

Fluorescent Magnesium Indicators

Introduction

Intracellular magnesium is important for mediating enzymatic reactions, DNA synthesis, hormone secretion, and muscle contraction. Molecular Probes offers several different fluorescent indicators based on the tricarboxylate APTRA chelator for investigating the influence of intracellular Mg^{2+} concentration on these processes and other cellular functions. They include furaptra,^{1,2} which we refer to as mag-fura-2 to denote the similarity of its structure and spectral response (Figure 1) to the Ca^{2+} indicator fura-2; mag-indo-1, with a structure and spectral response like that of indo-1; and mag-fura-5. For applications such as confocal laser scanning microscopy or flow cytometry, we offer a series of long wavelength-excitable Mg^{2+} indicators, including mag-fluo-4 and Magnesium Green™. The long wavelength-excitable indicators exhibit Mg^{2+} -dependent fluorescence increases without an accompanying spectral shift (Figure 2).

Molecular Probes' Mg^{2+} indicators are sensitive to Mg^{2+} concentrations from 0.1 to 10 mM. Intracellular free Mg^{2+} levels have been reported to be about 0.3 mM in synaptosomes,³ 0.37 mM in hepatocytes,⁴ and 0.5 to 1.2 mM in cardiac cells.⁵ Normal serum Mg^{2+} levels are 1.5 to 2.0 mM. Physiological changes in concentrations of intracellular magnesium are smaller and slower than calcium fluxes and are consequently more difficult to measure accurately.⁶ APTRA-based indicators also bind Ca^{2+} with high affinity (Table 1), with a spectral response that is almost indistinguishable from that of Mg^{2+} . Interference with Mg^{2+} measurements due to Ca^{2+} binding becomes significant when Ca^{2+} concentrations exceed about 1 μM .^{7,8} The Ca^{2+} -sensitivity of APTRA-based indicators can be exploited for detecting intracellular calcium levels in the micromolar range that would saturate the response of indicators such as fura-2. Such elevated calcium levels are associated with activation of smooth

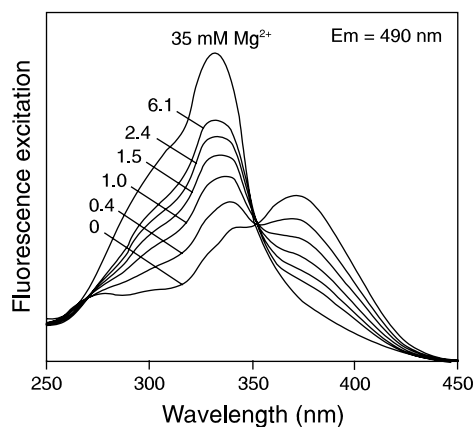


Figure 1. Fluorescence excitation response of mag-fura-2 to increasing magnesium concentrations.

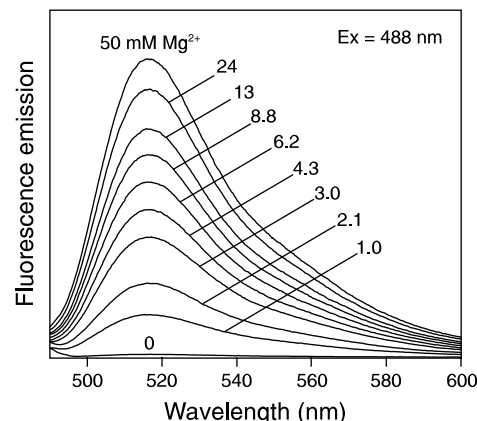


Figure 2. Fluorescence emission response of mag-fluo-4 to increasing magnesium concentrations.

muscle,⁹ neurons, and intracellular calcium stores.¹⁰ Furthermore, because APTRA-based indicators have high ion dissociation rates, they are more suitable for tracking rapid Ca^{2+} flux kinetics than indicators with $K_d(Ca^{2+}) < 1 \mu M$.^{9,11}

Storage and Handling

Upon receipt, the salt form indicators should be stored at $\leq -20^\circ C$, desiccated, and protected from light. Stock solutions may be prepared in distilled water or aqueous buffers. These solutions should be stable for at least six months if stored frozen and protected from light.

