$ MW : 1031.89
Soluble in DMSO
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see Indo-1FF salt (FP-AM630)
Membrane-permeant, ratiomeric dye.
AM hydrolysis leads to the highly fluorescent Indo-1FF (FP-AM630).

Description	Cat.#	Qty
Indo-1FF, AM	**FP-AM628A**	500 µg
	FP-AM628B	10 x 50 µg

abs em. Indo-1FF, K salt

$C_{31}H_{22}F_2K_5N_3O_{12}$ MW : 862.04
Soluble in water
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (10 mM Ca^{2+}) : 350/475 nm ; EC (360 nm) : 33 000 $M^{-1}cm^{-1}$
K_d : 33 µM
Membrane-impermeant but can be loaded into cells via microinjection, electroporation, or scrape loading.

Description	Cat.#	Qty
Indo-1FF, K salt	**FP-AM630A**	500 µg

Rhod-2FF

abs em. **Rhod-2FF, AM**

MW : 1146
Soluble in DMSO
Store at -20°C and protect from light
Cell-permeable, hydrolyzed in Rhod-2FF (FP- BB4140).

Description	Cat.#	Qty
Rhod-2FF, AM	**FP-BB4130**	10 x 50 µg

abs em. **Rhod-2FF, K salt**

MW : 891
Soluble in Water
Store at 4°C
A Ca^{2+} indicator which has a large fluorescent response upon Ca^{2+} binding. Lower autofluorescence than Fluo-3 is possible thanks to higher wavelength. Membrane-impermeant but can be loaded into cells via microinjection, electroporation, or scrape loading.

Description	Cat.#	Qty
Rhod-2FF, K salt	**FP-BB4140**	500 µg

Bapta-FF

Bapta-FF is a modified Bapta-2 eliciting similar properties but with higher K_d. Thus dedicated to higher Ca^{2+} concentrations

abs em. **Bapta-FF, AM**

MW : 766.00
Soluble in DMSO
Store at -20°C and protect from light

Description	Cat.#	Qty
Bapta-FF, AM	**FP-AM934A**	10 mg

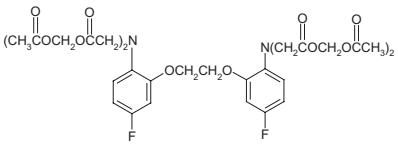
abs em. **Bapta-FF, free acid**

MW : 477.00
Soluble in water
Store at 4°C and protect from light
K_d : 65 µM
Membrane-impermeant

Description	Cat.#	Qty
Bapta-FF, free acid	**FP-AM932A**	10 mg

DF-Bapta

DF-BAPTA is rather used as chelating agent, because of its weak fluorescence in aqueous solutions ($\lambda_{em.}$ 363 nm, QY : 0.03).



abs ■ **DF-Bapta, AM**

5,5'difluoro-Bapta acetoxy methyl ester
$C_{34}H_{38}F_2N_2O_{18}$ MW : 800.68
Soluble in DMSO
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (EtOH) : 290/none
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see DF-BAPTA (FP-46743A)
Membrane-permeant.
Hydrolysis of AM group gives DF-Bapta free acid.

Description	Cat.#	Qty
DF-Bapta, AM	**FP-46742A**	10 mg

abs ■ **DF-Bapta, K salt**

$C_{22}H_{18}F_2K_4N_2O_{10}$ MW : 664.8
Soluble in water
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (pH 7.2) : 289/263 nm (weak)
K_d(pH7) : 635 nM
Membrane-impermeant.
Absorption spectrum is dependant on Ca^{2+}.
Main application is ^{19} NMR analysis of Ca^{2+} in live cells and tissues.

Description	Cat.#	Qty
DF-Bapta, K salt	**FP-46743A**	50 mg

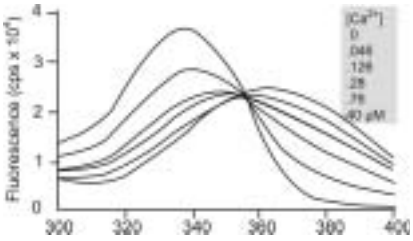
Other Bapta derivatives :
The most powerful Ca^{2+} chelator among these is 5,5'-dimethyl BAPTA, which is also available as its cell-permeant AM ester. with intermediate affinity for Ca^{2+}.
There's also the 5,5'-dibromo BAPTA, AM ester derivative of EGTA that can be passively loaded into cells to generate intracellular EGTA.
4-Trifluoromethyl BAPTA may also be a useful probe for 19F NMR analysis of intracellular Ca^{2+}.

