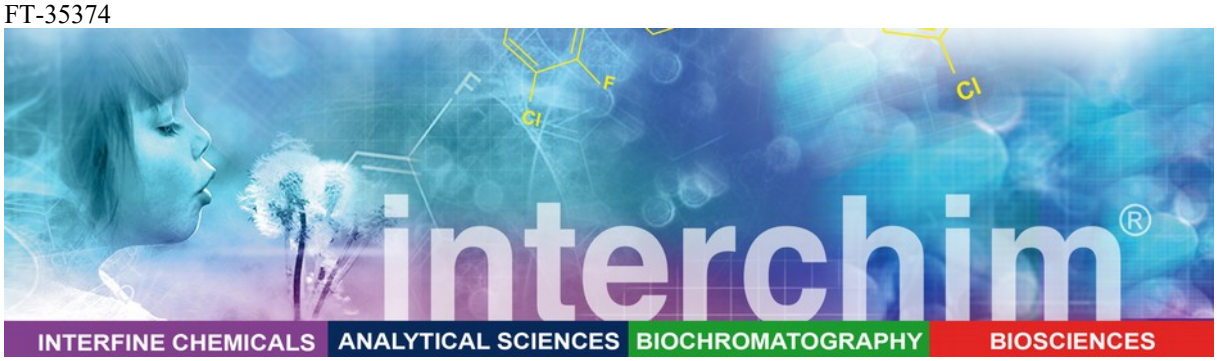
FluoProbes®

FT-35374



Mag-Fura-2

UV-excitable ratiometric fluorescent indicator for Mg^{2+}

Product Information

Name :	**Furaptra, AM ester (Mag-Fura-2, AM)**
Catalog Number :	FP-35374A, 1mg FP-35374B, 10x100µg FP-35374C, 20x50µg
Structure :	$C_{30}H_{30}N_2O_{19}$
Molecular Weight :	MW= 722.58
Soluble:	in DMSO
Absorption / Emission :	See FP-048111

Name :	**Furaptra, K salt (Mag-Fura-2)**
Catalog Number :	FP-048111, 1mg FP-048112 , 5x1mg
Structure :	$C_{18}H_{10}K_4N_2O_{11}$
Molecular Weight :	MW= 586.69
Soluble:	in water (pH>6)
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ (low [Ca2+]) = 369nm/511nm $\lambda_{exc}\backslash\lambda_{em}$ (high [Ca2+]) = 329nm/508nm $\lambda_{exc}\backslash\lambda_{em}$ (low [Mg2+]) = 369nm/511nm $\lambda_{exc}\backslash\lambda_{em}$ (high [Mg2+]) = 329nm/508nm
Extinction Coefficient :	ε (369nm, low Ca2+) = 22 000 $M^{-1}cm^{-1}$ ε (369nm, low Mg2+) = 22 000 $M^{-1}cm^{-1}$ ε (329nm, high Ca2+) = 26 000 $M^{-1}cm^{-1}$ ε (329nm, high Ca2+) = 24 000 $M^{-1}cm^{-1}$
K_d :	([Ca^{2+}])= 25 µM ([Mg^{2+}])= 19 mM

Name :	**Furaptra, Na salt**
Catalog Number :	FP-AK1681, 1mg
Structure :	$C_{18}H_{10}N_2Na_4O_{11}$
Molecular Weight :	MW= 522.25
Soluble:	in water (pH>6)
Absorption / Emission :	See FP-048111

Storage: **AM esters** can be stored desiccated and protected from light at –20°C. (M).
Indicator salts can be stored desiccated and protected from light at room temperature,+4°C) (L) or –20°C.

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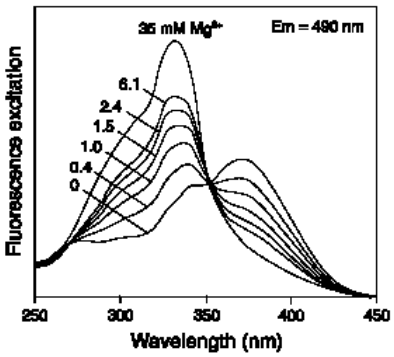
Introduction

Mag-Fura-2 is a UV-excitable ratiometric fluorescent indicator for magnesium with a Kd of 1.9 mM. Similar to Fura-2, the excitation wavelength of Mag-Fura-2 undergoes a blue shift from 369 nm to 330 nm. Mag-Fura-2 also responds to Ca^{2+} but with a significantly lower Kd than Fura-2 to Ca^{2+}. An important application of Mag-Fura-2 is its use in detecting high, transient Ca^{2+} concentration during Ca^{2+} spikes.

