# Mag-520™ AM

Catalog number: 20406
Unit size: 10x50 ug

| Component | Storage | Amount (Cat No. 20406) |
|---|---|---|
| Mag-520™ AM | Freeze (< -15 °C), Minimize light exposure | 10x50 ug |

## OVERVIEW

Intracellular magnesium is important for mediating enzymatic reactions, DNA synthesis, hormone secretion, and muscle contraction. A vast majority of the existing magnesium ion indicators are based on tricarboxylate APTRA chelator derived from the popular tetracarboxylate BAPTA calcium ion chelator. They include mag-fura-2, mag-indo-1, mag-fluo-4 and mag-rhod-3. However, all of them have higher affinity for calcium than magnesium although they were designed for detecting magnesium ion. Mag-520™ is the first commercial magnesium indicator that has higher affinity for magnesium than calcium. Its significantly improved selectivity can be used for magnesium signaling applications with fluorescence microscopy or flow cytometry. Mag-520™ AM is cell-permeable with almost identical spectra to those of FITC, making it a convenient fluorescent probe used with FITC filter set that is ubiquitously equipped with almost all fluorescence instruments.

## AT A GLANCE

**Protocol summary**

1. Grow cells as desired
2. Prepare and add Mag-520™ AM working solution to samples
3. Incubate samples at 37 °C for 15 to 45 minutes
4. Monitor the fluorescence intensity using flow cytometer with 530/30 nm filter (FITC channel) or using fluorescence microscopy with FITC filter set

## KEY PARAMETERS

**Flow cytometer**

| | |
|---|---|
| Emission | 530/30 nm filter |
| Excitation | 488 nm laser |
| Instrument specification(s) | FITC channel |

**Fluorescence microscope**

| | |
|---|---|
| Emission | FITC filter set |
| Excitation | FITC filter set |
| Recommended plate | Black wall/clear bottom |

## 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-520™ AM stock solution**

1. Reconstitute Mag-520™ AM by adding an appropriate volume of DMSO directly into the vial to achieve a final stock concentration of 2–5 mM. Mix thoroughly.

   **Note:** Mag-520™ AM working solution should be used promptly.

## PREPARATION OF WORKING SOLUTION

**Mag-520™ AM working solution**

1. Prepare a Mag-520™ AM working solution at a final concentration of 5–50 µM in the buffer of choice.

   **Note:** Mag-520™ AM working solution should be used promptly.

   **Note:** The concentration of the Mag-520™ AM should be optimized for different cell types and conditions.

## SAMPLE EXPERIMENTAL PROTOCOL

The following protocol can be used as a guideline and should be optimized according to the needs.

1. Grow cells as desired.
2. Remove the treatment and wash the cells with buffer of your choice such as DPBS.
3. Add Mag-520™ AM working solution and incubate the samples for 15-45 minutes at 37 °C incubator.

   **Note:** Optimal time for incubation needs to be determined carefully.

4. Remove the working solution and wash cells with buffer of your choice.
5. Resuspend cells in buffer of your choice.
6. Add stimulant to stimulate the cells and monitor the fluorescence intensity with flow cytometer using 530/30 nm filter (FITC channel) or fluorescence microscope with FITC filter set.

## EXAMPLE DATA ANALYSIS AND FIGURES

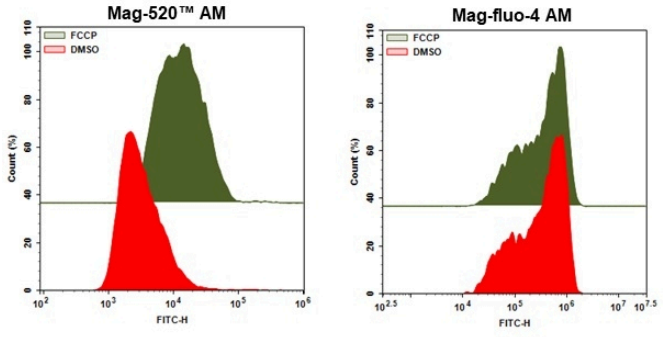


**Figure 1.** In vitro detection of $Mg^{+2}$. HL-60 cells were stained with AM form of Mag-520™ (5 uM) and mag-fluo-4 (5 uM) for 30 minutes and stimulated with FCCP (20 uM) for 30 minutes and response was recorded for end point analysis. Response was recorded using NovoCyte™ 3000 Flow Cytometer with 530/30 nm filter and 488

nm laser.

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