Near Membrane Ca^{2+} indicators

Measuring calcium in transient cells (as in myocytes) is hampered by rapid Ca^{2+} concentration changes near plasma membrane. An hydrophobic collar maintain the dye bound to membrane, but, unlike other hydrophobic dyes, the chelating portion measure Ca^{2+} in the gates and is not lost in the membrane.
The indo version **FIP18** got similar success. Now, the AM esters are available to perform non invasive experiments!

FFP18

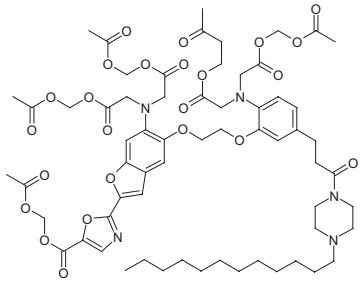
The FFP18 dye has been shown the best one made for transient cells (as myocytes). FFP18 measures rapid Ca^{2+} concentration changes near plasma membrane, thanks to its membrane association. It fluoresces in the blue region.



Reference :
Etter E. et al; Near membrane Ca^{2+} transients resolved using the Ca^{2+} indicator FFP18. Proceedings of National Academy of Sciences, 93, 5368-5373, 1996

abs em. **FFP18, AM**

$C_{62}H_{82}N_5O_{25}$ MW : 1297.76
Soluble in DMSO
Store at -20°C and protect from light
Spectra after hydrolysis as FFP18 salt (FP-AM605A)
Membrane-permeant (loading may need adjustement to cell type).
Fluorescent dye which selectively monitors near membrane calcium and has low calcium affinity.



Description	Cat.#	Qty
FFP18, AM	**FP-AM606A**	500 µg
	FP-AM606B	10 x 50 µg

abs em. **FFP18, K salt**

$C_{47}H_{57}N_5O_{15}K_5$ MW : 1127.51
Soluble in water
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (low $[Ca^{2+}]$) : 364/502 nm ; EC : 30 000 $M^{-1}cm^{-1}$
$\lambda_{exc.}/\lambda_{em.}$ (high $[Ca^{2+}]$) : 335/490 nm
Apparent K_d : 400 nM (pH 7.2)
Membrane impermeant.

Description	Cat.#	Qty
FFP18, K salt	**FP-AM605A**	250 µg

FIP18

FIP18 is similar to FFP18, but with blue shifted absorption and emission. It serves as ratiometric dye with the same fluorescent properties and similar applications as Indo-1, but selectively monitors near membrane Ca^{2+} and has low Ca^{2+} affinity.

abs em. **FIP18, AM**

$C_{60}H_{78}N_5O_{19}$ MW : 1173.3 1
Soluble in DMSO
Store at -20°C and protect from light
See FP-AM609A for spectra after hydrolysis
Membrane-permeant.

Description	Cat.#	Qty
FIP18, AM	**FP-AM608A**	500 µg
	FP-AM608B	10 x 50 µg

abs em. **FIP18, K salt**

$C_{50}H_{60}N_5O_{15}K_5$ MW : 1166.57
Soluble in water
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (low $[Ca^{2+}]$) : 340/475 nm ; EC : 32 000 $M^{-1}cm^{-1}$
$\lambda_{exc.}/\lambda_{em.}$ (high $[Ca^{2+}]$) : 330/408 nm
Apparent K_d : 450 nM (pH 7.2)

Description	Cat.#	Qty
FIP18, K sal	**FP-AM609A**	250 µg

Nomo and Momo

The following new dyes have the hydrophobic tail that allows them to stay in the plasma membrane, and still respond to calcium. The fluorescent properties are close to Fluo-3 and Fluo-4, with a slight lowering of affinity. Characterization are still under process.

abs em. Nomo™, AM

MW : 1424
Soluble in DMSO
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see product Nomo™ salt (FP-AY6771)
Membrane-permeant.

Description	Cat.#	Qty
Nomo™, AM	**FP-AY6741**	5 x 50 µg

abs em. Nomo™, K salt

MW : 1254
Soluble in water
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$: 506/525 mm ; EC 80 000 $M^{-1}cm^{-1}$
K_d : 800 nM
Non-ratiometric membrane permeant Ca^{2+} indicator.
Absorbance and Emission spectra are virtually the same with and without calcium.

