CALBIOCHEM®

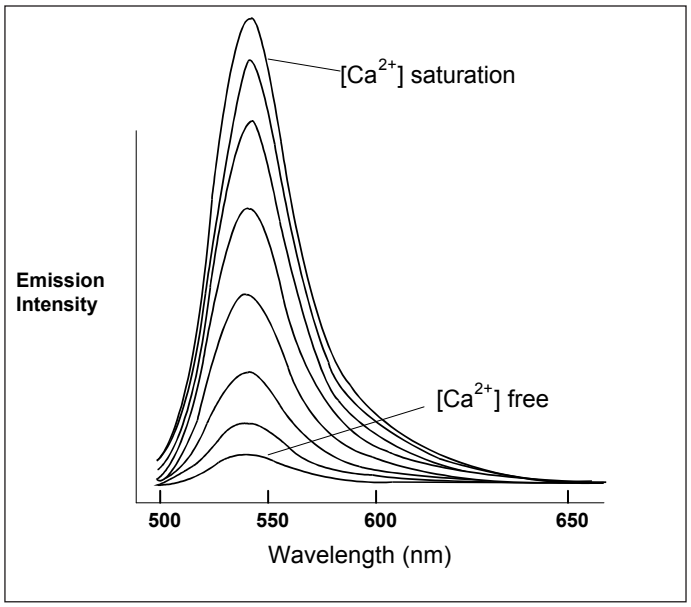
10394 Pacific Center Court
San Diego, CA 92121
(858) 450-9600

Customer Serve: (800) 854-3417
Technical Service: (800) 628-8470
Fax: (800) 776-0999

FLUORESCENT CALCIUM PROBES

Calcium plays a pivotal role in signal transduction and other cellular processes. Even minute changes in intracellular Ca^{2+} levels can have a major impact on cellular activities. Measurement of Ca^{2+} levels with fluorescent probes is one of the most sensitive techniques known. The method is based on the premise that these compounds display shifts in their excitation or emission spectra upon calcium binding.[1,2]

In 1980, Roger Tsien introduced a new generation of Ca^{2+} chelators that have backbones similar to EGTA and are capable of chelating Ca^{2+} with high affinity and specificity.[3] Molecules contain aromatic rings that interact electronically and sterically with the chelating backbone resulting in changes in their absorption and fluorescence properties upon calcium binding. The type of spectral changes which occur vary depending on the indicator used. It may be a shift in the absorbance spectra to a shorter wavelength with little change in the emission spectra (e.g., FURA 2); a shift in both absorbance and emission spectra to shorter wavelengths (e.g., INDO 1); or an increase or decrease in the quantum yield or efficiency of fluorescence (e.g., FLUO 3, see figure at right). These unique properties make these compounds highly suitable for use as sensitive probes for Ca^{2+}.



Fluo 3 Emission Spectra

Buffer: 10 mM MOPS, 100 mM KCl, 1 mM EGTA, pH 7.6. Emission Maximum: 526 nm. Excitation Wavelength: 488 nm. FLUO 3 Concentration: 5.0 μM in 1 ml. Titration Solution: 100 μM $CaCl_2$. Titration Aliquots: 1.0 μl.

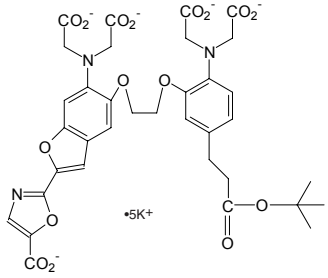
In general, the intensity of the fluorescent signal is dependent on the concentration of both the indicator and the ion. However, when Ca^{2+} binding results in a change in either the absorption or emission spectrum, it is possible to determine Ca^{2+} levels independently of the indicator concentration by using ratiometric techniques. In selecting the fluorescent probe appropriate for your application, it is important to consider both the expected intracellular calcium levels to be monitored and the nature of the spectral changes associated with calcium binding. The maximal optical response occurs near the dissociation constant of the indicator. Hence, it is recommended that the dissociation constant be matched to the expected intracellular Ca^{2+} concentration. Probes whose excitation spectra shift upon calcium binding are commonly used for fluorescent microscopy, while probes whose emission spectra shifts are better suited for use in flow cytometry. FLUO 3 is excited at longer wavelengths than many other probes, so it can be easily adopted for use in combination with caged compounds which release the active parent compound following irrradiation with UV light.

