Fluo-4 AM *Ultrapure Grade* *CAS 273221-67-3*

Catalog number: 20550, 20551, 20552
Unit size: 1 mg, 10x50 ug, 5x50 ug

Component	Storage	Amount (Cat No. 20550)	Amount (Cat No. 20551)	Amount (Cat No. 20552)
Fluo-4 AM *Ultrapure Grade* *CAS 273221-67-3*	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)	10x50 ug	5x50 ug

OVERVIEW

Calcium measurement is critical for numerous biological investigations. Fluorescent probes that show spectral responses upon binding calcium have enabled researchers to investigate changes in intracellular free calcium concentrations by using fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers. Fluo-3 and Fluo-4 are most commonly used among the visible light-excitable calcium indicators. Fluo-4 is an analog of Fluo-3 with the two chlorine substituents replaced by fluorines, which results in increased fluorescence excitation at 488 nm and consequently higher fluorescence signal levels. Cells may be loaded with the AM ester forms of these calcium indicators by adding the dissolved indicator directly to dishes containing cultured cells. However, Fluo-3 AM and Fluo-4 AM are only moderately fluorescent in live cells upon esterase hydrolysis, and require harsh cell loading conditions to maximize their cellular calcium responses. Fluo-8® and Cal-520® calcium dyes have been developed to improve cell loading and calcium response while maintaining the convenient Fluo-3 and Fluo-4 spectral wavelength of maximum excitation @ ~490 nm and maximum emission @ ~520 nm.

KEY PARAMETERS

Fluorescence microscope

Excitation	FITC
Emission	FITC
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Excitation	490
Emission	525
Cutoff	515
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-4 AM *UltraPure grade* Stock Solution
Prepare a 2 to 5 mM stock solution of Fluo-4 AM in high-quality, anhydrous DMSO.

PREPARATION OF WORKING SOLUTION

Fluo-4 AM *UltraPure grade* Stock Solution
On the day of the experiment, either dissolve Fluo-4 AM in DMSO or thaw an aliquot of the indicator stock solution to room temperature. Prepare a dye working solution of 2 to 20 μM in a buffer of your choice (e.g., Hanks and Hepes buffer) with 0.04% Pluronic® F-127. For most cell lines, Fluo-4 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-4 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 solution, 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-4 AM working solution into 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

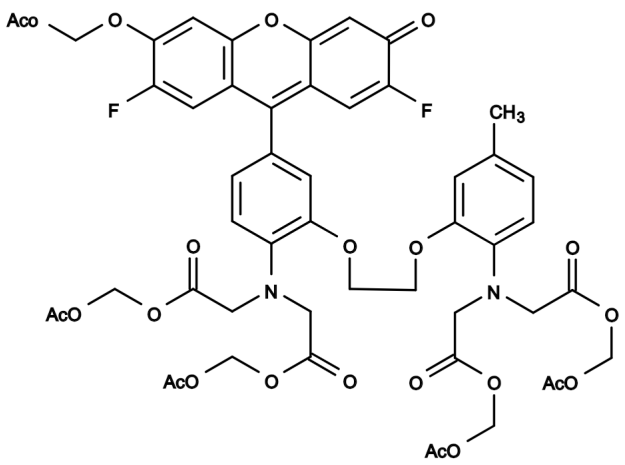

Figure 1. Chemical structure for Fluo-4 AM *Ultrapure Grade* *CAS 273221-67-3*

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.