Fura-2

Product Information

Product name Cat.number	MW ($g \cdot mol^{-1}$)	$\lambda_{exc}/\lambda_{em}$ max. Free Ca^{2+} (nm)	$\lambda_{exc}/\lambda_{em}$ max. High Ca^{2+} (nm)	mol. abs. ($M^{-1}cm^{-1}$)	Kd (nM)	Soluble
Fura-2, AM ester FP-42776A, 1mg FP-42776B, 10x100µg FP-42776C, 20x50µg FP-85312A, 1mM in dry DMSO	1001.87	363/512 (a)	335/505 (a)			DMSO
Fura-2, K salt FP-42777A	832.02	363/512	335/505	27 000 (b) 35 000 (c)	145 (d)	Water >pH6
Fura-2, NH_4 salt FP-AK166A	721.66	363/512	335/505	27 000 (b) 35 000 (c)	145 (d)	Water >pH6
Fura-2, Na salt FP-31493A	751.46	363/512	335/505	27 000 (b) 35 000 (c)	145 (d)	Water >pH6

(a) after hydrolysis
(b) low [Ca^{2+}]
(c) high[Ca^{2+}]
(d)> 250nM in presence of Mg^{2+} 1mM

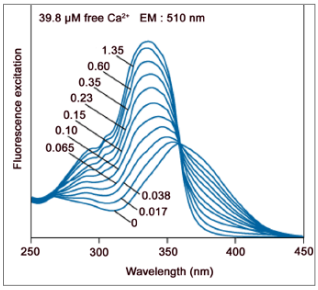
Storage: **Indicator salts** can be stored desiccated and protected from light at room temperature, +4°C or –20°C >1 year.
AM esters can be stored desiccated and protected from light at –20°C > 6 months.

Introduction

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 in calculating the intracellular concentrations.
Measurement of calcium concentration using this ratiometric measurements 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 is available as Acetoxymethyl ester. It is 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. 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 reactive to Ca^{2+}.
Fura-2 is also available as salts and are membrane-impermeant, but can be loaded into cells via microinjection or scrape loading.

FT-42776A



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

Directions for use

Handling and Storage

Stock solutions of the salts may be prepared in distilled water or aqueous buffers (pH>6) and stored at +4°C protected from light. They may be loaded into cells via microinjection, addition to patch pipette solutions, scrape loading, or using pinocytotic cell-loading reagent.

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 dessicated and protect from light.

Guidelines for use – cell loading

1. Prepare a 1-5mM stock solution in DMSO.
   Note: it is often more convenient and effective to add the non-ionic detergent Pluronic® 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®F127 in DMSO before dilution in the loading medium, making the final Pluronic®F127 concentration about 0.02%.
2. Prepare a 0.1-5µM (maximum) working solution in the appropriate buffered physiological medium.
   Note: Avoid amine-containing buffers such as Tris. Do not store for extended periods,due to hydrolysis.
3. Incubated one volume of cells with one volume of the AM ester for 15–60 minutes at +4°C/+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.

Example of specific applications

- on human neutrophils (Jaconi, 1990)
  The cells were suspended in Ca^{2+}-medium or in Ca^{2+} free medium and warmed to +37°C for 5 min.
  Fura-2/AM is added to a final concentration of 1 µm and incubated for 45 min.
  The cells were centrifugated and resuspended in Ca^{2+}-medium or in Ca^{2+} free medium for the experiment.

- on Settoli cells (Gorczynska, 1991)
  Fura-2 is dissolved in DMSO a 10 mg/ml f or a stock solution. For fluorescence microscopy: the cells were incubated with 20 µm Fura-2 AM (in appropriate medium) for 45 min at +37°C. Then they were washed and centrifugated 3 times in standard saline solution and resuspended in appropriate solution.
  For spectrofluorimetry: the cells were placed into a water-jacketed quartz cuvette maintained at +37°C. They were kept suspended by mixing and recordings were completed within 2 h of loading with Fura.

  See also reference with FluoProbes Fura-2 AM:

  Regulation of store-operated calcium entries and mitochondrial uptake by minidystrophin expression in cultured myotubes
  Vandebrouck A. *et al*. The FASEB Journal Express Article doi:10.1096/fj.04-3633fje (2005)

Contact our technical support for information in ratiometric method.

Related product(s)

- Pluronic® F-127, 10% in water, FP-379951
- Fura-2FF, AM ester FP-AM629A
- Fura-4F, AM ester #FP-R1237A
- Fura-5F, AM ester, #FP-Q9585A
- BIS Fura-2 AM #FP-BB4060
- Fura-2 LR, AM ester FP-AM603A
- Mag-Fura-2 AM (Furaptra AM), #FP-35374A
- ASANTE AM, Ratiometric Calcium Red, FJ2970

References

- **Bals S**., *et al.*, *Cell Calcium* ***11***, 385 (1990*)*
- **Bright G.R**., *et al,* in Fluorescence Microscopy of Living Cells in Culture, Part B, *Methods in Cell Biology, Academic Press,* **30,** 157 (1989)
- **Gorczynska E.**, *et al.*, "The role of calcium in follicle-stimulating hormone signal transduction in Sertoli cells", *J. Biol. Chem*., **266**, 23739 (1991) Article
- **Grynkiewicz G.**, *et al.,* "A new generation of Ca2+ indicators with greatly improved fluorescence properties", *J. Biol. Chem.,* **260**, 3340 (1985) Article
- **Jaconi. ME**, DP *et al.*, "Cytosolic free calcium elevation mediates the phagosome-lysosome fusion during phagocytosis in human neutrophils", *J. Cell Biol.,* **110**, 1555(1990) Article
- **Poirier N.** *et al*., Inducing CTLA-4–Dependent Immune Regulation by Selective CD28 Blockade Promotes Regulatory T Cells in Organ Transplantation, *Science Translational Medicine*, Vol. 2, Issue 17, p. 17ra10 *(*2010) Abstract
- **Sabourin J.** *et al*., Regulation of TRPC1 and TRPC4 Cation Channels Requires an α1-Syntrophin-dependent Complex in Skeletal Mouse Myotubes, *J. Biol. Chem*., 284: 36248 - 36261 (2009) Abstract
- **Vandebrouck A**., *et al.*, Regulation of capacitative calcium entries by alpha1-syntrophin: association of TRPC1 with dystrophin complex and the PDZ domain of alpha1-syntrophin*, FASEB J* ***10.1096/fj.06-6683***, (2007*)*
- **Ward C.A**. *et al., J. Mol. Cell. Cardiol.* **24**, 937(1992)

Ordering information

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

Fura-2, AM ester	FP-42776A	1 mg
Fura-2, AM ester	FP-42776B	10x100 µg
Fura-2, AM ester	FP-42776C	20x50 µg
Fura-2, AM ester, FluoProbes Pure Grade	FP-42776D	1 mg
Fura-2, AM ester	FP-85312A	1 ml at 1mM in dry DMSO
Fura-2, K salt	FP-42777A	1 mg
Fura-2, NH_4 salt	FP-AK166A	1 mg
Fura-2, Na salt	FP-31493A	1 mg

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

REV : E1104