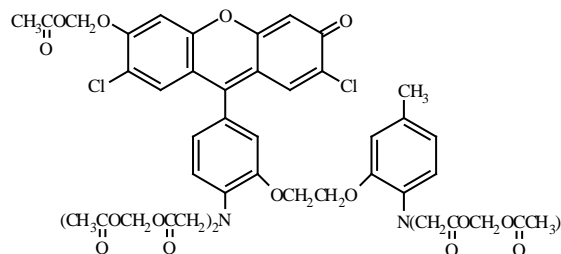


PRODUCT AND SAFETY DATA SHEET

PRODUCT NAME: FLUO-3, AM ester, 1mM in anhydrous DMSO**CATALOG #:** 50015**MOLECULAR INFORMATION:** C₅₁H₅₀Cl₂N₂O₂₃
MWt: 1129.9
[121714-22-5]**PROPERTIES:**

Color & Form Orange red liquid
Solubility N/A
Absorption/Emission For Fluo-3: 506 nm/526 nm (low or high [Ca²⁺])
Extinction Coefficient For Fluo-3: 86,000 M⁻¹cm⁻¹ (506 nm)

STORAGE AND HANDLING:

The DMSO stock solution should be tightly sealed and frozen at -20 °C upon receipt. 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:

Fluo-3 is a widely used long wavelength fluorescent calcium indicator developed by professor Roger Tsien and colleagues.^{1,2} The indicator absorbs at 506 nm and thus can be efficiently excited by the 488 argon-ion laser. Fluo-3 is essentially non-fluorescent without Ca²⁺ present, but the fluorescence (at 526 nm) increases at least 40 times on Ca²⁺ binding. Unlike fura-2 and indo-1, neither the excitation nor the emission maximum of fluo-3 shifts significantly before and after Ca²⁺ binding. As a result, the ratioing technique for measuring [Ca²⁺] is not applicable to fluo-3. Also, because fluo-3 binds Ca²⁺ more weakly (K_d = 450 nM) than do fura-2 and indo-1, it is more useful for measuring high transient Ca²⁺ concentration during Ca²⁺ spikes.

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. 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. 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. Biotium also offers FREE Pluronic F-127 (**59000**) that facilitates AM ester solubilization. A 20% Pluronic F-127

**APPLICATION
(Continued)**

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.³

Calcium concentration and fluorescence are related according to the equation:

$$[Ca^{2+}] = K_d [(F - F_{min}) / (F_{max} - F)]$$

where F is the fluorescence of the indicator at experimental calcium concentration, F_{min} is the fluorescence in the absence of calcium and F_{max} is the fluorescence of the indicator at saturated calcium concentration.

The K_d for fluo-3 was reported to be 450 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 more detailed information on calibration, please refer to the literature. 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.* **264**, 8171(1989); 2) *J. Biol. Chem.* **264**, 8179(1989); 3) *Meth. in Enzymol.* **192**, 38 (1990).

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.

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