

Data Table — 20.5 Aequorin: A Bioluminescent Ca²⁺ Indicator

Cat #	MW	Storage	Soluble	Abs	EC	Em	Solvent	Notes
C-2944	423.47	FF,D,LL,AA	MeOH	429	7,500	see Notes	pH 7	1, 2, 3
C-6776	457.53	FF,D,LL,AA	MeOH	431	9,000	see Notes	MeOH	1, 2
C-6779	425.46	FF,D,LL,AA	MeOH	437	8,700	see Notes	MeOH	1, 2
C-6780	407.47	FF,D,LL,AA	MeOH	437	9,500	see Notes	MeOH	1, 2
C-14260	415.49	FF,D,LL,AA	MeOH	430	7,000	see Notes	MeOH	1, 2
C-14261	399.49	FF,D,LL,AA	MeOH	433	10,000	see Notes	MeOH	1, 2

For definitions of the contents of this data table, see “How to use This Book” on page viii.

Notes

- Coelenterazine complexes with apoaequorin emit calcium-dependent bioluminescence. Bioluminescence emission maxima (relative intensity at 100 nM Ca²⁺) are as follows: C-2944, 466 nm (1); C-6776, 468 nm (0.15); C-6779, 472 nm (20); C-6780, 466 nm (16); C-14260, 442 nm (28); C-14261, 445 nm (500) (Cell Calcium 14, 373 (1993)).
- Do NOT dissolve in DMSO.
- Aqueous solutions of coelenterazine (>1 mM) can be prepared in pH 7 buffer containing 50 mM 2-hydroxypropyl-β-cyclodextrin (Biosci Biotechnol Biochem 61, 1219 (1997)).

Product List — 20.5 Aequorin: A Bioluminescent Ca²⁺ Indicator

Cat #	Product Name	Unit Size
A-6785	AquaLite® aequorin (aequorin) *recombinant*	25 µg
C-2944	coelenterazine	250 µg
C-14260	coelenterazine <i>cp</i>	250 µg
C-6779	coelenterazine <i>f</i>	250 µg
C-6780	coelenterazine <i>h</i>	250 µg
C-14261	coelenterazine <i>hcp</i>	250 µg
C-6776	coelenterazine <i>n</i>	250 µg
C-6777	Coelenterazine Sampler Kit *coelenterazine and five analogs, 25 µg each*	1 kit

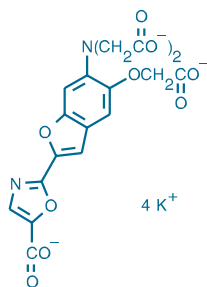


Figure 20.62 M-1290 mag-fura-2, tetrapotassium salt.

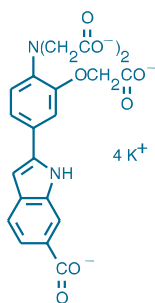


Figure 20.63 M-1293 mag-indo-1, tetrapotassium salt.

20.6 Fluorescent Mg²⁺ Indicators

Intracellular Mg²⁺ is important for mediating enzymatic reactions, DNA synthesis, hormonal secretion and muscular contraction. To facilitate the investigation of magnesium's role in these and other cellular functions, Molecular Probes offers several different fluorescent indicators for measuring intracellular Mg²⁺ concentration. They include furaptra,^{1,2} which we refer to as mag-fura-2 to denote the similarity of its structure (Figure 20.62) and spectral response with the Ca²⁺ indicator fura-2; mag-indo-1, with a structure (Figure 20.63) and spectral response similar to that of indo-1; and mag-fura-5. For applications such as confocal laser-scanning microscopy and flow cytometry, we offer the Magnesium Green and mag-fluo-4 indicators. The various methods for measuring intracellular Mg²⁺ have been reviewed.^{1,3,4}

Mg²⁺ indicators are generally designed to maximally respond to the Mg²⁺ concentrations commonly found in cells, typically ranging from about 0.1 mM to 6 mM.^{5,6} Intracellular free Mg²⁺ levels have been reported to be ~0.3 mM in synaptosomes,⁶ 0.37 mM in hepatocytes⁷ and 0.5–1.2 mM in cardiac cells,⁴ whereas the concentration of Mg²⁺ in normal serum is ~0.44–1.5 mM.⁸ Mg²⁺ indicator salts have frequently been microinjected in skeletal muscle.^{9–12} Measurements using fluorescent Mg²⁺ indicators are somewhat more demanding than intracellular Ca²⁺ determinations because physiological changes in Mg²⁺ concentration are relatively small.¹³ Compartmentalization and binding to proteins can also be a problem in use of these indicators in cells.¹⁴ Mg²⁺ indicators also bind Ca²⁺; however, typical physiological Ca²⁺ concentrations (10 nM–1 µM) usually do not interfere with Mg²⁺ measurements because the affinity of these indicators for Ca²⁺ is low. Although Ca²⁺ binding by Mg²⁺ indicators can be a complicating factor in Mg²⁺ measurements,^{15,16} this property can also be exploited for measuring high Ca²⁺ concentrations (1–100 µM);^{17–19} see Section 20.2 and Section 20.3 for further examples.

