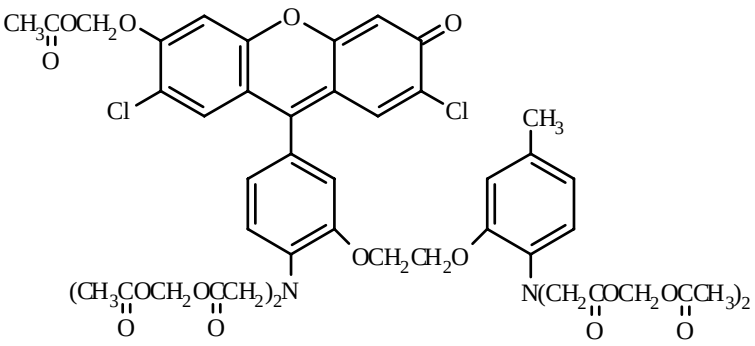
Fluo-3 AM (special packaging)

PromoKine

1-[2-Amino-5-(2,7-dichloro-6-hydroxy-3-oxo-9-xanthenyl)phenoxy]-2-(2-amino-5-methylphenoxy)ethane-N,N,N',N'-tetraacetic acid, acetomethyl ester

Instruction Manual

Catalog Number	PK-CA707-50013
Description	Fluo-3 is a long wavelength calcium probe having its absorption maximum at 506 nm, thus making it excitable by the argon-ion laser. Unlike Fura-2 and Indo-1, neither the excitation nor the emission maximum of the sensor shifts significantly before and after Ca^{2+} binding. As a result, the ratioing technique is not applicable to Fluo-3. Fluo-3 is essentially nonfluorescent without Ca^{2+} present, but the fluorescence increases at least 40 times on Ca^{2+} binding. Also, because Fluo-3 binds Ca^{2+} more weakly (higher Kd) than do Fura-2 and Indo-1, it is more useful for measuring high transient Ca^{2+} concentration during Ca^{2+} spikes. The long wavelength of the fluorescent signal is also convenient for minimizing photodamage to sample cells. Fluo-3 is also useful for caged calcium and others that are cleaved by the photo-irradiation in the UV region. Fluo-3 AM is an acetoxymethyl ester derivative of Fluo-3 that can be easily loaded into cells by incubation. Fluo-3 AM itself does not respond to calcium. However, once inside the cells it is readily hydrolyzed to Fluo-3 free acid by nonspecific esterases.
Quantity	10 x 100 µg
Excitation / Emission Maxima	λex\λem (DNA) = 506/526 nm (low or high [Ca^{2+}]); Extinction Coefficient: 86,000 $M^{-1}cm^{-1}$ (506 nm)
Molecular Structure	
Molecular Weight / Molecular Formula	1129.9 Da; $C_{51}H_{50}Cl_2N_2O_{23}$
Purity	>95% (as determined by HPLC)
Appearance / Formulation / Solubility	Orange red solid; soluble in DMSO.
Intended Use	For in vitro research use only. Not for diagnostic or therapeutic procedures.
Storage & Stability	Fluo-3 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 Fluo-3 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 Fluo-3 AM ester is a membrane-permeant form of Fluo-3 and can be loaded into most of cells by incubation with dilute aqueous solutions of the AM ester. The following procedure serves as an approximate guide for loading Fluo-3 AM into cells: a) prepare cells in suspension or on a slide; b) prepare a 1-5 mM stock solution using anhydrous DMSO; c) dilute an aliquot of the DMSO stock solution to a concentration of 1-5 µM with a suitable buffer. The final concentration of the dye should be as low as possible 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. A 20% Pluronic F-127 solution in DMSO can be used to substitute for pure DMSO for making the AM ester stock solution if solubility problem occurs; d) mix equal volumes of aqueous AM ester and cell suspension and incubate for 15 to 60 minutes at 20°C to 37°C; e) wash the cells twice with buffer. For more detailed information on loading the dye, please refer to the literature. Calcium concentration and fluorescence are related according to the equation: $[Ca^{2+}] = Kd [(F (Fmin)/(Fmax (F)]$ where F is the fluorescence of the indicator at experimental calcium concentration, Fmin is the fluorescence in the absence of calcium and Fmax is the fluorescence of the indicator at saturated calcium concentration. The Kd for Fluo-3 was reported to be 450 nM in cell-free media (325 nM in defined buffer). 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 more detailed information on calibration, please refer to the literature. 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 Human T cells)* Reagents: - 2 mM Fluo 3-AM/DMSO (1 mg Fluo 3-AM in 442 µl DMSO) - Pluronic F127 - Hanks·balanced salt solution (HBSS) - HEPES buffer saline (10 mM HEPES, 1 mM Na_2HPO4, 137 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM glucose, 0.1% BSA, pH 7.4) Protocol: 1. Add 16.5 mg Pluronic F127 to Fluo 3-AM/DMSO solution. Pluronic F127 prevents aggregation of Fluo 3-AM in HBSS and helps uptake with cells. 2. Dilute the Fluo 3-AM solution with HBSS to prepare 4 mM Fluo 3-AM working solution. 3. Add the Fluo 3-AM working solution to the cells, and incubate at 37°C for 20 minutes. 4. Add 5 volumes of HBSS containing 1% fetal calf serum, and continue the incubation for another 40 minutes. 5. Wash the cells 3 times with HEPES buffer saline. Then resuspend the cells to prepare $1x10^5$ cells/ml solution using HEPES buffer saline. 6. Incubate at 37°C for 10 minutes. Then use the cells for fluorescent calcium ion detection. 7. Monitor the fluorescence at 528 nm (excitation: 490-500 nm). *Cell staining conditions differ by cell types, so it is necessary to optimize the conditions for each experiment.
References	1) J. Biol. Chem. 264, 8171(1989) 2) J. Biol. Chem. 264, 8179(1989) 3) Meth. in Enzymol. 192, 38 (1990)
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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