Indo-1, AM *CAS 112926-02-0*

Catalog number: 21030, 21033
Unit size: 1 mg, 50 mg

Component	Storage	Amount (Cat No. 21030)	Amount (Cat No. 21033)
Indo-1, AM *CAS 112926-02-0*	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)	1 vial (50 mg)

OVERVIEW

Calcium measurements are critical for numerous biological investigations. Fluorescent probes that show spectral responses upon binding to Ca2+ have enabled researchers to investigate changes in intracellular free Ca2+ concentrations by using fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers. This cell-permeant Indo-1 AM, is a UV light excitable, emission ratioable Ca2+ indicator. Upon binding to Ca2+, the emission maximum of Indo-1 AM shifts from 480 nm to 400 nm. Indo-1 is preferred for flow cytometry, in which it is more practical to use a single laser for excitation, such as the 351-364 nm spectral lines of the argon-ion laser.

KEY PARAMETERS

Fluorescence microscope

Emission	Indo-1 filter set
Excitation	Indo-1 filter set
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Cutoff	Ex/Em = 340/400, no cut off. Ex/Em = 340/475, cut off 455
Emission	400, 475
Excitation	340
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

Indo-1 AM Stock Solution

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

PREPARATION OF WORKING SOLUTION

Indo-1 AM Working Solution

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

2. Prepare a 2 to 20 µM Indo-1 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, Indo-1 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 Indo-1 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 Indo-1 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 1 hour 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 an Indo-1 filter set or a fluorescence plate reader containing a programmable liquid handling system such as a FlexStation, at Ex/Em_1 = 340/400 nm no cutoff and Ex/Em_2 = 340/475 cutoff 455 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

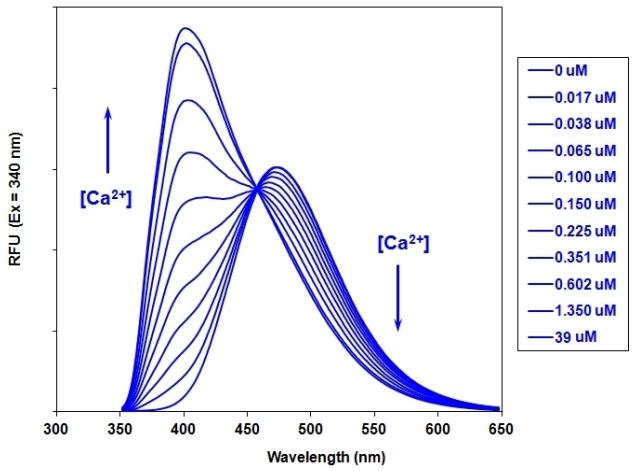

Figure 1. Fluorescence emission spectra of Indo-1 in solutions conctaining 0 to 39uM free Ca2+.

DISCLAIMER

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