Many of these probes are provided both as a lipophilic acetoxymethylester (AM) and as an alkali salt. The AM derivatives are cell-permeable. They are converted to free indicator by the hydrolytic activity of intracellular esterases.[5,6] The alkali salt forms of these indicators are loaded into cells by microinjection or by permeabilization techniques. Microinjection is achieved by using pressure injection techniques with microelectrodes and ionophoresis electrodes.[7,8] Chemical agents, such as digitonin, may also be used to permeabilize the cells.

References Cited:

1. Tsien, R.Y. 1989. *Methods in Cell Biology* **30,** 127.
2. Szmacinski, H., et al. 1993. *Photochem. Photobiol.* **58,** 341.
3. Tsien, R.Y. 1980. *Biochemistry* **19,** 2396.
4. Haugland, R. 1993. in *Fluorescent and Luminescent Probes for Biological Activity* (Mason, W.T., ed.) p. 34, Academic Press, San Diego.
5. Grynkiewicz, G., et al. 1985. *J. Biol. Chem.* **260,** 3340.
6. Minta, A., et al. 1989. *J. Biol. Chem.* **264,** 8171.
7. William, D.A., et al. 1993. in *Fluorescent and Luminescent Probes for Biological Activity* (Mason, W.T., ed.) p 320, Academic Press, San Diego.
8. Williams, D.A., et al. 1990. *J. Physiol.* **428,** 243.

FF6, Pentapotassium Salt. A fluorescent calcium probe with properties similar to FURA 2 (Cat. No. 344900). Exhibits greater hydrophobicity and therefore associates with membranes more than FURA 2. *Purity: >90% by HPLC.* M.W. 946.1.

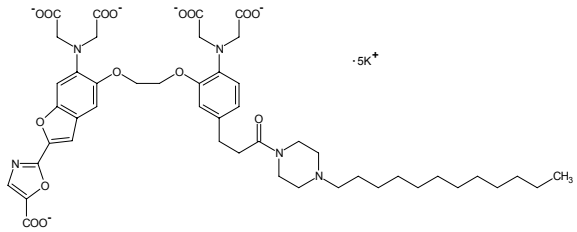


Cat. No. 341520-Y 500 g

FF6/AM. A cell permeable pentaacetoxymethyl (AM) derivative of FF6 (Cat. No. 341520) that exhibits spectral properties similar to its parent compound. *Purity: >95% by HPLC.* M.W. 1116.0.

Cat. No. 341522-Y 1 mg

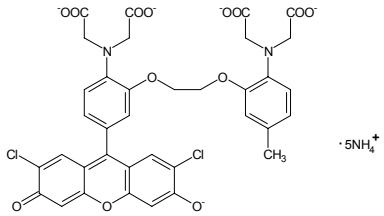
FFP-18, Pentapotassium Salt. An amphipathic FURA-2 analog with a lipophilic tail that functions as a near-membrane calcium indicator. *Purity: 90% by TLC.* M.W. 1126.5.



Ref.: Fay, F.S., et al. 1992. Biophysical Society Meeting Poster, Washington, D.C.

