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

Catalog number: 21010
Unit size: 1 mg

Component	Storage	Amount (Cat No. 21010)
Fluo-3, AM *CAS 121714-22-5*	Freeze (< -15 °C), Minimize light exposure	1 vial (1 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

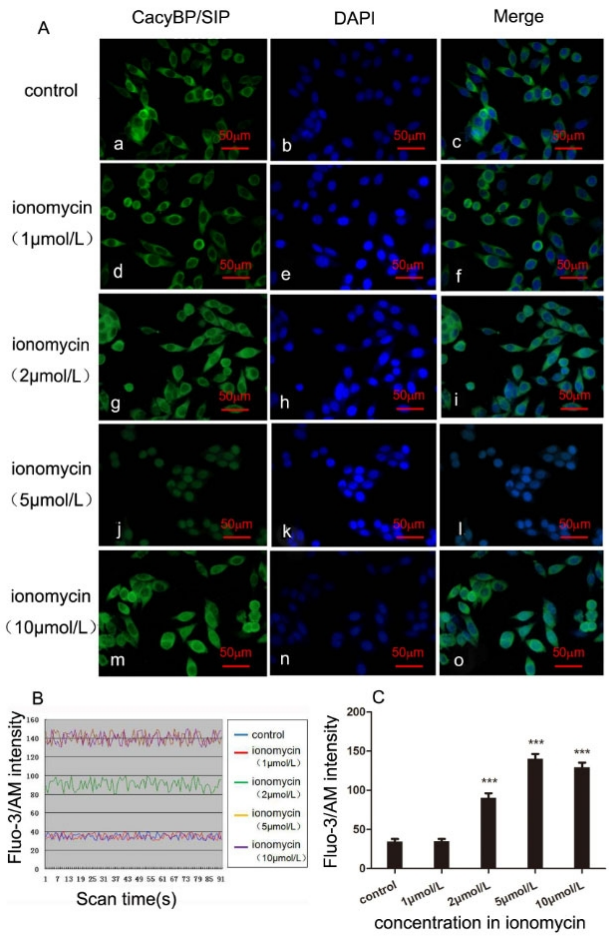

Figure 1. Effect of increased [Ca^{2+}]i on the subcellular localization of CacyBP/SIP in colon cancer SW480 cells. (A) Effect of different concentrations of ionomycin on the localization of endogenous CacyBP/SIP. Cells were treated with ionomycin for 30 min, followed by immunostaining using anti-CacyBP/SIP, and were imaged with confocal microscopy. CacyBP/SIP was translocated to the perinuclear region in SW480 cells. After stimulation with an increasing amount of ionomycin (0, 1, 2, 5, 10 μmol/L) for 30 min at 37°C, SW480 cells were fixed and immunostained using CacyBP/SIP MAb (panels a, d, g, j, and m), and nuclei were labelled with DAPI (panels b, e, h, k, and n). The merged images are shown in panels c, f, i, l, and o. The scale bar represents 50 μm. (B) The intensity of cytosolic free intracellular Ca^{2+} fluorescence in SW480 cells treated with ionomycin (0, 1, 2, 5, 10 μmol/L). The Fluo-3 fluorescence intensity in SW480 cells reached a plateau at 5 μmol/L and 10 μmol/L of ionomycin. SW480 cells were loaded with 20 μmol/L of Fluo-3/AM for 45 min under a confocal microscope (495 nm). The fluorescence was captured every 2 sec and recorded for 3 min. (C) The bar chart shows the intracellular Fluo-3 intensity. Ca^{2+} concentration is increased by treatment with 2, 5, and 10 μmol/L of ionomycin (***P<0.001). Source: **The effect of S100A6 on nuclear translocation of CacyBP/SIP in colon cancer cells** by Shanshan Feng et al., *PLOS*, March 2018.

DISCLAIMER

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