Description	Cat.#	Qty
Nomo™, K salt	**FP-AY6771**	5 x 50 µg

abs em. Momo™, AM

MW : 1391
Soluble in DMSO
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see product Momo™ salt (FP-AY6801)
Membrane-permeant. Non-ratiometric
Stay close to the membrane and is a good Ca^{+} membrane dye.
Excitation and emission wavelengths are the same as Fluo-3.

Description	Cat.#	Qty
Momo™, AM	**FP-AY6781**	500 µg

abs em. Momo™, K salt

MW : 1222
Soluble in water
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$: 419/515 mm ; EC 80 000 $M^{-1}cm^{-1}$
K_d : 710 nM
Membrane impermeant.

Description	Cat.#	Qty
Momo™, K salt	**FP-AY6801**	500 µg

Other Fluorescent Ca^{2+} indicators

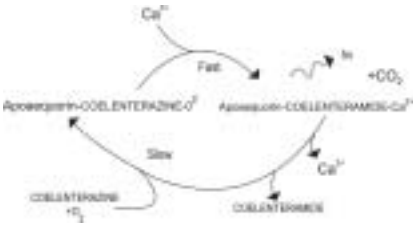
Several other Ca^{2+} indicators are available that provides absorption/emission maxima and affinity (K_d) that may suit better your application :

- Derivatives of previous standard indicators
- Original indicators, i.e. BTC, a low affinity ratiometric dye that measures Ca^{2+} in high concentrations more accurately than Fura-2 (lower sample autofluorescence).

Please inquire for any specific need.

Coelenterazines (Luminescent Ca^{2+} indicators)

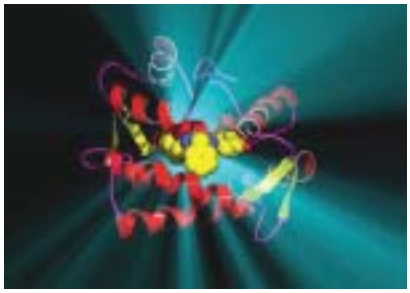
Coelenterazine (native form) is a bioluminescent substrate for enzymes apoaequorin and Renilla luciferase. FluoProbes® offers a large selection of coelenterazine analogs, with each of them giving unique luminescent properties. All these analogs are highly purified (purity : >98%) to ensure optimal results in bioassays. Careful, selection of a coelenterazine derivative may be necessary to best suit your application.



The aequaporin / coelenterazine system involves a ca 22Kda complex that contains apoaequorin protein (a protein from Aequorea victoria jelly fish), molecular oxygen, and the luminogen coelenterazine. It releases carbon dioxide and blue light upon oxidation in presence of Ca^{2+}.

1) Using coelenterazine / aequorin complex for Ca^{2+} study.
Compared with fluorescent calcium indicators, aequaporin complex/coelenterazin system has several advantages in detecting calcium :

- The Ca^{2+}/aequorin complex can detect a broad range of calcium concentrations, from ~0.1 µM to >100 µM,
- Background is lower than with fluorescence, and no autofluorescence of sample occurs,
- Although signal is lower, higher signal/noise ratios can be obtained with imaging equipments,
- The aequorin complex is not exported from cells, allowing to follow calcium concentration changes in cells for hours to days.



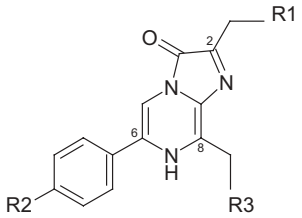
Ca^{2+} - Aequaporin photo system "in action" according X-Ray studies.

Coelenterazine has two emission peaks at 405 and 465 nm, respectively, allowing to measure calcium concentration via the ratio of emission intensities.[17]

2) Using coelenterazine / aequorin complex for ROS species
Coelenterazine products are widely used for superoxide and peroxynitrite detect via chemiluminescence [13,11]. See page E100.

