Fura-2, AM *UltraPure Grade* *CAS 108964-32-5*

Catalog number: 21021, 21023
Unit size: 1 mg, 20x50 ug

Component	Storage	Amount (Cat No. 21021)	Amount (Cat No. 21023)
Fura-2, AM *UltraPure Grade* *CAS 108964-32-5*	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)	20x50 ug

OVERVIEW

Among the ratiometric calcium indicators, Fura-2 and Indo-1 are most commonly used. Fura-2 is excitation-ratioable while Indo-1 is emission-ratioable. Fura-2 is preferred for ratio-imaging microscopy, in which it is more practical to change excitation wavelengths than emission wavelengths. Upon binding Ca2+, Fura-2 exhibits an absorption shift that can be observed by scanning the excitation spectrum between 300 and 400 nm, while monitoring the emission at ~510 nm. Fura-2, AM is a cell-permeable calcium indicator that is emission-ratiometric and UV light'excitable. This AM ester form can be loaded into live cells noninvasively.

KEY PARAMETERS

Fluorescence microscope

Emission	Fura 2 filter set
Excitation	Fura 2 filter set
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Cutoff	475
Emission	510
Excitation	340, 380
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

Fura-2 AM *UltraPure Grade* Stock Solution

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

PREPARATION OF WORKING SOLUTION

Fura-2 AM *UltraPure Grade* Working Solution

1. On the day of the experiment, either dissolve Fura-2 AM in DMSO or thaw an aliquot of the indicator stock solution to room temperature.

2. Prepare a 2 to 20 µM Fura-2 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, Fura-2 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 Fura-2 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 Fura-2 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 a Fura 2 filter set or a fluorescence plate reader containing a programmable liquid handling system such as a FlexStation, at Ex/Em_1 = 340/510 nm cutoff 475 nm and Ex/Em_2 = 380/510 nm cutoff 475 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

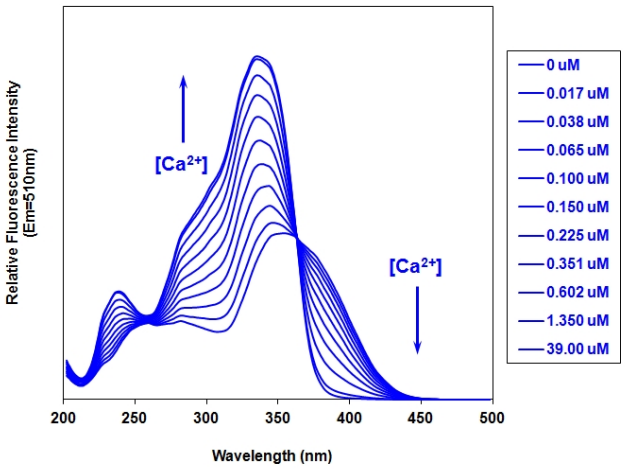

Figure 1. Fluorescence excitation spectra of Fura-2 in solutions conctaining 0 to 39uM free Ca2+.

DISCLAIMER

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