For intracellular calibration of Mg²⁺ indicators, Molecular Probes offers the ionophores A-23187 and the nonfluorescent 4-bromo A-23187 (A-1493, B-1494; Section 20.8), which

are preferred over ionomycin (I-24222, Section 20.8) because they transport Mg^{2+} more effectively.^{2,11} Solutions used to calibrate Mg^{2+} indicators should be initially free of heavy metals such as Mn^{2+} that can interact with the indicators. These metals can be removed by treating the solution with the divalent cation chelator TPEN (T-1210, Section 20.8).

Magnesium Indicators Excited by UV Light

The dissociation constants for Mg^{2+} of mag-fura-5 and mag-indo-1 are 2.3 mM and 2.7 mM, respectively, slightly higher than that of mag-fura-2, which is 1.9 mM. Mag-fura-2 was first used to detect Mg^{2+} fluctuations in embryonic chicken heart cells²⁰ and rat liver cells.² The lower-affinity mag-fura-5 and mag-indo-1 indicators are sensitive to somewhat higher spikes in intracellular Mg^{2+} .^{10,13,21} The affinities of mag-fura-2 and mag-indo-1 for Mg^{2+} are reported to be essentially invariant at pH values between 5.5 and 7.4 and at temperatures between 22°C and 37°C.²² A detailed study of the photo-physics of mag-fura-2 has been published.²³ Comparisons of intracellular and solution dissociation constants for mag-fura-2 have been published by Hurley and co-workers¹⁵ and by Tashiro and Konishi.¹¹

As with their Ca^{2+} -indicating analogs, mag-fura-2 undergoes an appreciable shift in excitation wavelength upon Mg^{2+} binding (Figure 20.64), and mag-indo-1 exhibits a shift in both its excitation and emission wavelengths (Figure 20.65). Equipment, optical filters (Table 24.8) and calibration methods are very similar to those required for the Ca^{2+} indicators. The excitation-ratioable mag-fura-2 and mag-fura-5 indicators are most useful for fluorescence microscopy, whereas the emission-ratioable mag-indo-1 indicator is preferred for flow cytometry. Simultaneous flow cytometric measurements of Ca^{2+} and Mg^{2+} have been made using fluo-3 and mag-indo-1.²⁴ Researchers have used mag-fura-2 to measure intracellular Mg^{2+} in a wide variety of cells, organelles and tissues, including:

- Mouse distal convoluted tubule cells^{25–27}
- Jurkat cells²⁸
- Rat hepatocytes²⁹
- Smooth muscle^{11,30}
- Cortical neurons³¹
- Platelets^{32,33}
- Isolated mitochondria^{34,35}

Mag-fura-2 and mag-fura-5 have also been used to measure levels of intracellular free Zn^{2+} ^{36,37} (Section 20.7).

In addition to the cell-impermeant potassium salts (M-1290, M-3103, M-1293), we offer the cell-permeant AM esters of mag-fura-2, mag-fura-5 and mag-indo-1 as a set of 20 vials, each containing 50 μ g (M-1292, M-3105, M-1295). The special packaging is recommended when small quantities of the dyes are to be used over a long period of time. Mag-fura-2 AM is also available in a single vial containing 1 mg (M-1291).

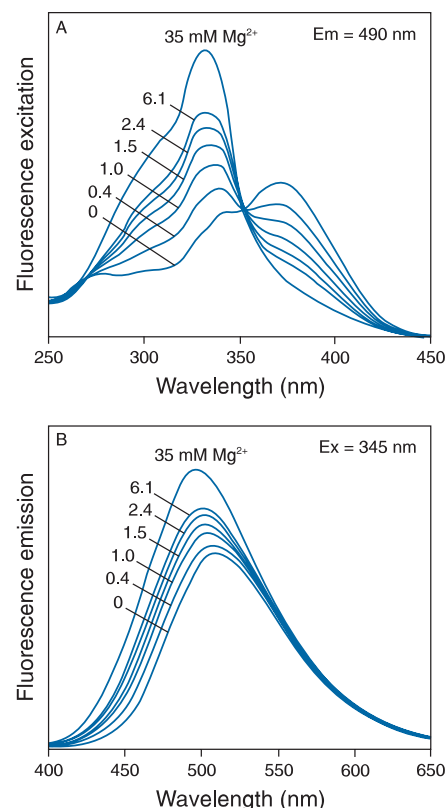


Figure 20.64 A) Fluorescence excitation and B) fluorescence emission spectra of mag-fura-2 (M-1290) in solutions containing 0–35 mM Mg^{2+} .