1) Meth. Cell Biol. 40, 305(1994)
2) Meth. Enzymmol. 172, 164, (1989)
3) J. BioChem. 105, 473(1989)
4) J. Chem. Soc. Chem. Commun. 21, 1566(1986)
5) Meth. Enzymol. 57, 271(1978)
6) Tetrahedron Lett. 31, 2963(1973)
7) Nature 256, 236(1975)
8) Anal. BioChem. 219, 169(1994)
9) proc. Natl. Acad. Sci. U SA 96, 151(1999)
10) Free Radic. Biol. Med. 28, 1232(2000)
11) Circ. Res. 84, 1203(1999)
12) Immunol. Today 15, 7(1994)
13) Anal. BioChem. 206, 273(1992)
14) BioChem. Pharmacol. 60, 471(2000)
15) J. Clin. Chem. Clin. BioChem. 25, 23(1987)
16) Bioluminescence and Chemiluminescence : Current Status (Stanley, P.E. and Kricka, L.J., Eds), pp.511-514, Wiley, Chichester
17) BioChem. J. 251, 405(1988)
18) BioChem. Biophys. Res. Commun. 233, 349(1997)

3) Using coelenterazine for reporter systems
The development of aequaporin vectors prompted many gene reporter assays, as well aequaporin tagged recombinant protein reporter assays to investigate Ca^{2+} (see this section) and reactive Oxygen species (ROS) assays. Recombinant proteins can even be targetted in definite cell compartments, for fine measurements.



* All datas from BioChem. J. 261, 913(1989) ; other data can be found in O.Shimoraura Cell Calcium 14, 373 (1993)
§ Luminescence capacity is the total light emission of aequorin in saturating Ca^{2+}. Intensity of luminescence in saturating Ca^{2+} measured at max emission wavelength. Half-rise time is the delay elapsed to get 50% of the maximum emission.
substituant groups R1, R2 and R3, in positions 2, 6 and 8, are hydrogen (H), hydroxyl (OH), Phenyl (Phe), CycloPentyl (CP), 2-propionyl (2P), Napthyl (Naph), methyl (Met). Coelenterazine e has a -CH_2CH- bridge between the 6-phenyl-OH and position 2 of the imidazopyrazinone core.

Technical tip

Bioluminescence is the light produced in a biochemical reaction involving the oxidation of a substrate by an enzyme.

This phenomenon has been used extensively in different formats for life science research and drug discovery owing to its extremely high sensitivity and non-hazardous nature. Among examples of bioluminescence applications :

- Calcium detection in live cells or tissues [1-7]
- Reporter gene assays [8]
- ELISA, bioluminescence resonance energy transfer (BRET) for protein interaction studies 9
- Superoxide anion detection [10-14]
- Drug high throughput screening.

The key enzymes involved in most of these applications are, for Ca+ detections, apoaequorin and, for gene reporter assays, various luciferases including Renilla luciferase and Firefly luciferase (also combined with alkaline phosphatase[15] or ß-galactosidase[16] in assays).

See also Luciferins/Firefly luciferase (section E49) for gene reporter assays.

Table of Luminescent Properties of Coelenterazine Products with Apoaequorin*

Coelenterazine	Cat.#	R1 (2) #	R2 (6) #	R3 (8) #	λ max. Emission (nm)	Relative Luminescence capacity §	Relative Intensity §	Half-rise Time(s) §
Coelenterazine Native	**FP-97233A**	OH	OH	Phe	465	1.00	1.00	0.4-0.8
Coelenterazine cp	**FP-R3079A**	OH	OH	CP	442	0.95	20	0.15-0.3
Coelenterazine e	**FP-T8677A**	OH	OH#	Phe	405 and 465	0.5	4	0.15-0.3
Coelenterazine f	**FP-43876A**	F	OH	Phe	473	0.80	18	0.4-0.8
Coelenterazine fcp	**FP-R4711A**	F	OH	CP	452	0.57	135	0.4-0.8
Coelenterazine h	**FP-R3078A**	H	OH	Phe	464	0.82	10	0.4-0.8
Coelenterazine hcp	**FP-08353A**	H	OH	CP	444	0.67	190	0.15-0.3
Coelenterazine i	**FP-R3080A**	I	OH	Phe	476	0.70	0.03	8
Coelenterazine ip	**FP-R4712A**	I	OH	2P	441	0.54	47	1
Coelenterazine n	**FP-39819A**	Naph	OH	Phe	467	0.26	0.01	5

Coelenterazine Native

$C_{26}H_{21}N_3O_3$ MW : 423.47
Store at 20°C and protect from light

Description	Cat.#	Qty
Coelenterazine Native	**UP972331**	50 µg
	UP972333	1 mg
	FP-97233B	250 µg

Coelenterazine cp

$C_{28}H_{23}N_3O_3$ MW : 415.48
Store at 20°C and protect from light

Description	Cat.#	Qty
Coelenterazine cp	**UPR30791**	50 µg
	UPR30793	1 mg
	FP-R3079B	250 µg

