

SUMMARY

shipped at room temperature; store at -20 °C

CAS Number: 108964-32-5

$C_{44}H_{47}N_3O_{24}$, **Molecular Weight:** 1001.85

Unit: 1 mg

For research use only

Chemical Name: 1-[6-Amino-2-(5-carboxy-2-oxazolyl)-5-benzofuranyloxy]-2-(2-amino-5-methylphenoxy) ethane-N,N,N',N'-tetraacetic acid, pentaacetoxymethyl ester.

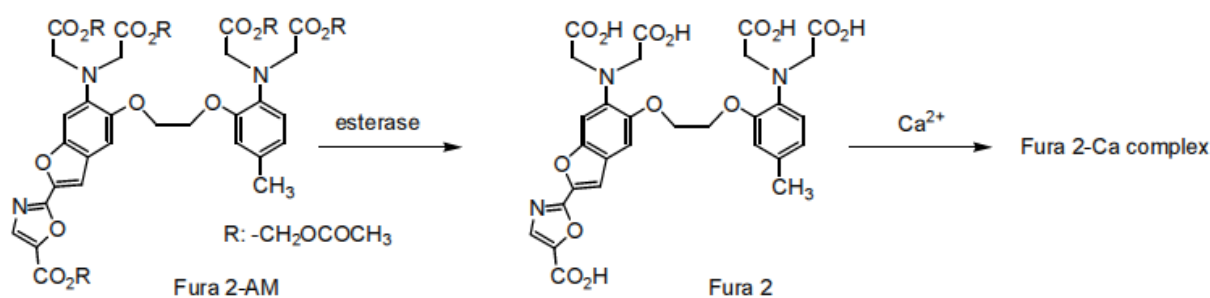
Appearance: yellow or yellowish orange, solid

Purity: > 98.0 % (HPLC)

Fluorescent spectrum: pass test
 λ_{ex} : 380 nm, λ_{em} : 510 nm (free)
 λ_{ex} : 340 nm, λ_{em} : 510 nm (Ca complex)

Solubility: 4 mg/ml water

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Hydrolysis of the AM ester

Properties:

Fura-2 was developed to improve the fluorescent properties of Quin-2. The signal intensity in 1 mM of loaded Fura-2 corresponds to that of 30 mM of loaded Quin-2. This allows an experiment at a lower concentration of indicator using Fura-2 as compared to Quin-2. Fura-2 is one of the most widely used calcium indicators for ratiometric measurement. Many types of instrumentation are now available for experiments using Fura-2. It is especially suitable for digital imaging microscopy. It is less susceptible to photobleaching than Indo-1. Changes in the cell shape can sometimes affect the fluorescent ratio at 340 nm and 380 nm. For example, fluorescent signal intensities at these wavelengths sometimes decrease simultaneously with smooth muscle contraction. For blood vessels, however, the increase of the signal intensity at 340 nm tends to be smaller on contraction, while the decrease of the signal intensity at 380 nm tends to be larger with its contraction. Fura-2 AM is an acetoxymethyl ester derivative of Fura-2 that can be easily loaded into cells by incubation.

General Protocol for Fura-2-AM (for NG 108-15 / Neuronal Cell Line)*

* Cell staining conditions differ by cell types, so it is necessary to optimize the conditions for each experiment.

Reagents:

- 1 mM Fura-2 AM/DMSO (1 mg Fura-2 AM in 1 ml DMSO)
- Hanks•balanced salt solution (HBSS) HEPES buffer saline (20 mM HEPES, 115 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, 0.8 mM MgCl₂, 13.8 mM glucose, pH 7.4)

Procedure:

1. Culture cells on a glass-bottom dish using DMEM containing 5% fetal calf serum.
2. Change the medium to 1 mM dibutyl cAMP/DMEM, and culture the cells for 3 - 4 days to induce dendrites.
3. Dilute 1 mM Fura-2 AM DMSO solution with HEPES buffer saline to prepare 1 mM Fura-2 AM working solution (not storable!).
4. Remove the culture medium, and add 0.5 ml of the Fura-2 AM working solution to the cells.
5. Incubate for 20 min. Then remove the Fura-2 AM working solution.
6. Wash the cells once with HEPES buffer saline. Then incubate the cells for 1 hour in the HEPES buffer saline.
7. Use the cells for fluorescent calcium ion detection.
8. Monitor the excitation spectra at 380 nm (calcium free) and 340 nm (calcium complex) with fixed emission at 510 nm.