Cat. No. 341510-Y 250 g

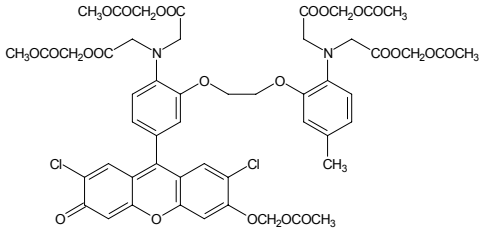
FLUO 3, Pentaammonium Salt. Almost nonfluorescent at resting Ca^{2+} levels, but fluorescence increases 40-fold upon Ca^{2+} binding. Since its excitation maximum is at 506 nm, this compound can be used in conjunction with caged compounds, which are released by UV light. Its relatively high K_d for Ca^{2+} facilitates the measurement of higher peaks of Ca^{2+} transients than is possible with FURA 2. *Purity: >90% by HPLC.* M.W. 854.7.



Ref.: Zucker, R.S. 1992. *Cell Calcium* **13,** 29; Merritt, J.E., et al. 1990. *Biochem. J.* **269,** 513; Rijkers, G.T., et al. 1990. *Cytometry* **11,** 923; Saavedra-Molina, A., et al. 1990. *Biochem. Biophys. Res. Commun.* **167,** 148.

Cat. No. 343244-Y 500 g
1 mg

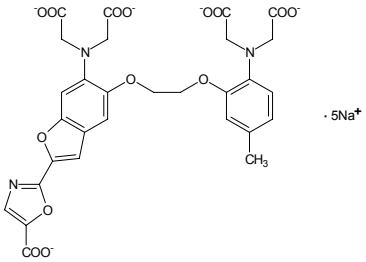
FLUO 3/AM. A membrane-permeable version of FLUO 3 that exhibits spectral properties similiar to its parent compound. *Purity: 85% by HPLC.* M.W. 1129.9.
Note: 1 set = 10 x 100 g.



Ref.: Schuman, M.A., et al. 1993. *J. Biol. Chem.* **268,** 2134.

Cat. No. 343242-Y 1 set
500 g
1 mg

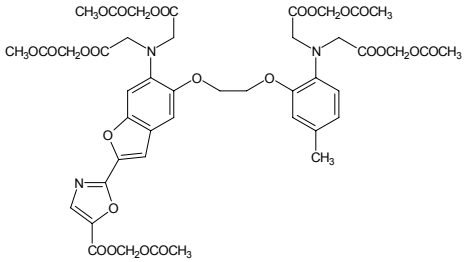
FURA 2, Pentasodium Salt. Offers 30 times the fluorescent intensity of QUIN 2 (Cat. No. 551826). This higher sensitivity allows a much smaller amount of indicator to be loaded into cells and, therefore, greatly reduces toxic effects and other artifacts. Particularly well-suited for use in fluorescent microscopy. *Purity: 95% by HPLC.* M.W. 751.5.



Ref.: Bals, S., et al. 1990. *Cell Calcium* **11,** 385; Goldman, W.F., et al. 1990. *Cell Calcium* **11,** 221; Hisayama, T., et al. 1990. *Br. J. Pharmacol.* **100,** 677; Ozaki, H., et al. 1988. *J. Biol. Chem.* **263,** 14074.

Cat. No. 344900-Y 250 g
1 mg
5 mg

FURA 2/AM. Cell-permeable ester form of FURA 2. Hydrolysis by non-specific esterases traps the indicator in the cytosol. *Purity: 95% by HPLC.* M.W. 1001.9.
Note: 1 set = 10 x 100 g.



Ref.: Davis, M.J., et al. 1992. *Am. J. Physiol.* **263,** 1292; Ward, C.A., and Moffet, M.P. 1992. *J. Mol. Cell. Cardiol.* **24,** 937.

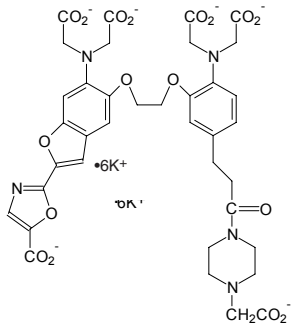
Cat. No. 344905-Y 1 set
250 g
1 mg
5 mg

FURA 2/AM in Solution. A 1 mM solution of FURA 2/AM (Cat. No. 344905) in anhydrous DMSO. *95% by HPLC.* M.W. 1001.9.