Acetoxymethyl (AM) esters are susceptible to hydrolysis and should be stored at $\leq -20^\circ C$, desiccated, and protected from light. When stored properly, they are expected to be stable for at least six months. The AM esters should be reconstituted in dimethylsulfoxide (DMSO). Concentrations of about 1–5 mM (molecular weights are given in Table 1) are generally suitable for these stock solutions. Once prepared, it is preferable to use DMSO stock solutions as soon as possible to avoid decomposition and a resulting loss of ability to load cells. Stock solutions of AM esters should be stored frozen, desiccated, and protected from light. Because the integrity of AM esters is primarily dependent on minimizing their exposure to water, the use of high-quality *anhydrous* DMSO is recommended. **AVOID REPEATED FREEZING AND THAWING OF DMSO STOCK SOLUTIONS.**

Table 1. Chemical and spectroscopic properties of magnesium indicators.

Indicator	MW*		Zero Magnesium			High Magnesium			K_d (Mg ²⁺) (mM)	K_d (Ca ²⁺) (μ M)
	Salt	AM	λ_A † (nm)	ϵ_{max} ‡ (cm ⁻¹ M ⁻¹)	λ_F § (nm)	λ_A † (nm)	ϵ_{max} ‡ (cm ⁻¹ M ⁻¹)	λ_F § (nm)		
mag-fura-2	587	723	369	22,000	511	330	24,000	491	1.9	25
mag-fura-5		737	369	23,000	505	332	25,000	482	2.3	28
mag-indo-1	595	731	349	38,000	480	330	33,000	417	2.7	35
mag-fluo-4	682	818	490	74,000	NA**	493	75,000	517	4.7	22
Magnesium Green™	916	1026	506	77,000	531	506	75,000	531	1.0	6
mag-rhod-2		845	547	68,000	NA**	549	69,000	577	ND	70
mag-X-rhod-1		949	575	82,000	NA**	578	82,000	603	10.7	45

* Molecular weight. † Absorption maximum. ‡ Molar extinction coefficient. § Fluorescence emission maximum. Spectroscopic data and K_d (Mg²⁺) values measured at 22°C in 115 mM KCl, 20 mM NaCl, 10 mM Tris, pH 7.05, with 0 mM ("Zero") to 35 mM ("High") Mg²⁺. ** Fluorescence of ion-free indicator is extremely weak; ND = not determined; NA = not applicable.

Properties

A summary of the chemical and spectroscopic properties of Molecular Probes' magnesium indicators is presented in Table 1. In general, the ion-binding affinities of fluorescent ion indicators can vary markedly depending on environmental factors such as pH, temperature, ionic strength, protein binding, and viscosity, such that the effective K_d inside a cell may be somewhat different from that determined *in vitro*.⁹ There are several published comparisons of intracellular and solution K_d (Mg²⁺) values for mag-fura-2.^{7,12}

Applications

Cell Loading with AM Esters

The following protocol for loading cell-permeant AM esters of Mg²⁺ indicators is representative of those reported in the literature. Optimal loading conditions may vary and must be determined empirically for each cell type. Loading temperatures range from room temperature to 37°C; higher temperatures promote more rapid loading and lower temperatures tend to minimize compartmentalization of the dye. The detergent Pluronic® F-127 is sometimes used to promote dispersion of the AM ester in the loading buffer.

Materials Required

- **Cell incubation medium:** any appropriate physiological medium may be used, e.g., 120 mM NaCl, 20 mM HEPES, 4.7 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 1.25 mM CaCl₂, 10 mM glucose, pH 7.4.
- **Indicator stock solution:** AM ester derivative of the Mg²⁺ indicator at 1–5 mM in high-quality *anhydrous* DMSO.
- **Pluronic F-127** (optional) available from Molecular Probes in three forms: 1 mL of a 20% (w/v) solution in DMSO (P3000MP), 30 mL of a 10% (w/v) solution in water (P6866) and 2 g solid (P6867).

1. Preincubate cells for 10 minutes at the required temperature (25–37°C) to allow stabilization and equilibration of ion gradients.

2. Prepare a dispersion of the AM ester by vigorously mixing 4 μ L of indicator solution and (optionally) 5 μ L of 20% (w/v) Pluronic F-127 in 1 mL of medium.