Our fluorescent indicators for Mg^{2+} are extensively used to measure high-level Ca^{2+} transients in cells. The spectral responses and ratiometric capabilities of the mag-fura-2 and mag-indo-1 indicators to Mg^{2+} are virtually identical to those of the fura-2 and indo-1 indicators to Ca^{2+} . The Magnesium Green and mag-fluo-4 indicators are the best green-fluorescent indicators available for Mg^{2+} and relatively high levels of intracellular Ca^{2+} . Their fluorescence responses, however, are not ratioable unless combined with a metal-insensitive dye standard.

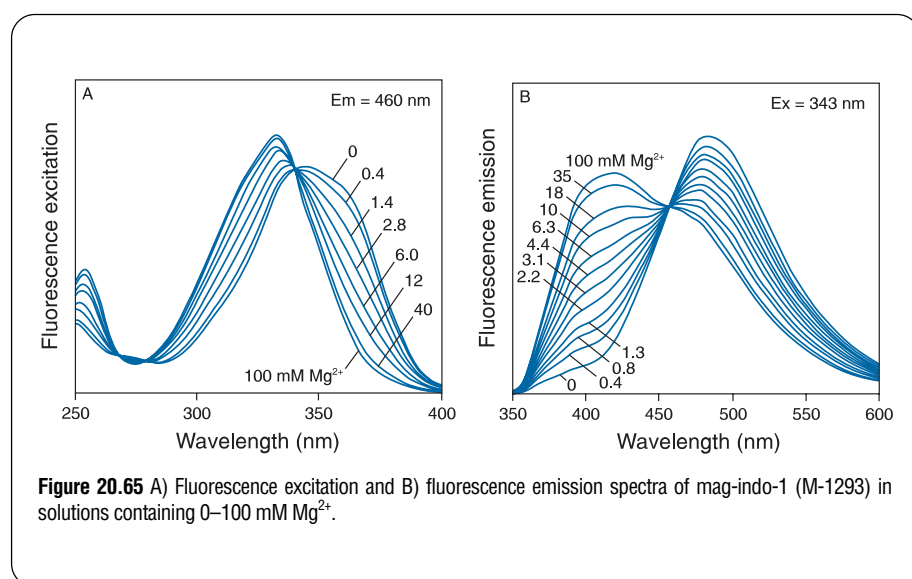


Figure 20.65 A) Fluorescence excitation and B) fluorescence emission spectra of mag-indo-1 (M-1293) in solutions containing 0–100 mM Mg^{2+} .

Magnesium Indicators Excited by Visible Light

Molecular Probes offers several visible light-excitable Mg^{2+} indicators, including the Magnesium Green and mag-fluo-4 indicators. As with mag-fura-2, mag-fura-5 and mag-indo-1, these visible light-excitable Mg^{2+} indicators can also be used as low-affinity Ca^{2+} indicators (Section 20.3) and may be useful as indicators for Zn^{2+} and other metals (Section 20.7).

Magnesium Green Indicator

Our Magnesium Green indicator (Figure 20.45) exhibits a higher affinity for Mg^{2+} ($K_d \sim 1.0$ mM) than does mag-fura-2 ($K_d \sim 1.9$ mM) or mag-indo-1 ($K_d \sim 2.7$ mM); this indicator also binds Ca^{2+} with moderate affinity (K_d for Ca^{2+} in the absence of $Mg^{2+} \sim 6 \mu M$).³⁸ The spectral properties of the Magnesium Green indicator are similar to those of the Calcium Green indicators. Upon binding Mg^{2+} , Magnesium Green exhibits an increase in fluorescence emission intensity without a shift in wavelength (Figure 20.66). The Magnesium Green indicator has been used to investigate the binding of free Mg^{2+} by the bacterial SecA protein³⁹ and by protein tyrosine kinases.⁴⁰ By exploiting the fact that ATP has greater Mg^{2+} -binding affinity than ADP, researchers have used Magnesium Green to detect ATP hydrolysis in spontaneously contracting cardiomyocytes.^{41,42} Magnesium Green is available as a cell-impermeant potassium salt (M-3733) or as a cell-permeant AM ester (M-3735).

