Fura-8™, AM

Catalog number: 21055, 21056
Unit size: 1 mg, 10x50 ug

Component	Storage	Amount (Cat No. 21055)	Amount (Cat No. 21056)
Fura-8™, AM	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)	10x50 ug

OVERVIEW

Although Fura-2 has become the preferred excitation-ratioable calcium indicator of choice, it has certain limitations (e.g., lower sensitivity than single wavelength calcium indicators such as Fluo-8® and Cal-520®). To address these concerns, AAT Bioquest has devoted considerable efforts to the development of Fura™ 8, a high-affinity ratiometric calcium indicator with improved sensitivity and higher signal-to-noise ratios. The fluorescence emission of Fura™ 8 is red-shifted to a longer visible wavelength, facilitating the detection of Fura™ 8 by common filter sets. Fura™ 8, AM is membrane-permeant and is excited at 355 nm and 415 nm and emits 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-8™ AM Stock Solution
Prepare a 2 to 5 mM stock solution of Fura-8™ AM in high-quality, anhydrous DMSO.

PREPARATION OF WORKING SOLUTION

Fura-8™ AM Working Solution
On the day of the experiment, either dissolve Fura-8™ 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-8™ 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-8™ 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-8™ 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

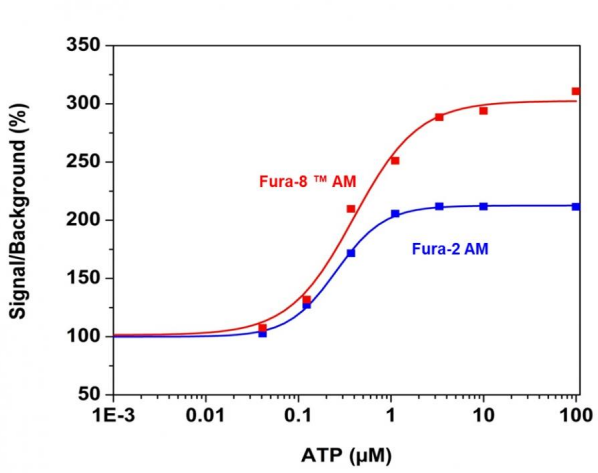

Figure 1. ATP Dose response in CHO-K1 cells measured with Fura-2 AM and Fura-8™ AM respectively. CHO-K1 cells were seeded overnight at 40,000 cells/100 μL/well in a black wall/clear bottom 96-well plate. The cells incubated with Fura-2 AM or Fura-8 AM calcium assay dye-loading solution respectively for 1 hour. ATP Dose was added by Flexstation.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.