Ref.: Davis, M.J., et al. 1992. *Am. J. Physiol.* **263,** H1292; Ward, C.A., and Moffet, M.P. 1992. *J. Mol. Cell. Cardiol.* **24,** 937.

Cat. No. 344906-Y 1 ml

FURA-PE3, Hexapotassium Salt. A fluorescent calcium probe with applications and fluorescent properties similar to FURA 2 (Cat. No. 344900). However, because it is protonated in the cytosol, it cannot easily diffuse out of the cell. FURA-PE3 remains trapped intracellularly longer than FURA 2. *Purity: 90% by HPLC.* M.W. 1054.3.



Ref.: Abe. F., et al. 1995. *Br. J. Pharmacol.* **116**, 3000; Vorndran, C., et al. 1995. *Biophys. J.* **69**, 312; Poenie, M., et al. 1994. *Proc. 52nd Annual Meeting of Microscopy Soc. Am.* 168.

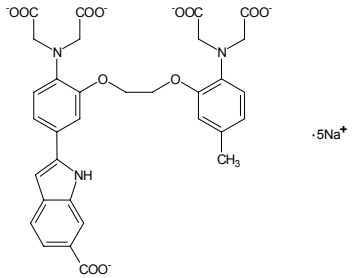
Cat. No. 344910-Y 500 g

FURA-PE3 AM. Acetoxymethyl (AM) derivative of FURA-PE3 (Cat. No. 344910) with spectral properties similar to the parent compound. Can be loaded into cells in a manner similar to FURA 2/AM (Cat. No. 344905). Hydrolysis by non-specific esterases traps the indicator in the cytosol. *Purity: 95% by HPLC.* M.W. 1258.1. Note: 1 set = 5 x 50 g.

Ref.: Abe. F., et al. 1995. *Br. J. Pharmacol.* **116**, 3000; Vorndran, C., et al. 1995. *Biophys. J.* **69**, 312; Poenie, M., et al. 1994. *Proc. 52nd Annual Meeting of Microscopy Soc. Am.* 168.

Cat. No. 344911-Y 1 set

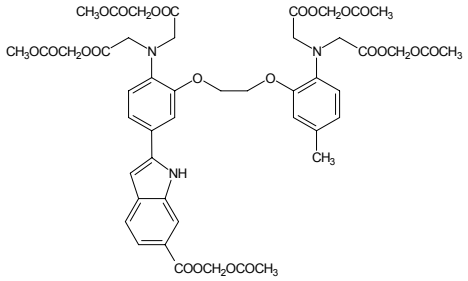
INDO 1, Pentasodium Salt. Useful for spectrofluorometric determinations of calcium by ratiometric techniques because of the shift in fluorescence emission from 482 nm to 398 nm upon Ca^{2+} binding. Commonly used in flow cytometry. *Purity: >90% by TLC.* M.W. 759.5.



Ref.: Brunkhorst, B.A., et al. 1991. *J. Biol. Chem.* **266,** 13035; Wahl, M., et al. 1990. *Cell Calcium* **11,** 487.

Cat. No. 402095-Y 1 mg
5 mg

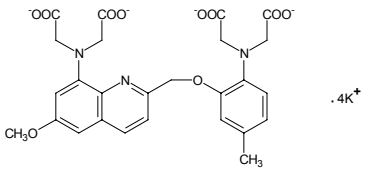
INDO 1/AM. A cell-permeable ester derivative of INDO 1. It is rapidly hydrolyzed by cytosolic esterases and remains trapped within cell. *Purity: 95% by HPLC.* M.W. 1009.9. Note 1 set = 10 x 100 g.



