Fura-PE3 AM (Fura-2 LR)

Product Information

Name :	**FURA-PE3 AM (Fura-2 Leakage Resistant)**
Catalog Number :	FP-AM603A, 1mg FP-AM603B, 20x50 µg
Structure :	$C_{55}H_{63}N_5O_{29}$
Molecular Weight :	1258.13 MW
Solubility:	Soluble in DMSO
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ (low [Ca^{2+}]) = 364 nm / 502 nm(*) $\lambda_{exc}\backslash\lambda_{em}$ (high [Ca^{2+}]) = 335 nm / 495 nm(*)

(*) after hydrolysis

Name :	**Fura-2 LR, K salt.**
Catalog Number :	FP-AM604A, 1mg
Structure :	$C_{37}H_{33}N_5O_{17}K_6$
Molecular Weight :	1054.3 MW
Solubility:	Soluble in water pH>6
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ (low [Ca^{2+}]) = 364 nm / 502 nm $\lambda_{exc}\backslash\lambda_{em}$ (high [Ca^{2+}]) = 335 nm / 495 nm.
Extinction Coefficient :	ε (high [Ca^{2+}]) =33 000 $M^{-1}cm^{-1}$
K_d :	250 nM at pH7.2

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 LR is a calcium indicator derivated of Fura-2 with similar properties. But it resists rapid leakage.
One of the problems associated with the other indicators is their tendency to leak out of cells and accumulates in organelles. The leakage can be so high in some cell types that it becomes difficult to load enough dye into cells for measurements. Cells initially load evenly but soon leak out with the remaining fluorescence largely associated with organelles.

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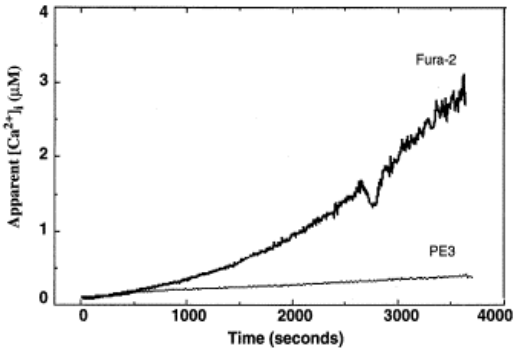

Fura-2 LR resolved this problem: the figure illustrated a leakage comparaison from 322 T lymphoma cells loaded with Fura-2 LR and the Fura-2

Fura-2 LR 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 Ca2+. However, once they have entered the cells, they are rapidly hydrolyzed by intracellular esterases into the parent Ca2+ indicators, thus becoming reactive to Ca2+.
Fura-2 LR is also available as salts and is membrane-impermeant, but can be loaded into cells via microinjection, addition to patch pipette solutions or scrape loading, or using pinocytotic cell-loading reagent.

Instruction 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. These solutions should be stable for at three months.

AM esters should be reconstituted in anhydrous 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

The following protocol are some suggestion and should be optimised for each dye and cell type.

1. Prepare cells in suspension or on a slide.
2. Prepare a 1-10 mM in DMSO stock solution of the AM ester.
   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%.
3. Prepare a 1–5 µM working solution in the appropriate buffered physiological medium.
   Note: Avoid amine-containing buffers such as Tris.
4. Mix equal volumes of AM ester and cell suspension and incubate for 15 to 60 min at +4°C to +37°C.
5. Remove the AM ester solution by washing in indicator-free medium and incubate cells 30 min to allow complete de-esterification of intracellular AM esters.
   Note: eventual indicator leakage may be quenched if needed by addition of anti-fluorescein antibody

Related products

- Fura-2, AM ester FP-42776A
- Fura-2 LowAff, AM ester FP-AM629A
- Fluo-8 NW, FP-CJ2560
- Pluronic® F-127 FP-37361A 1 g
- Pluronic® F-127, 20% FP-69806A 1 ml

References

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- **Sangly P**. *et al*., « Lysosomal Ca^{2+} Stores in Bovine Corneal Endothelium », *Investigative Ophthalmology and Visual Science.***43**, 2341 (2002) Article
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- **Zu-Cheng Ye**, *et al.,* **«** Glioma Cells Release Excitotoxic Concentrations of Glutamate », *Cancer Research* **59**, 4383, (1999) article

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