Fluo-3, AM *Bulk package* *CAS 121714-22-5*

Catalog number: 21012
Unit size: 50 mg

Component	Storage	Amount (Cat No. 21012)
Fluo-3, AM *Bulk package* *CAS 121714-22-5*	Freeze (< -15 °C), Minimize light exposure	1 vial (50 mg)

OVERVIEW

Calcium measurement is critical for numerous biological investigations. Fluorescent probes that show spectral responses upon binding Ca2+ have enabled researchers to investigate changes in intracellular free Ca2+ concentrations by using fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers. Fluo-3 and Rhod-2 are most commonly used among the visible light-excitable calcium indicators. Fluo-3 indicators are widely used in flow cytometry and confocal laser-scanning microscopy. More recently, Fluo-3, AM has been extensively used in cell-based high-throughput screening assays for functional GPCR assays. Fluo-3 is essentially nonfluorescent unless bound to Ca2+ and exhibits a quantum yield at saturating Ca2+ of ~0.14 and a Kd for Ca2+ of 390 nM.

KEY PARAMETERS

Fluorescence microscope

Emission	FITC
Excitation	FITC
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Cutoff	515
Emission	525
Excitation	490
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Bottom read mode/Programmable liquid handling

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Fluo-3 AM Stock Solution

1. Prepare a 2 to 5 mM stock solution of Fluo-3 AM in high-quality, anhydrous DMSO.

PREPARATION OF WORKING SOLUTION

Fluo-3 AM Working Solution

1. On the day of the experiment, either dissolve Fluo-3 AM in DMSO or thaw an aliquot of the indicator stock solution to room temperature.

2. Prepare a 2 to 20 μM Fluo-3 AM working solution in a buffer of your choice (e.g., Hanks and Hepes buffer) with 0.04% Pluronic® F-127. For most cell lines, Fluo-3 AM at a final concentration of 4-5 μM is recommended. The exact concentration of indicators required for cell loading must be determined empirically.

   Note: The nonionic detergent Pluronic® F-127 is sometimes used to increase the aqueous solubility of Fluo-3 AM. A variety of Pluronic® F-127 solutions can be purchased from AAT Bioquest.

   Note: If your cells contain organic anion-transporters, probenecid (1-2 mM) may be added to the dye working solution (final in well concentration will be 0.5-1 mM) to reduce leakage of the de-esterified indicators. A variety of ReadiUse™ Probenecid products, including water-soluble, sodium salt, and stabilized solutions, can be purchased from AAT Bioquest.

SAMPLE EXPERIMENTAL PROTOCOL

Following is our recommended protocol for loading AM esters into live cells. This protocol only provides a guideline and should be modified according to your specific needs.

1. Prepare cells in growth medium overnight.

2. On the next day, add 1X Fluo-3 AM working solution to your cell plate.

   Note: If your compound(s) interfere with the serum, replace the growth medium with fresh HHBS buffer before dye-loading.

3. Incubate the dye-loaded plate in a cell incubator at 37 °C for 30 to 60 minutes.

   Note: Incubating the dye for longer than 2 hours can improve signal intensities in certain cell lines.

4. Replace the dye working solution with HHBS or buffer of your choice (containing an anion transporter inhibitor, such as 1 mM probenecid, if applicable) to remove any excess probes.
5. Add the stimulant as desired and simultaneously measure fluorescence using either a fluorescence microscope equipped with a FITC filter set or a fluorescence plate reader containing a programmable liquid handling system such as an FDSS, FLIPR, or FlexStation, at 490/525 nm cutoff 515 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

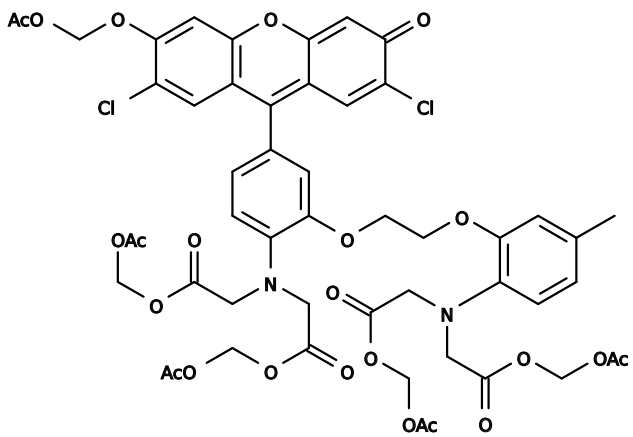

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Figure 1. Chemical structure for Fluo-3, AM *Bulk package* *CAS 121714-22-5*

DISCLAIMER

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