Ref.: Grierson, J.P., et al. 1992. *J. Neurophysiol.* **67,** 704; Lopez, M., et al. 1989. *Cytometry* **10,** 165.

Cat. No. 402096-Y 1 set
1 mg
5 mg

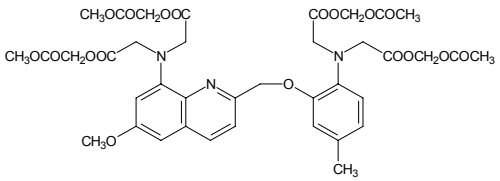
QUIN 2, Tetrapotassium Salt. Binding of Ca^{2+} leads to a major shift in UV absorption spectrum, and a twenty-fold enhancement in fluorescence quantum yield. *Purity: >98% by UV.* M.W. 693.9.



Ref.: Komada, H., et al. 1989. *Cell Struct. Funct.* **14,** 141; Vijayaraghavan, S., and Hoskins, D. 1989. *Cell Calcium* **10,** 241; Arslan, P., et al. 1985. *J. Biol. Chem.* **260,** 2719; Tsien, R.Y., et al. 1982. *J. Cell Biol.* **94,** 325.

Cat. No. 551826-Y 50 mg

QUIN 2/AM. A cell-permeable form of QUIN 2 used as a fluorescent, highly selective Ca^{2+} ion buffer and indicator. Hydrolyzed to QUIN 2 (Cat. No. 551826) by cytoplasmic esterases. Useful for monitoring cytoplasmic free Ca^{2+}. *Purity: 98% by TLC.* M.W. 829.8.



Cat. No. 551828-Y 10 mg
25 mg

Spectral Properties of Selected Fluorescent Calcium Probes*

Indicator	Excitation Max./ Emission Max. (nm) [Low Ca^{2+}]	Excitation Max./ Emission Max. (nm) [High Ca^{2+}]	Extinction Coefficient [Low Ca^{2+}] M^{-1} cm^{-1}	K_d-Ca^{2+} (nM)
FF6	360/510	340/505	33,000	135
FFP-18	364/502	335/490	32,000	415
FLUO 3	506/526	506/526	79,000	450
FURA 2	362/512	335/505	27,000	224
FURA-PE3	364/508	335/495	33,000	264
INDO 1	349/482	331/398	34,000	250
QUIN 2	354/510	332/505	5,000	79

* Approximate values are given.

Solubility

The alkali and ammonium salts of these indicators are soluble in water. The AM esters are soluble in anhydrous DMSO and other organic solvents, but are insoluble in water. Pluronic F-127 (Cat. No. 540025), a mild, low toxicity detergent, can be used to aid the dispersion and solubilization of AM esters.

Stability

Direct stability studies have not been conducted, but the following observations have been made during the production and handling of these products. As solids, the salt and the AM esters are stable for several months. The solutions are also quite stable at -20°C, if protected from light and air.

Storage

For prolonged storage, these products should be placed in a freezer (-20°C), under a dry, inert atmosphere and protected from light. Stock solutions should also be stored in a similar manner.

Convenient Sets

For your convenience, some indicators are available in sets where the product is subdivided into 10 tubes for easy storage and use.

For your research needs CALBIOCHEM® offers highly specific probes for Zn^{2+}.

Zn^{2+} Indicators	Cat. No.
TFL-Zn, Free Acid	585200
TFL-Zn, Potassium Salt	585202

Please call our Technical Service Department or your local sales office for more information on these products.

United Kingdom
Tel (0115) 943 0840
Fax (0115) 943 0951
customer.service@cnuk.co.uk

USA, Canada, & Mexico
Tel (800) 854-3417
Fax (800) 776-0999
technical@calbiochem.com

Germany
Tel (06196) 63955
Fax (06196) 62361
customer.service@calbiochem-novabiochem.de

CB0594-1197

Printed on Recycled Paper