Fura-8FF™, AM

Catalog number: 20620
Unit size: 10x50 ug

Component	Storage	Amount
Fura-8FF™, AM	Freeze (< -15 °C), Minimize light exposure	10x50 ug

OVERVIEW

The cell-permeant Fura-8FF AM is an analog of Fura-8 AM with much lower calcium binding affinity, Kd ~10 µM. Fura-8FF has its emission shifted into longer visible wavelength that is compatible with the common filter sets. Fura-8FF™ AM is more sensitive to calcium than Fura-2FF AM with higher signal/background ratio than that of Fura-2FF AM. e., calculating the excitation intensity ratios at 354 nm and 415 nm by monitoring emission intensity at 530 nm.

KEY PARAMETERS

Fluorescence microscope

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

Fluorescence microplate reader

Excitation	355, 415
Emission	530
Cutoff	475
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-8FF™ AM Stock Solution
Prepare a 2 to 5 mM stock solution of Fura-8FF™ AM in high-quality, anhydrous DMSO.

PREPARATION OF WORKING SOLUTION

Fura-8FF™ AM Working Solution
On the day of the experiment, either dissolve Fura-8FF™ 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, Fura-8FF™ 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-8FF™ 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 Fura-8FF™ 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 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 = 355/530 nm cutoff 475 nm and Ex/Em_2 = 415/530 nm cutoff 475 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

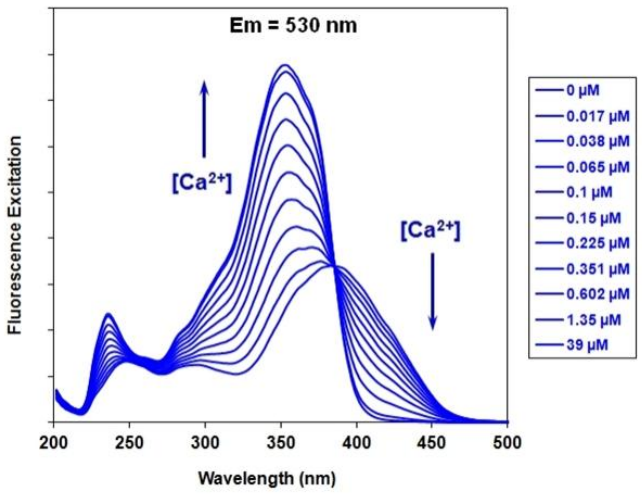

Figure 1. Fluorescence excitation spectra of Fura-8™ in the presence of 0 to 39 µM free Ca2+.

DISCLAIMER

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