3. Add 0.25 mL of this dispersion per 0.75 mL of cell containing medium. Mix well. This will give a final concentration for the indicator of 1–5 μ M, depending on the initial stock concentration.

4. Continue incubation 15–60 minutes as required.

5. Wash the cells three times in the required final incubation medium and then incubate for a further 30 minutes to allow complete de-esterification of intracellular AM esters before commencing fluorescence measurements.

Response Calibration

Calibration methods for Mg²⁺ indicators are generally similar to those required for Ca²⁺ indicators.^{13,14} Note that the assumption is made that all the indicator is de-esterified and available for ion binding. The calibration consists of fluorescence measurements for the completely ion-free and completely ion-saturated indicator relative to externally controlled Mg²⁺ concentrations. Intracellular calibration may be achieved either by releasing the indicator into the surrounding medium via detergent lysis of the cells (digitonin is commonly used) or by manipulating the intracellular Mg²⁺ using an ionophore without releasing the indicator. Ratioable indicators like mag-fura-2 and mag-indo-1 may also be calibrated in cell-free solutions, although calibration *in situ* is preferred since the Mg²⁺ affinity of the indicator may be dependent on the cytosolic environment (see *Properties*). The ionophore A-23187 (A1493) or the nonfluorescent 4-bromo A-23187 (B1494) are preferred over ionomycin for Mg²⁺ calibrations since they transport Mg²⁺ more effectively.^{1,12} Solutions used to calibrate magnesium indicators should be initially free of heavy metal ions such as manganese that can interact with the magnesium indicators. These can be removed by treatment of solutions with the divalent cation chelator TPEN (T1210).

For indicators exhibiting ion-dependent spectral shifts, such as mag-fura-2 and mag-indo-1, the K_d value (or the Mg^{2+} concentration if K_d is known) can be obtained from the following equation:

$$[Mg^{2+}] = K_d Q \frac{(R - R_{\min})}{(R_{\max} - R)}$$

where R represents the fluorescence intensity ratio ($F_{\lambda_1}/F_{\lambda_2}$) in which λ_1 and λ_2 are the fluorescence detection wavelengths for the ion-complexed and free indicator, respectively. Ratios corresponding to the titration end points are denoted by subscripts

indicating the minimum and maximum ion concentration. Q is the ratio F_{\min}/F_{\max} at λ_2 and K_d is the dissociation constant for the ion-indicator complex.

For indicators such as mag-fluo-4, Magnesium Green™, mag-rhod-2 and mag-X-rhod-1 K_d may be determined using the following equation, in which F denotes fluorescence intensity measured at a single wavelength:

$$[Mg^{2+}] = K_d \frac{(F - F_{\min})}{(F_{\max} - F)}$$

In the above equations, note that values of F are dependent on the concentration of indicator, whereas values of R are not.

References

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Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
A1493	A-23187 free acid (calcimycin).....	10 mg
B1494	4-bromo A-23187, free acid.....	1 mg
M1290	mag-fura-2, tetrapotassium salt *cell impermeant*	1 mg
M1291	mag-fura-2, AM *cell permeant*	1 mg
M1292	mag-fura-2, AM *cell permeant* *special packaging*	20 x 50 µg
M1295	mag-indo-1, AM *cell permeant* *special packaging*	20 x 50 µg
M14205	mag-fluo-4, tetrapotassium salt *cell impermeant*	500 µg
M14206	mag-fluo-4, AM *cell permeant* *special packaging*	10 x 50 µg
M14214	mag-rhod-2, AM *cell permeant* *special packaging*	10 x 50 µg
M14216	mag-X-rhod-1, AM *cell permeant* *special packaging*	10 x 50 µg
M3105	mag-fura-5, AM *cell permeant* *special packaging*	20 x 50 µg
M3733	Magnesium Green™, pentapotassium salt *cell impermeant*	1 mg
M3735	Magnesium Green™, AM *cell permeant* *special packaging*	20 x 50 µg
M6890	Magnesium Orange™, tripotassium salt *cell impermeant*	1 mg
T1210	tetrakis-(2-pyridylmethyl)ethylenediamine (TPEN)	100 mg

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