- Intracellular magnesium is important for mediating enzymatic reactions, DNA synthesis, hormone secretion and muscle contraction.
- Several different fluorescent indicators based on the tricarboxylate APTRA chelator are available for investigating the influence of intracellular Mg^{2+} concentration on these processes and other cellular functions. They include furaptra,also called mag-fura-2 in similarity to the Ca^{2+} indicator fura-2.
- Such Mg^{2+} indicators are sensitive to Mg^{2+} concentrations from 0.1 to 10 mM. Intracellular free Mg^{2+} levels have been reported to be about 0.3 mM in synaptosomes, 0.37 mM in hepatocytes r and 0.5 to 1.2 mM in cardiac cells r. Normal serum Mg^{2+} levels are 1.5 to 2.0 mM. Physiological changes in concentrations of intracellular magnesium are smaller and slower than calcium fluxes and are consequently more difficult to measure accurately.
- APTRA-based indicators also bind Ca^{2+} with high affinity, with a spectral response that is almost indistinguishable from that of Mg^{2+}. Interference with Mg^{2+} measurements due to Ca^{2+} binding becomes significant when Ca^{2+} concentrations exceed about 1 µM. The Ca^{2+}-sensitivity of APTRA-based indicators can be exploited for detecting intracellular calcium levels in the micromolar range that would saturate the response of indicators such as fura-2. Such elevated calcium levels are associated with activation of smooth muscle r,neurons and intracellular calcium stores r. Furthermore, because APTRA-based indicators have high ion dissociation rates, they are more suitable for tracking rapid Ca^{2+} flux kinetics than indicators with Kd (Ca^{2+}) <1 mM r.



Available forms of Mg^{2+} indicators

These 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.
Salts form are membrane-impermeant, but can be loaded into cells via microinjection or scrape loading.

- Ion-binding affinities of fluorescent ion indicators can vary markedly depending on environmental factors such as pH, temperature, ionic strength, protein binding and viscosity, such that the effective Kd inside a cell may be somewhat different from that determined in vitro, leaing to different measurments (review) .
- Pluronic® F-127 is sometimes used to promote dispersion of the AM ester in the loading buffer.

Directions 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.

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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. The average concentration is 1-5 mM.

Protocol 1 (r)

Guidelines for use – on 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® in DMSO before dilution in the loading medium, making the final Pluronic® concentration about 0.02%.

2. Cells were incubated in appropriate medium for 10 min at the temperature of the experiment to allow equilibration.
3. Incubated cells with the AM ester for 15–60 minutes at +20/37°C so that the final concentration is 1–5 µM.

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.
Notes: 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 three times in indicator-free medium and incubating for 30 minutes to allow complete de-esterification of intracellular AM esters.
5. Begin fluorescence measurement.

Magnesium Calibration

Calibration is performed measuring the ratio of furaptra fluorescence intensities with excitation at 369 and 329 nm.
A range of concentration is realized by mixing a buffer containing K/CaEGTA and a stock solution of Mag-Fura-2.
The following protocol is an example : the range concentration, the working volume, the temperature, the PH should be optimized according the experiment.

1. Prepare a stock solution of the Ca^{2+} indicator (salt form) in any Ca^{2+}- and K^{+} EGTA-free buffer at approximately 100–500 times the concentration required for the measurements (typically 0.2–1 mM).

2. Prepare a “zero Ca^{2+} sample”: to 2 ml of component A, add an adequate volume of indicator stock solution to a final concentration in the range of 1–10 µM.

3. Prepare a “high Ca^{2+} sample”: to 6 ml of component B, add three time the volume of indicator stock solution (added in the “zero Ca^{2+} sample”).
   Check that the pH of both solution are the same.

4. The absorption / emission are recorded as follow: prepare reciprocal dilutions of both samples from 1 to 9mM Ca^{2+} by replacing sequentially the volume indicated in following table starting from 2ml of the “zero Ca^{2+} sample with the same volume of the “high Ca^{2+} sample”. (see table below).
   Example: for the 1mM CaEGTA, remove 0.2 ml of the above zero calcium solution and replace this with 0.2 ml of the “high calcium solution”. Record the spectrum. The remove 0.22ml from the above 1 mM CaEGTA solution and replace it with 0.22 ml of the “high calium solution”…

5. A calibration curve is plotted and used to determine the $[Ca^{2+}]$free of an unknown calcium solution.

Note: for ratiometric indicators such as fura-2 and indo-1 (excitation or emission spectra shifting with varying calcium concentration), the ration of the excitation or emission at two wavelengths is plotted against $[Ca^{2+}]_{free}$.

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Related products

- A-23187 ionophores (Calcimycin), FP-28362B
- TPEN divalent cation chelator, FP-44736A
- Mg-EDTA, T31380, 25 g

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Ordering information

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

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