

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

Catalog number: 20383  
Unit size: 10x50 ug

Component	Storage	Amount (Cat No. 20383)
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

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

#### Mag-Fura-2 AM Stock Solution

1. 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

1. 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.
2. Prepare a 2 to 20 µM Mag-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, Mag-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 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 solutions, can

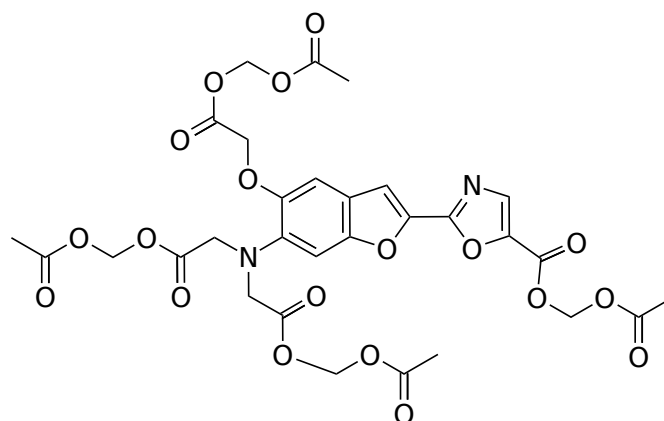
be purchased from AAT Bioquest.

### 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 Mag-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<sub>1</sub> = 340/510 nm cutoff 475 nm and Ex/Em<sub>2</sub> = 380/510 nm cutoff 475 nm.

### EXAMPLE DATA ANALYSIS AND FIGURES



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

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