Mag-Fura-2, AM *Cell-permeant*

Catalog number: 20383
Unit size: 10x50 ug

Component	Storage	Amount
Mag-Fura-2, AM *Cell-permeant*	Freeze (< -15 °C), Minimize light exposure	10x50 ug

OVERVIEW

Mag-Fura-2, AM is an intracellular magnesium indicator that is ratiometric and UV light-excitable. It has the spectral properties that closely match Fura-2. This acetoxymethyl (AM) ester form is useful for noninvasive intracellular loading. It is also used for measuring high level of calcium ion in live cells.

KEY PARAMETERS

Fluorescence microscope

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

Fluorescence microplate reader

Excitation	340, 380
Emission	510
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.

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

PREPARATION OF WORKING SOLUTION

Mag-Fura-2 AM Working Solution
On the day of the experiment, either dissolve Mag-Fura-2 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, Mag-Fura-2 AM at a final concentration of 4 to 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 Mag-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 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 Mag-Fura-2 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 $_1$ = 340/510 nm cutoff 475 nm and Ex/Em $_2$ = 380/510 nm cutoff 475 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

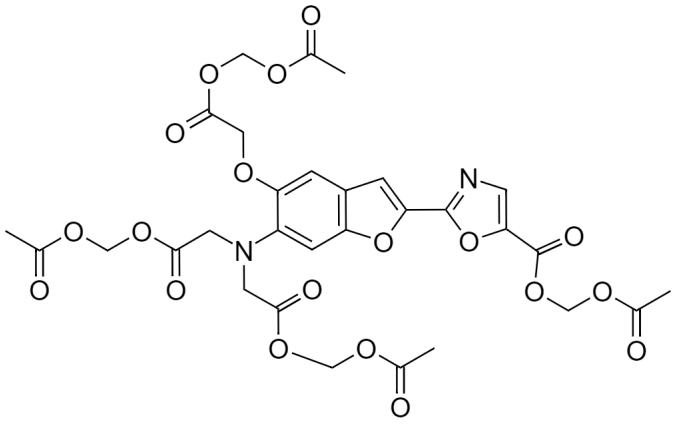

Figure 1. Chemical structure for Mag-Fura-2, AM *Cell-permeant*

DISCLAIMER

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