Coelenterazine e

$C_{25}H_{25}N_3O_3$ MW : 449.51
Store at 20°C and protect from light

Description	Cat.#	Qty
Coelenterazine e	**UPT86770**	50 µg
	FP-43876B	250 µg

Coelenterazine f

$C_{26}H_{20}N_3O_2F$ MW : 425.45
Store at 20°C and protect from light

Description	Cat.#	Qty
Coelenterazine f	**UP438761**	50 µg
	UP438763	1 mg
	FP-43876B	250 µg

Coelenterazine fcp

$C_{25}H_{24}FN_3O_2$ MW : 417.49
Store at 20°C and protect from light

Description	Cat.#	Qty
Coelenterazine fcp	**UPR47110**	50 µg
	UPR47111	1 mg
	FP-R4711B	250 µg

Coelenterazine h

$C_{26}H_{21}N_3O_2$ MW : 407.49
Store at 20°C and protect for light

Description	Cat.#	Qty
Coelenterazine h	**UPR30782**	50 µg
	UPR30783	1 mg
	FP-R3078B	250 µg

Coelenterazine hcp

$C_{25}H_{25}N_3O_2$ MW : 399.49
Store at 20°C and protect from light

Description	Cat.#	Qty
Coelenterazine hcp	**UP083532**	50 µg
	UP083534	1 mg
	FP-08353B	250 µg

Coelenterazine i

$C_{26}H_{20}IN_3O_2$ MW : 533.36
Store at 20°C and protect from light

Description	Cat.#	Qty
Coelenterazine i	**UPR30801**	50 µg
	UPR30803	1 mg
	FP-R3080B	250 µg

Coelenterazine ip

$C_{23}H_{23}N_3O_3$ MW : 389.45
Store at 20°C and protect from light

Description	Cat.#	Qty
Coelenterazine ip	**UPR47120**	50 µg
	UPR47122	1 mg
	FP-R4712B	250 µg

Coelenterazine n

$C_{30}H_{23}N_3O_3$ MW : 457.54
Store at 20°C and protect from light

Description	Cat.#	Qty
Coelenterazine n	**UP398192**	50 µg
	UP398193	1 mg
	FP-39819B	250 µg

2-methyl Coelenterazine

Coelenterazin 2-methyl analog
$C_{20}H_{17}N_3O_2$ MW : 331.4
Store at 20°C and protect from light

Description	Cat.#	Qty
2-methyl Coelenterazin	**UPT88890**	50 µg
	UPT88891	1 mg

Coelenterazine 400a

$C_{26}H_{21}N_3O$ MW : 391.48
Store at 20°C and protect from light

Description	Cat.#	Qty
Coelenterazine 400a	**UPBB8391**	50 µg
	UPBB8392	1 mg
	FP-BB839B	250 µg

Coelenterazine Sampler Kit

Contains 25 µg of each nine coelenterazine analogs : coelenterazine, coelenterazine *cp*, coelenterazine *f*, coelenterazine *fcp*, coelenterazine *h*, coelenterazine *hcp*, coelenterazine *i*, coelenterazine *ip*, and coelenterazine *n*.

Description	Cat.#	Qty
Coelenterazine Sampler Kit	**FP-42176C**	1 kit (9 x 25 µg)

* All coelenterazines are soluble in MeOH ; DO NOT DISSOLVE IN DMSO
Aquous solutions >1 mM can be prepared at in pH 7 buffer containing 50 mM 2-hydroxypropyl cyclodextrin.

Related products :
- ADVASEP and FluoCD™ technology

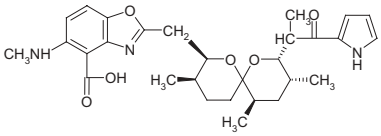
Ask for other quantities.

Other reagents useful for Ca^{2+} studies

Ca^{2+} studies require not only valuable indicators. Interchim provides several buffers and detergents for Ca^{2+} calibration and fluorescent dyes loading in cells, as ionophores blocker ionic channels to assess signal measurement specificity. Concanavalin A may also be useful as it require Ca^{2+} and Mg^{2+} for binding to oligosaccharides.

