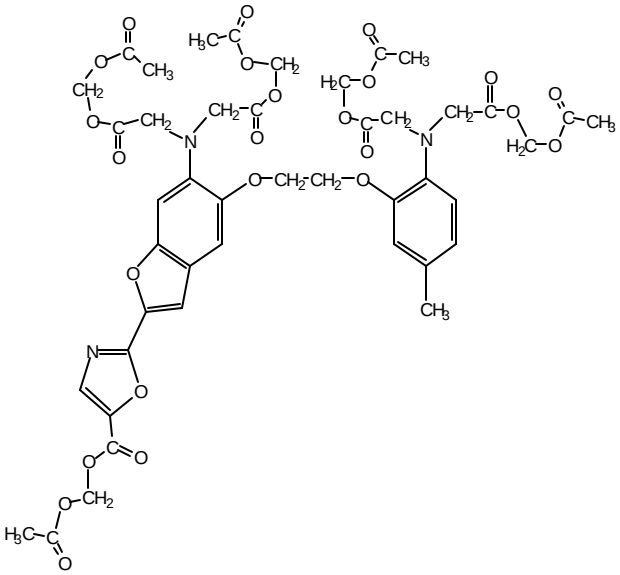
SIGMA-ALDRICH

3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

FURA 2-AM
Sigma Prod. No. **F0888**

CAS NUMBER: 10894-32-5



PHYSICAL DESCRIPTION:

Appearance: Yellow powder,
sometimes with greenish cast
Molecular formula: $C_{44}H_{47}N_3O_{24}$
Molecular weight: 1001.9
Fluorescence data:[2] Maxima for dominant peaks at longest wavelength, at 22 " 2°C in 100 mM KCl. Number in parentheses is the millimolar extinction coefficient.

Absorption (Excitation)	Emission
Free ester:	
$?_{ex}$ = 369 nm (30)	$?_{em}$ = 478 nm
Ca2+ complex:	
$?_{ex}$ = 335 nm (33)	$?_{em}$ = 505 nm (510)

STORAGE / STABILITY AS SUPPLIED:

Fura 2-AM should be stored at -20°C with desiccant in the dark. Under these conditions Fura 2-AM should be stable at least a year.

SOLUBILITY / SOLUTION STABILITY:

Fura 2-AM dissolves in acetone up to 10 mg/mL, and is also soluble in chloroform.[1] The product is also soluble in anhydrous DMSO.[4] It should be stored desiccated at -20°C or lower. Store solutions under inert atmosphere, frozen and away from light. Solubility in aqueous systems is very poor, and may be enhanced by adding a small amount of a nonionic detergent only to the working stock solution (Pluronic F-127, Sigma No. P2443, has been suggested).[3]

GENERAL REMARKS:
Fura 2-AM is the acetoxymethyl ester of the fluorescent calcium probe Fura 2, which is much better than a similar chelator "Quin 2". The excitation peak for Fura 2 shifts from 362 nm for the calcium-free chelator to about 335 nm for the calcium-saturated form.

GENERAL REMARKS: (continued)

It has been reported that 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.[8] 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 cencentration 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] During incubation for 30-40 minutes cells will take up the Fura 2-AM. Intracellular esterases cleave intracellular Fura 2-AM into the active Fura 2. 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 when excited 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 wash 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).
5. Goldman, W.F. et al., *Cell Calcium*, 11, 221-231 (1990).
6. Tsien, Rin and Poenie, *Cell Calcium*, 6, 145-157 (1985).

REFERENCES: (continue)

7. *Molecular Imaging in Neurosciences: A Practical Approach*, N.A. Sharif, Ed., (IRL Press at Oxford University Press, 1993), 172-175.
8. Almers and Neher, *FEBS Letters*, 192, 13 (1985).

ADDITIONAL REFERENCES:

DeBernardi, M.A. and Brooker, G., *Proc. Natl. Sci.* USA, 93, 4577-4582 (1996). "Single Cell Ca^{2+}/cAMP cross-talk monitored by simultaneous Ca^{2+}/cAMP fluorescence ratio imaging."

Goldman, W.F. et al., *Cell Calcium*, 11, 221 (1990). Measurement of intracellular Ca^{2+} in cultured arterial smooth muscle cells using FURA-2 and digital imaging microscopy.

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

Marchetti, J. et al., *Pflügers Arch.,* 416, 561-567 (1990). "Cholinergic agonists increase cell calcium in rat medullary collecting tubules."