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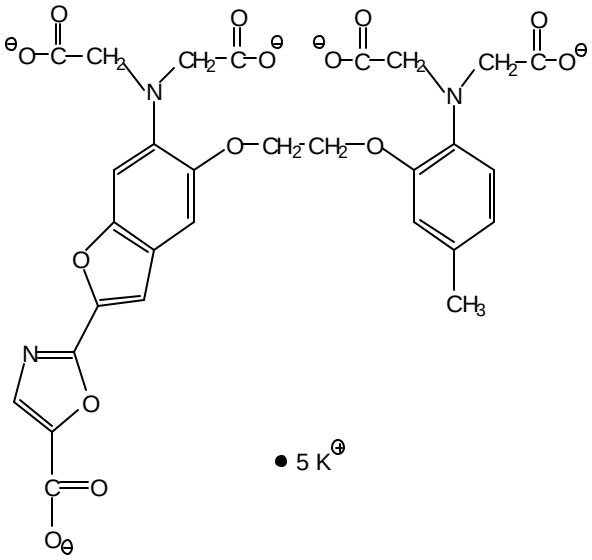
ProductInformation

FURA 2
Sigma Prod. No. **F0763**

CAS NUMBER: 11369-64-7
SYNONYMS: 1-(2-(5'-carboxyoxazol-2'yl)-6-aminobenzofuran-5-oxy)-2-(2'-amino-5'-methylphenoxy)ethane-N,N,N,N'-tetraacetic acid, pentapotassium salt



PHYSICAL DESCRIPTION:

Appearance: Yellow powder or film
Molecular formula: $C_{29}H_{22}N_3O_{14}K_5$
Molecular weight: 832.0
Fluorescence data:[2] Maxima for peak at longest wavelength, at 22 " 2°C in 100 mM KCl. Number in parentheses is the millimolar extinction coefficient.

Absorption (excitation)	Emission	Quantum efficiency of fluorescence
Free anion:		
$?_{ex}$ = 362 nm (27)	$?_{em}$ = 512 nm (518)	0.23
Ca^{2+} complex:		
$?_{ex}$ = 335 nm (33)	$?_{em}$ = 505 nm (510)	0.49

STORAGE / STABILITY AS SUPPLIED:

Fura 2 should be stored at -20°C with desiccant in the dark. Samples stored two years showed no significant change by TLC compared with new samples.

SOLUBILITY / SOLUTION STABILITY:

Fura 2 dissolves in 0.1 M NaOH up to 10 mg/mL, but also in methanol or water at much lower concentrations.[1] This salt is reportedly water-soluble, but somewhat unstable and undergoes decomposition whether dry or in solution. For Fura 2-AM, Sigma Prod. No. F0888, DMSO has been used for stock solutions of 1 mM.[4,5] Stock solutions should be stored at -20° at pH>10 in an inert atmosphere and protected from light. (The ester F0888 is more stable both as a solid and in solution.)

GENERAL REMARKS:
Fura 2 chelates calcium ion, much better than a similar chelator "Quin 2". The excitation peak shifts from 362 nm for the calcium-free chelator to about 335 nm for the calcium-saturated form. Fura 2 has fluorescence about 30 times brighter than that of Quin 2, due to a 6-fold higher extinction coefficient and a 5-fold higher quantum efficiency. It is significantly more resistant to photobleaching; measurements of fluorescence can usually be made over a period of an hour without significant loss from leakage or bleaching. A final cell concentration of 10-50 µM results in less acidification and toxicity due to acetic acid and formaldehyde released by hydrolysis of the acetoxymethyl (AM) ester.[3]

The form of the chelator usually introduced to cells is Fura 2-AM, which is a cell permeant, whereas the salt form, Fura 2, is not.[2] (Intracellular esterases convert Fura 2-AM into the active Fura 2. Cells must first be permeabilized in order to use Fura 2 directly.) The spectral characteristics of Fura 2-AM are the same as those of unbound Fura 2, but only Fura 2 is sensitive to calcium ion. Addition of [Ca^{2+}] increases the fluorescence of Fura 2 from excitation at 330-350 nm and decreases the fluorescence from excitation at 380 nm. Monitoring the decreasing signal generated by 380 nm excitation will indicate the extent of cleavage of the Fura 2-AM and subsequent binding of Ca^{2+} ion.[5,6]

A number of calcium-sensitive probes have been reported, some with dual excitation, some with dual emission wavelengths.[7]

A suggested cell loading procedure:[3]

1. Prepare viable cells in suspension or on a slide.
2. Prepare a 1 mM stock solution of Fura 2-AM (F0888) in DMSO.
3. Dilute an aliquot of the indicator 100 to 500-fold into a suitable buffer. Use the minimum concentration of AM ester to obtain an adequate signal, to reduce artifacts resulting from incomplete enzymatic hydrolysis of the ester. (This aqueous solution should not be stored for extended periods of time due to spontaneous hydrolysis.)
4. Add the aqueous indicator solution to an equal volume of cells.
5. Incubate for 15-60 minutes at 25°C to 37°C, then was cells twice with buffer.

REFERENCES:

1. Sigma quality control.
2. Grynkiewicz, Poenie and Tsien, *J. Biol. Chem.,* 260, 3440-3450 (1985).
3. Supplier information.
4. Bals, S. et al., *Cell Calcium,* 11, 385-396 (1990). "Spontaneous and experimentally evoked [Ca^{2+}]$_i$-transients in cardiac myocytes measured by means of a fast Fura-2 technique."
5. Goldman, W.F. et al., *Cell Calcium*, 11, 221-231 (1990). "Measurement of intracellular Ca^{2+} in cultured arterial smooth muscle cells using Fura-2 and digital imaging microscopy."
6. Tsien, Rink and Poenie, *Cell Calcium*, 6, 145-157 (1985). "Measurement of cytosolic free calcium in individual small cells using fluorescence microscopy with dual excitation wavelengths."
7. *Molecular Imaging in Neurosciences: A Practical Approach*, N.A. Sharif, Ed., (IRL Press at Oxford University Press, 1993), 172-175.

ADDITIONAL REFERENCES:

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Hisayama, T. et al., *Br. J. Pharmacol.,* 100, 677-684 (1990). "Ryanodine reveals multiple contractile and relaxant mechanisms in vascular smooth muscle..."

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