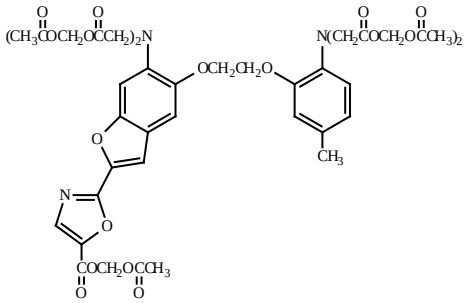
Biotium
Supplier of fluorescent and related biochemical reagents for life science research and drug discovery
Glowing Products for Science™
46117 Landing Parkway, Fremont, CA 94538 U.S.A.
Tel: 1-510-265-1027; Fax: 1-510-265-1352
www.biotium.com

PRODUCT AND SAFETY DATA SHEET

PRODUCT NAME: **FURA-2, AM Ester** (Fura-2-pentakis(acetoxymethyl) ester)

CATALOG #: **50033/50033-1/50034**

MOLECULAR INFORMATION: $C_{44}H_{47}N_3O_{24}$
MWt: 1001.9
[108964-32-5]



PROPERTIES:

Color & Form	Light yellow solid
Purity	$\geq$ 98% by HPLC
Solubility	Soluble in DMSO
Absorption/Emission	363 nm/512 nm (no Ca^{2+}); 335 nm/505 nm (high Ca^{2+})
Extinction Coefficient	27,000 $M^{-1}cm^{-1}$ (363nm, no Ca^{2+}); 35,000 M^{-1} cm^{-1}(high Ca^{2+})

STORAGE AND HANDLING: Fura-2 AM ester should be stored desiccated at –20 °C upon receipt. We recommend DMSO as the solvent for making stock solution. To dissolve the material, both DMSO and Fura-2 AM should be warmed to room temperature before mixing. Allow sufficient time for the AM ester to dissolve since the dissolution can be kinetically slow. For long term storage, anhydrous DMSO should be used and the DMSO stock solution should be tightly sealed and frozen at –20 °C. Also, the stock solution should be warmed to room temperature each time before opening to avoid moisture condensation, which may hydrolyze the AM ester during long term storage.

APPLICATION: Fura-2 is a widely used UV-excitable fluorescent calcium indicator developed by professor Roger Tsien.[1] It has been used in many cellular systems and applications particularly in microscopic imaging. Upon calcium binding, the fluorescent excitation maximum of the indicator undergoes a blue shift from 363 nm (Ca^{2+}-free) to 335 nm (Ca^{2+}-saturated), while the fluorescence emission maximum is relatively unchanged at ~510 nm. The indicator is typically excited at 340 nm and 380 nm respectively and the ratio of the fluorescent intensities corresponding to the two excitations is used in calculating the intracellular concentrations. Measurement of calcium concentration using this RATIOING METHOD avoids interference due to uneven dye distribution and photobleaching.[2]

Fura-2 AM ester is a membrane-permeant form of Fura-2 and can be loaded into most of cells by incubation with dilute aqueous solutions of the AM ester. Fura-2 AM itself does not respond to calcium. However, once inside the cells it is readily hydrolyzed to Fura-2 by nonspecific esterases. The following procedure serves as an approximate

46117 Landing Parkway, Fremont, CA 94538 U.S.A.
Tel: 1-510-265-1027; Fax: 1-510-265-1352
www.biotium.com

APPLICATION (Continued)

guide for loading Fura-2 AM into cells:

a) prepare cells in suspension or on a slide;
b) prepare a 1-5 mM DMSO stock solution of the AM ester;
c) dilute an aliquot of the DMSO stock solution into a suitable buffer;
NOTE: Normally, a relatively low concentration (as low as 0.1 μM) is sufficient to achieve an adequate fluorescent signal since the dye is enriched intracellularly. In general, the concentration of the AM ester in the buffer should not exceed 5 μM in order to minimize background fluorescence and nonspecific staining. Biotium also offers Pluronic F-127 (**59000**) that facilitates AM ester solubilization if problem occurs.
d) mix equal volumes of aqueous AM ester and cell suspension and incubate for 15 to 60 minutes at 4 °C to 37 °C;
e) wash the cells twice with buffer.

The K_d for Fura-2 was reported to be 224 nM in cell-free media. However, the K_d is usually affected by a number of factors in cells including pH, proteins concentrations, ionic strength, temperature and viscosity. Thus, calibration of the K_d is necessary for accurate measurement of intracellular calcium concentrations. For details on calibration, we recommend that you consult the references listed at the end of this document (See refs 2-8). Biotium offers A-23187(**59001**), an ionophore that is commonly used for intracellular calibration of calcium indicators.

Biotium also offers EDC (**59002,** also known as EDAC), which can be used to fix calcium indicators in cells, if post histochemical studies are desired following physiological experiments.

Ref: 1) *J. Biol. Chem.* **260**, 3440(1985); 2) Bright, G.R., et al, in *Fluorescence Microscopy of Living Cells in Culture, Part B, (Methods in Cell Biology, Vol. 30)*, Academic Press (1989) p. 157; 3) *Am. J. Physiol.* **261**, C1107(1991); 4) *Biophys. J.* **54**, 1089(1988); 5) *Biochem. Biophys. Res. Comm.* **177**, 184(1991); 6) *Cell Calcium* **11**, 85(1990); 7) *Cell Calcium* **12**, 279(1991); 8) *Neuropharmacol.* **34**, 1423(1995)

TOXICITY: Unknown

FIRST AID:	Potentially harmful. Avoid prolonged or repeated exposure. Avoid getting in eyes, on skin, or on clothing. Wash thoroughly after handling. If eye or skin contact occurs, wash affected areas with plenty of water for 15 minutes and seek medical advice. In case of inhaling or swallowing, move individual to fresh air and seek medical advice immediately.