References

- A. Malgeroli, D. Milani, J. Meldolesi, T. Possan, *J. Cell. Biol.*, **105**, 1045 (1987).
A. V. Tepikin, J. Llopis, V. A. Snitsarev, D. V. Gallacher, O. H. Petersen, *Pfluegers Arch.*, **428**, 664 (1994).
C. G. Gourin, L. Gan, P. Vichi, T. R. Tritton, D. N. Krag, *Cell. Pharmacol.*, **2**, 115 (1995).
D. T. Clarkson, C. Brownlee, S.M. Ayling, *J. Cell. Sci.*, **91**, 71 (1998).
G. Gryniewicz, M. Poenie, R. Y. Tsien, *J. Biol. Chem.*, **260**, 3440 (1985).
H. Tatsumi, Y. Katayama, *J. Neurosci. Methods*, **53**, 209 (1994).
H. Szmajdzinski, J. R. Lakowicz, *Cell Calcium*, **18**, 64 (1995).
H. Yamada, G. Seki, S. Taniguchi, S. Uwatoko, K. Suzuki, K. Kurokawa, *Cell. Physiol. Biochem.*, **5**, 286 (1995).
J. Eberle, A. M. Reichlmayr-Lais, M. Kirchgessner, *J. Anim. Physiol. Anim. Nutr.*, **73**, 96 (1995).
K. R. Sipido, G. Callewaert, *Cardiovasc. Res.*, **29**, 717 (1995).
M. E. Kargacin, G. J. Kargacin, *Am. J. Physiol.*, **267**, C1 145 (1994).
M. Poenie, J. Alderton, R. Y. Tsien, R. A. Steinhardt, *Nature*, **315**, 148 (1985).
M. Sanlon, D. A. Sanlon, D. A. Williams, F. S. Fay, *J. Biol. Chem.*, **262**, 6308 (1987).
P. A. Iredale, J. M. Dickenson, *Methods Mol. Biol. (Totowa, N. J.)*, **41**, 203 (1995).
P. J. Helm, O. Franksson, K. Carlsson, *Pfluegers Arch.*, **429**, 672 (1995).
P. L. Becker, F. S. Fay, *Am. J. Physiol.*, **253**, C613 (1987).
R. Y. Tsien, T. J. Rink, M. Poenie, *Cell Calcium*, **6**, 145 (1985).
S. K. Ambler, M. Poenie, R. Y. Tsien, P. Taylor, *J. Biol. Chem.*, **263**, 1952 (1988).
S. T. McCarthy, J. P. Younger, W. G. Owen, *Biophys. J.*, **67**, 2076 (1994).
T. Munsch, J. W. Deitmer, *J. Neurosci. Methods*, **57**, 195 (1995).
T. Stevens, B. Fouty, D. Cornfield, D. M. Rodman, *Cell Calcium*, **16**, 404 (1994).
V. Van den Bergh, N. Boens, F. C. De Schryver, M. Ameloot, P. Steels, J. Gallay, M. Vincent, A. Kowalczyk, *Biophys. J.*, **68**, 1110 (1995).
W. Almer, E. Neher, *FEBS Lett.*, **192**, 13 (1985).
W. G. Regehr, P. Atluri, *Biophys. J.*, **68**, 2156 (1995). 24. D. T. Clarkson, C. Brownlee, S.M. Ayling, *J. Cell. Sci.*, **91**, 71 (1998).

Order Information, Shipping and Storage

Order#	Product	Quantity
MFP-F201	Fura-2 AM	1 mg
shipped at room temperature; store at -20 °C		

Contact and Support

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