Mag-Fluo-4

Mag-fluo-4 (Figure 20.67) is an analog of fluo-4 with a K_d for Mg^{2+} of 4.7 mM and a K_d for Ca^{2+} of 22 μM ,³⁸ making it useful as an intracellular Mg^{2+} indicator as well as a low-affinity Ca^{2+} indicator (Section 20.3). Mag-fluo-4 has a much more sensitive fluorescence response to Mg^{2+} binding than does our Magnesium Green indicator. Because physiological fluctuations of intracellular Mg^{2+} concentration are typically small, this increased sensitivity is a considerable advantage. Like fluo-4, mag-fluo-4 is essentially nonfluorescent in the absence of divalent cations and exhibits strong fluorescence enhancement with no spectral shift upon binding Mg^{2+} (Figure 20.68). Mag-fluo-4 is available as a cell-impermeant potassium salt (M-14205) or as a cell-permeant AM ester (M-14206).

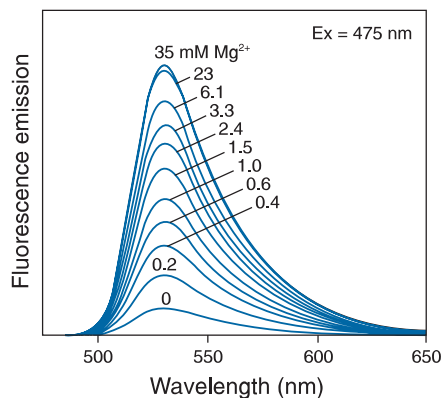


Figure 20.66 Mg^{2+} -dependent fluorescence emission spectra of Magnesium Green (M-3733).

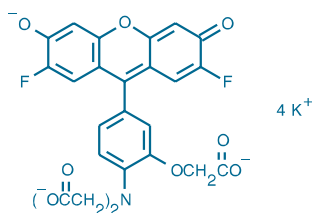


Figure 20.67 M-14205 mag-fluo-4, tetrapotassium salt.

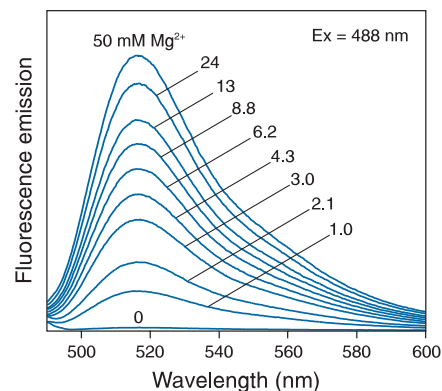


Figure 20.68 Fluorescence emission spectra of mag-fluo-4 (M-14205) in solutions containing 0–50 mM Mg^{2+} .

The flux of Mg^{2+} in live cells is far less than that of intracellular Ca^{2+} , making Mg^{2+} measurements more demanding and far less common than those of intracellular Ca^{2+} . Intracellular Mg^{2+} concentrations, however, have an effect on the dissociation constants of all indicators for intracellular Ca^{2+} .

References

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Data Table — 20.6 Fluorescent Mg²⁺ Indicators

Cat #	MW	Storage	Soluble	Low Mg ²⁺				High Mg ²⁺				Product	K _d	Notes
				Abs	EC	Em	Solvent	Abs	EC	Em	Solvent			
M-1290	586.68	L	pH >6	369	22,000	511	H ₂ O	330	24,000	491	H ₂ O/Mg ²⁺	M-1290	1.9 mM	1, 2, 3, 4
M-1291	722.57	F,D,L	DMSO	366	31,000	475	EtOAc					M-1290		
M-1292	722.57	F,D,L	DMSO	366	31,000	475	EtOAc					M-1290		
M-1293	594.74	L	pH >6	349	38,000	480	H ₂ O	330	33,000	417	H ₂ O/Mg ²⁺	M-1293	2.7 mM	1, 2, 3, 4
M-1295	730.63	F,D,L	DMSO	354	37,000	472	MeOH					M-1293		
M-3103	600.70	L	pH >6	369	23,000	505	H ₂ O	332	25,000	482	H ₂ O/Mg ²⁺	M-3103	2.3 mM	1, 2, 3, 4
M-3105	736.60	F,D,L	DMSO	365	31,000	461	EtOAc					M-3103		
M-3733	915.90	L	pH >6	506	77,000	531	H ₂ O	506	75,000	531	H ₂ O/Mg ²⁺	M-3733	1.0 mM	1, 2, 3, 4, 5
M-3735	1025.71	F,D	DMSO	302	16,000	none	MeOH					M-3733		
M-14205	681.77	D,L	pH >6	490	74,000	see Notes	H ₂ O	493	75,000	517	H ₂ O/Mg ²⁺	M-14205	4.7 mM	1, 2, 3, 4, 6
M-14206	817.66	F,D,L	DMSO	457	25,000	see Notes	MeOH					M-14205		7