Ionophores and channel blockers

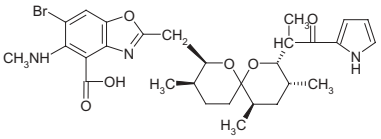
Ionophores are able to open ion channels in membranes. They are used to equilibrate certain ion concentrations for loading or control experiments.



abs em. **A23187(Ca) (calcimycin; Calcium Ionophore III; A-23187 free acid)**

$C_{29}H_{37}N_3O_6$ MW : 523.63
Soluble in DMSO and ethanol
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$: 378/438 nm ; EC : 8 900 $M^{-1}cm^{-1}$
A-23187 is a calcium ionophore that rapidly equilibrates intracellular and extracellular Ca^{2+} and Mg^{2+} concentrations. It is used to quench intracellular fluorescence of Mg^{2+} indicators for calibration purpose, notably for visible light–excitable indicators, including BAPTA, Fluo-3, Fluo-4, Rhod-2, X-Rhod-1 and Fura Red™. However, for Ca^{2+} (but not for Mg^{2+}), 4-Br-A23187 (FP-37222A) is preferred because it does not fluoresce, suiting also for Fura2, Indo-1 and Quin-2.
Has also been used to equilibrate intracellular Zn^{2+}.
Caron-Leslie, L.A., et al. FASEB J. 8, 639 (1994)

Description	Cat.#	Qty
A23187(Ca)	**FP-28362A**	10 mg



abs em. **4-Br A23187, free acid**

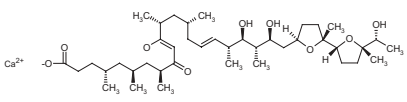
4-Bromo calcimycin free acid
$C_{29}H_{36}BrN_3O_6$ MW : 602.54
Soluble in DMSO and ethanol
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$: 289 nm/negligible ; EC : 20000 $M^{-1}cm^{-1}$
4-Br-A23187 is an analog of A23187 but does not fluoresce making it preferable for most Ca^{2+} and Mg^{2+} calcium indicator calibrations, suiting notably also for Fura2, Indo-1 and Quin-2.

Description	Cat.#	Qty
4-Br A23187, free acid	**FP-37222A**	1 mg

abs em. **Ionomycin, calcium salt**

$C_{41}H_{70}CaO_9$ MW : 747.09
Soluble in DMSO and ethanol
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$: 300 nm/negligible ; EC : 22 000 $M^{-1}cm^{-1}$
An effective Ca^{2+} ionophore that is commonly used both to modify intracellular Ca^{2+} concentrations and to calibrate fluorescent Ca^{2+} indicators. Ionomycin also transports other ions (Pb^{2+} and some other divalent cations, several lanthanide trivalent cations).

Description	Cat.#	Qty
Ionomycin, calcium salt	**FP-53989A**	1 mg



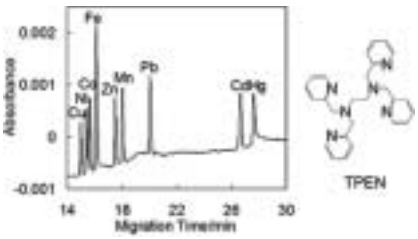
abs em. **Thapsigargin**

$C_{34}H_{50}O_{12}$ MW : 650.77
Soluble in DMSO and ethanol
Store at 4°C and protect from light
Cell-permeable tumor promoter which enhances the discharge of Ca^{2+} from intracellular stores by specifically inhibiting endoplasmic reticulum Ca^{2+}-ATPase.

Description	Cat.#	Qty
Thapsigargin	**FP-42759A**	1 mg

Technical tip

"**Caged compounds**" biologically active molecules that contain a photolabile "cage" that realease the active parent compound that upon photolysis. Caging is thus used to deliver active compound to definite site or in definite timing, and also to impart greater membrane permeability to the parent compounds. Loading in cells may be direct, or need as well microinjection or permeabilization techniques. Irradiation with short pulses of light produces light achieves
quantity-specific jumps in concentration, allowing the study of associated physiological responses in a much shorter time frame (milliseconds) than was previously possible. I.e. the use of Caged ATP and Ca^{2+} indicators eliminated the resolution limitation inherent in diffusion techniques.
Most caged (Ca^{2+}) chelators are based on the photochemistry of the o-nitrobenzyl group. The photolysis of this group alters the affinity of these agents for Ca^{2+}. Such photolabile calcium chelators have binding affinity for calcium that is decreased by irradiation with light. They are used to control the concentration of Ca^{2+} in the cell, especially those that are very small or otherwise not amenable to microinjection.



