

**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 Ca<sup>2+</sup> have enabled researchers to investigate changes in intracellular free Ca<sup>2+</sup> 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-exitable 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 Ca<sup>2+</sup> and exhibits a quantum yield at saturating Ca<sup>2+</sup> of ~0.14 and a Kd for Ca<sup>2+</sup> 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.
3. Incubate the dye-loaded plate in a cell incubator at 37 °C for 30 to 60 minutes.
 

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

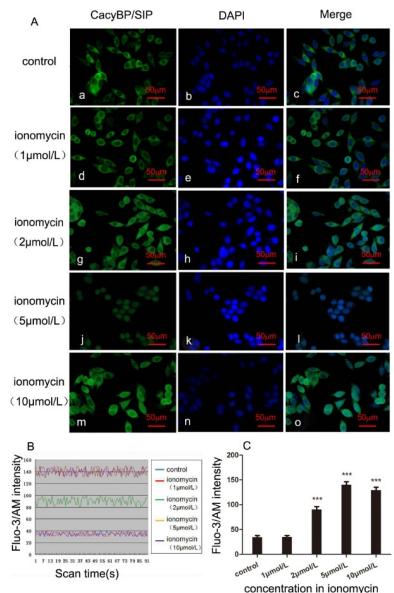


Figure 1A shows the localization of endogenous CacyBP/SIP in SW480 cells treated with increasing concentrations of ionomycin (0, 1, 2, 5, and 10  $\mu\text{mol/L}$ ) for 30 min. The images are arranged in a 5x3 grid. The columns are labeled 'CacyBP/SIP', 'DAPI', and 'Merge'. Rows are labeled 'control', 'ionomycin (1  $\mu\text{mol/L}$ )', 'ionomycin (2  $\mu\text{mol/L}$ )', 'ionomycin (5  $\mu\text{mol/L}$ )', and 'ionomycin (10  $\mu\text{mol/L}$ )'. Each panel contains a scale bar representing 50  $\mu\text{m}$ .

Figure 1B: Time course of Fluo-3/AM intensity in SW480 cells treated with increasing concentrations of ionomycin (0, 1, 2, 5, and 10  $\mu\text{mol/L}$ ) for 30 min. The intensity plateaus at 5  $\mu\text{mol/L}$  and 10  $\mu\text{mol/L}$  ionomycin.

Figure 1C: Bar chart showing the intracellular Fluo-3 intensity in SW480 cells treated with increasing concentrations of ionomycin (0, 1, 2, 5, and 10  $\mu\text{mol/L}$ ) for 30 min. The intensity increases significantly with increasing ionomycin concentration (\* $P < 0.001$ ).

**Figure 1. Effect of increased  $[\text{Ca}^{2+}]_{\text{i}}$  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  $\mu\text{mol/L}$ ) 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  $\mu\text{m}$ . (B) The intensity of cytosolic free intracellular  $\text{Ca}^{2+}$  fluorescence in SW480 cells treated with ionomycin (0, 1, 2, 5, 10  $\mu\text{mol/L}$ ). The Fluo-3 fluorescence intensity in SW480 cells reached a plateau at 5  $\mu\text{mol/L}$  and 10  $\mu\text{mol/L}$  of ionomycin. SW480 cells were loaded with 20  $\mu\text{mol/L}$  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.  $\text{Ca}^{2+}$  concentration is increased by treatment with 2, 5, and 10  $\mu\text{mol/L}$  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.

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