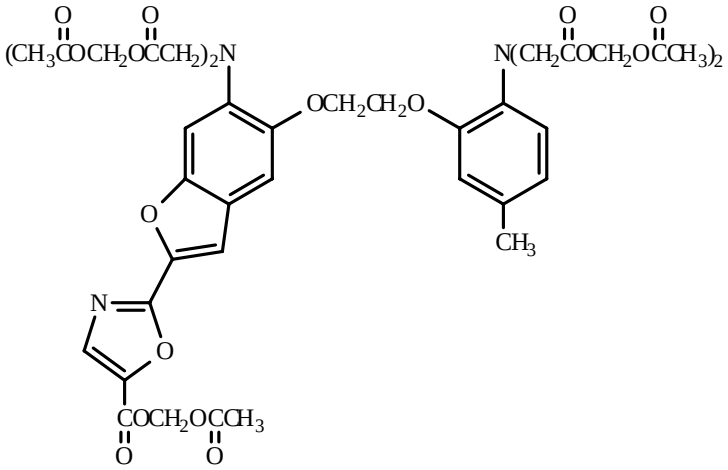
Fura-2 AM (special packaging)

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

PromoKine

Instruction Manual

Catalog Number	PK-CA707-50033-1
Description	Fura-2 is a widely used UV-excitable fluorescent calcium indicator developed by professor Roger Tsien. 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. Fura-2 AM is an acetoxymethyl ester derivative of Fura-2 that can be easily loaded into cells by incubation.
Quantity	20 x 50 µg
Excitation / Emission Maxima	λex\λem = 363/512 nm (no Ca^{2+}); λex\λem = 335/505 nm (high Ca^{2+}); Extinction Coefficient: 27,000 $M^{-1}cm^{-1}$ (363 nm, no Ca^{2+}); 35,000 $M^{-1}cm^{-1}$ (high Ca^{2+})
Molecular Structure	
Molecular Weight / Molecular Formula	1001.9 Da; $C_{44}H_{47}N_3O_{24}$
Purity	>95% (as determined by HPLC)
Appearance / Formulation / Solubility	Light yellow solid; soluble in DMSO.
Intended Use	For in vitro research use only. Not for diagnostic or therapeutic procedures.
Storage & Stability	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.

Applications	The fluorescent calcium indicator 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 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. PromoKine also offers Pluronic F-127 (PK-CA707-59000/-59004/-59005) 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 Kd for Fura-2 was reported to be 224 nM in cell-free media. However, the Kd is usually affected by a number of factors in cells including pH, proteins concentrations, ionic strength, temperature and viscosity. Thus, calibration of the Kd 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). PromoKine offers A-23187 (PK-CA707-59001), an ionophore that is commonly used for intracellular calibration of calcium indicators. PromoKine also offers EDC (PK-CA707-59002, also known as EDAC), which can be used to fix calcium indicators in cells, if post histochemical studies are desired following physiological experiments. General Protocol (for NG 108-15/ Neuronal Cell Line)* 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 $CaCl_2$, 0.8 mM $MgCl_2$, 13.8 mM glucose, pH 7.4) Protocol: 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. 4. Remove the culture medium, and add 0.5 ml of the Fura-2 AM working solution to the cells. 5. Incubate for 20 minutes. 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. *Cell staining conditions differ by cell types, so it is necessary to optimize the conditions for each experiment.
References	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)
Caution	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.

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