A high resolution detection of doubly charged first row transition and heavy metal ions by capillary electrophoresis (CE) in biological samples with sub-micromol sensitivity using TPEN (The Analyst, 2005, 130(5), 659 – 663).

Caged compounds

Caged compounds (Caged Ca^{2+}, Caged Chelators) are used to scavenge or to release defined ions rapidly upon a photoactivation pulse. Intracellular Ca^{2+} concentration changes can thus be created and their physiological effects observed.

Chelators

abs em. **TPEN**

Tetrakis-(2-pyridylmethyl)ethylenediamine
$C_{26}H_{28}N_6$ MW : 424.55
Soluble in ethanol
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$: 261 nm/negligible ; EC : 14 000 $M^{-1}cm^{-1}$
K_d for Zn^{2+} = 2.6 x10^{-16} M
K_d for Fe^{2+} = 2.4 x10^{-15} M
K_d for Mn^{2+} = 5.4 x10^{-11} M
K_d for Ca^{2+} = 4.0 x10^{-5} M
K_d for Mg^{2+} = 2.0 x10^{-2} M
TPEN selectively chelates intracellular heavy metal ions such as Zn^{2+}, Cu^{2+} Mn^{2+} and Fe^{2+} without disturbing Ca^{2+} and Mg^{2+} concentrations. Used to set the zero reference of Zn^{2+} level. Applications :
- Discrimination of Ca^{2+}/heavy metal ions effects
- Study of Zn^{2+} effect on enzymes or protein structure

Description	Cat.#	Qty
TPEN	**FP-44736A**	100 mg

TCPP

$C_{48}H_{30}N_4O_8$ MW : 790.79
tetrakis-(4-carboxyphenyl)porphine
Soluble in pH > 6 or DMSO

Description	Cat.#	Qty
TCPP	**FP-83201A**	5 mg

TSPP

$C_{44}H_{30}N_4O_{12}S_4$ MW : 9354.98
tetrakis-(4-sulfophenyl)porphine

Description	Cat.#	Qty
TSPP	**FP-80234A**	5 mg

GEDTA (EGTA)

O,O'-Bis(2-aminoethyl)ethyleneglycol-N,N,N',N'-tetraacetic acid
$C_{14}H_{24}N_2O_{10}$ MW : 380.35
EGTA is the most widely used calcium selective chelator. The calcium complex of EGTA is 100 000 times more stable than its Mg complex. It is utilized to prepare calcium buffers and control the calcium ion concentration.

Description	Cat.#	Qty
GEDTA (EGTA)	**T31560**	10 g
	T31561	100 g

abs em. **BAPTA and its derivates**

BAPTA is useful as chelating agent for the preparation of buffers, and even superior to EDTA, because it is very selective for Ca^{2+} over Mg^{2+}. Several derivatives are available for more specific buffering applications. See page E40.

abs **5,5'-dibromo BAPTA, AM**

$C_{34}H_{38}Br_2N_2O_{18}$ MW : 922.48
Store at -20°C and protect from light
$\lambda_{exc.}$: 296 nm ; EC : 6 900 $M^{-1}cm^{-1}$
$\lambda_{exc.}$ (hydrolyzed) : see diBromo-BAPTA Soluble in EtOAc
Membrane permeant.
Intracellular AM hydrolysis yields diBromo-Bapta (FP-96301A).

Description	Cat.#	Qty
5,5'-dibromo BAPTA, AM	**FP-48338A**	25 mg

abs 5,5'-dibromo BAPTA, K salt

$C_{22}H_{18}Br_2K_4N_2O_{10}$ MW : 786.62
Soluble in water pH>6
$\lambda_{exc.}$: 263 nm (dependant on Ca^{2+}) ; EC : 18 000 $M^{-1}cm^{-1}$
K_d : 1.6 µM
Has an intermediate affinity for Ca^{2+}. Has been applied for buffering, as well as for Ca^{2+} binding studies, or blockage of certain cell physiology processes.

