**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 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.

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.

**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/Em₁ = 340/510 nm cutoff 475 nm and Ex/Em₂ = 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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