For definitions of the contents of this data table, see "How to use This Book" on page viii.

Notes

1. Dissociation constants are known to vary considerably depending on the temperature, pH, ionic strength, viscosity, protein binding, presence of other ions (especially polyvalent ions), instrument setup and other factors. It is strongly recommended that these values be verified under user-specific experimental conditions.
2. This indicator binds Ca²⁺ with higher affinity than Mg²⁺, producing a similar spectral response.
3. K_d(Mg²⁺) values have been determined at Molecular Probes in 115 mM KCl, 20 mM NaCl, 10 mM Tris, pH 7.05, 0–35 mM Mg²⁺ at 22°C.
4. Spectra measured in aqueous buffers containing 0 or 35 mM Mg²⁺, indicated as H₂O and H₂O/Mg²⁺, respectively.
5. This indicator exhibits fluorescence enhancement in response to ion binding, with essentially no change in absorption or emission wavelengths.
6. Fluorescence of the free indicator is very weak and is enhanced >100-fold on binding Mg²⁺.
7. Fluorescence of this AM ester derivative is very weak and is enhanced only after hydrolytic cleavage followed by binding of divalent cations to the anionic indicator.

Product List — 20.6 Fluorescent Mg²⁺ Indicators

Cat #	Product Name	Unit Size
M-14206	mag-fluo-4, AM *cell permeant* *special packaging*	10 x 50 µg
M-14205	mag-fluo-4, tetrapotassium salt *cell impermeant*	500 µg
M-1291	mag-fura-2, AM *cell permeant*	1 mg
M-1292	mag-fura-2, AM *cell permeant* *special packaging*	20 x 50 µg
M-1290	mag-fura-2, tetrapotassium salt *cell impermeant*	1 mg
M-3105	mag-fura-5, AM *cell permeant* *special packaging*	20 x 50 µg
M-3103	mag-fura-5, tetrapotassium salt *cell impermeant*	1 mg
M-1295	mag-indo-1, AM *cell permeant* *special packaging*	20 x 50 µg
M-1293	mag-indo-1, tetrapotassium salt *cell impermeant*	1 mg
M-3735	Magnesium Green™, AM *cell permeant* *special packaging*	20 x 50 µg
M-3733	Magnesium Green™, pentapotassium salt *cell impermeant*	1 mg

20.7 Fluorescent Indicators for Zn²⁺ and Other Metal Ions

Not only do certain metal ions play an important role in biological structure and activity, but they can also serve as useful probes of biological processes, including ion transport through Ca²⁺ channels.^{1,2} Metal contamination, however, can cause ecological problems and present significant risks to health. For example, Hg²⁺ at submicromolar concentrations can cause a rapid and sustained increase in intracellular Ca²⁺ levels in rat T-lymphocytes³ and modify the depolarization- and agonist-stimulated Ca²⁺ signals in neuroadrenergic PC12 cells.⁴ Furthermore, Pb²⁺ can mimic Ca²⁺ in important cellular processes; rat astrocytes exposed to Pb²⁺ show significant increases in the levels of intracellular inositol 1,4,5-triphosphate.⁵

Measuring heavy metal ion concentrations in cells and environmental samples with indicators originally designed for detection of Ca²⁺ and Mg²⁺ has been hampered by competitive binding of other, more abundant, cations.⁶ Molecular Probes has made a strong commitment to the development of fluorescence-based methods for detecting biologically relevant metals, as well as for sensing low concentrations of heavy metal ions in environmental samples.⁷ The goal of this research is to develop new sensor molecules that will

Molecular Probes has the most extensive assortment of indicators available for Zn²⁺ and other heavy metals. However, measurements of these ions in cells using fluorescent ion indicators are complicated by metal binding to thiols and other sites. Our FuraZin, IndoZin, FluoZin and RhodZin indicators are very sensitive to Zn²⁺, with essentially no interference by Ca²⁺ or Mg²⁺.