Description	Cat.#	Qty
5,5'-dibromo BAPTA, K salt	**FP-96301A**	100 mg

abs 5,5'-difluoro- BAPTA, AM

$C_{34}H_{38}F_2N_2O_{18}$ MW : 800.68
Store at -20°C and protect from light
$\lambda_{exc.}$: 290 nm ; EC : 5 700 $M^{-1}cm^{-1}$
$\lambda_{exc.}$ (hydrolyzed) : see diFluoro-BAPTA
Membrane permeant.
Intracellular AM hydrolysis yields diFluoro-Bapta (FP-46743A)

Description	Cat.#	Qty
5,5'-difluoro- BAPTA, AM	**FP-46742A**	25 mg

abs 5,5'-difluoro- BAPTA, K salt

$C_{22}H_{18}F_2K_4N_2O_{10}$ MW : 664.78
$\lambda_{exc.}$: 290 nm ; EC : 5 100 $M^{-1}cm^{-1}$
K_d : 635nM
Has the lower affinity for Ca^{2+}
used for NMR analysis of Ca^{2+} in live cells and tissues.

Description	Cat.#	Qty
5,5'-difluoro- BAPTA, K salt	**FP-46743A**	50 mg

abs 5,5'-dimethyl BAPTA, AM
(MAPTA, AM)

$C_{36}H_{44}N_2O_{18}$ MW : 792.75
Store at -20°C and protect from light
$\lambda_{exc.}$: 291 nm ; EC : 5 900 $M^{-1}cm^{-1}$
$\lambda_{exc.}$ (hydrolyzed) : see diMethyl-BAPTA
Membrane permeant.
Intracellular AM hydrolysis yields diFluoro-Bapta (FP-46743A).

Description	Cat.#	Qty
5,5'-dimethyl BAPTA, AM	**FP-46778A**	25 mg

abs 5,5'-dimethyl BAPTA, K salt
(MAPTA, K salt)

$C_{24}H_{24}K_4N_2O_{10}$ MW : 656.87
Soluble in water pH>6
$\lambda_{exc.}$: 291nm ; EC : 5 100 $M^{-1}cm^{-1}$
K_d: 40nM
This chelator has the higher affinity for Ca^{2+}.

Description	Cat.#	Qty
5,5'-dimethyl BAPTA, K salt	**FP-46779A**	100 mg

Other chelators available.

Loading buffers and Loading/clearing agents

Calcium Calibration kit

Includes all buffers and the method to load cells with defined Ca^{+} concentrations.
This kit contains a 10 mM K_2EGTA buffered solution and a 10 mM CaEGTA buffered solution.
The kit can be used to generate calcium concentrations from zero up to 40 mM.

Description	Cat.#	Qty
Calcium Calibration kit	**FP-21527A**	1 kit

Pluronic® F-127

A non-ionic detergent useful for solubilizing large dye molecules such as Fura-2/AM, Fluo-3/AM, Indo-1/AM and Rhod-2/AM to facilitate cell loading. Pluronic F-127 is usually dissolved in DMSO or water up 20% w/v by gentle heating.

References : Poenie, M. et al. Science 233, 886(1986); Drummond, I.A.S., et al. J. Biol. Chem. 262, 12801(1987).

Description	Cat.#	Qty
Pluronic® F-127	**FP-37361A**	1 g

Pluronic®F-127, 20% solution

in DMSO

Description	Cat.#	Qty
Pluronic®F-127, 20% solution (in DMSO)	**FP-69806A**	1 ml

FluoCD™

See ready to use formulation DiOc C6(3)-FluoCD™ (page E131)
FCM washing buffer
Our proprietary Cyclodextrin is very useful for loading several fluorescent probes
Enhances the fluorochrome aqueous solubility
- Protects it in its micro-environment
- Creates and maintain stable homogeneous distributions
- Provides more convenient physical forms (suspension to solution, oil to solid)

ADVASEP-7

A dextrin based compound used with styryl dyes to ease cells washing of membrane bound dye. See section.

Description	Cat.#	Qty
ADVASEP-7	**FP-AM305A**	250 mg

Other reagents for Ca^{2+} studies

Several **crosslinkers** are available for in situ fixation of amino-containing dyes (see section crosslinkers), the most widely used being EDAC.

EDAC (EDC, Carbodiimide)

$C_8H_{18}ClN_3$ MW : 191.7
Soluble in water
Useful for fixing in situ chelators, including the fluorescent ion indicators in this catalogue. The fixation of ion indicators makes it useful for post histological studies following the physiological experiments. EDC is also a widely used reagent that activates carboxy groups for amine couplings.

Reference : Tymianski, M. et al. Cell Calcium 21(3), 175(1997)

Description	Cat.#	Qty
EDAC (EDC, Carbodiimide)	**FP-52005D**	1 g

See also Ion Channel Cell Lines.