Fluorescent Indicators for Zinc

Table 1. Contents and storage information.

Material	Amount*	Storage	Stability
Water-soluble indicator salts	1 mg or 500 µg	• ≤–20°C • Desiccate • Protect from light	When stored as directed, this product is stable for at least 6 months.
Acetoxymethyl (AM) and acetate ester derivatives	1 mg, 100 µg, or 50 µg		
*See product label for details. **Note:** Avoid repeated freezing and thawing of DMSO stock solutions.			
Approximate fluorescence excitation/emission maxima: See Table 2.			

Introduction

Zinc is an important divalent cation in biological systems, influencing DNA synthesis, microtubule polymerization, gene expression, apoptosis, immune system function, and the activity of enzymes such as carbonic anhydrase and matrix metalloproteinases (MMP). Zn^{2+} is also functionally active in synaptic transmission, and is a contributory factor in neurological disorders including epilepsy and Alzheimer's disease.[1,2] The intracellular concentration of free Zn^{2+} is extremely low in most cells (<1 nM), with the remainder being bound to proteins or nucleic acids.[3]

Zinc concentrations in the 1–100 nM range can be measured using fluorescent indicators nominally designed for Ca^{2+} detection (*e.g.*, fura-2, K_d (Zn^{2+}) = 3 nM).[4-6] The FluoZin™-1 and FluoZin™-2 indicators are designed for detection of higher Zn^{2+} concentrations that are present in synaptic vesicles and released in response to electrical stimulation or excitotoxic agonists.[1,7] These indicators based on the N-(2-methoxyphenyl)iminodiacetate chelator (Figure 1) are designed for detection of Zn^{2+} in the 0.05–50 µM range with minimal interfering Ca^{2+} sensitivity. FluoZin™-3 (K_d(Zn^{2+}) ~ 15 nM) is suitable for detection of Zn^{2+} concentrations in the 1–100 nM range and it has shown to be the most Zn^{2+}-sensitive and Zn^{2+}-specific of the three FluoZin™ Zn^{2+} indicators. The cell permeant AM-ester is useful for detecting low intracellular Zn^{2+} levels and small concentration changes.[19]

RhodZin™-3 shows promise as an indicator for Zn^{2+} in the mitochondria.[20] Exhibiting pH-insensitive fluorescence similar to tetramethylrhodamine, this indicator may prove useful for monitoring changes in Zn^{2+} levels associated with pro-apoptosis.

The Newport Green™ DCF indicator has moderate zinc-binding affinity (K_d(Zn^{2+}) ~1 µM) but is essentially insensitive to Ca^{2+} (K_d(Ca^{2+}) >100 µM), making this a valuable probe for detecting Zn^{2+} influx into neurons through voltage- or glutamate-gated channels.[8-11] When used alongside dyes with dual Ca^{2+}/Zn^{2+} sensitivity such as fura-2 and mag-fura-2, Newport Green™ DCF provides confirmation that changes in Zn^{2+} levels, and not Ca^{2+} or Mg^{2+}, are

being detected.[8,12] Newport Green™ PDX incorporates the same di-(2-picolyl)amine chelator as Newport Green™ DCF but has a higher Zn^{2+} dissociation constant (Table 1) and a larger Zn^{2+}-free to Zn^{2+}-saturated fluorescence intensity increase.

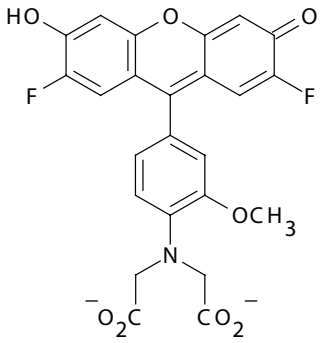

Figure 1. Structure of FluoZin™-1.

Table 2. Fluorescent indicators for zinc.

Indicator	Water-soluble form	Permeant ester form	Ex/Em*	K_d (Zn^{2+})**
FluoZin™-1	F24180	F24181	495/515	8 µM
FluoZin™-2	—	F24189	495/525	2 µM
FluoZin™-3	F24194	F24195	494/516	15 nM[§]
RhodZin™-3	R36350	R36351	550/575	65 nM
Newport™ Green DCF	N7990	N7991	505/535	1 µM
Newport™ Green PDX	N24190	N24191	495/520	30 µM

*Ex/Em: Fluorescence excitation and emission maxima in nm. **Dissociation constant of indicator/Zn^{2+} complex measured in 50 mM MOPS pH 7.0 at 22°C. [§]Kd determined in 135 mM NaCl, 1.1 mM EGTA, 20 mM HEPES, pH 7.4, 0 to 10 µM free Zn^{2+} at 22°C.

Properties

Indicator spectroscopic properties and Zn^{2+} binding affinities are summarized in Table 2. The other indicators listed in Table 2 all exhibit fluorescence intensity increases on binding Zn^{2+} with no accompanying spectral shift. All of the indicators have negligible sensitivity to Ca^{2+} concentrations up to at least 100 µM.

Before Starting

Preparing Stock Solutions and Storage

Prepare the stock solutions of water-soluble indicator salts in distilled water or aqueous buffers, and store frozen (≤–20°C) and **protected from light**. When stored properly, the solutions are stable for at least six months.

Acetoxymethyl (AM) and acetate ester derivatives are susceptible to hydrolysis and should be stored at ≤–20°C, desiccated, and **protected from light**. When stored under these conditions, these compounds are stable for at least six months. Reconstitute ester derivatives in dimethylsulfoxide (DMSO). Concentrations of about 1–5 mM (molecular weights (MW) are printed on the product label) are generally suitable for these stock solutions. Once prepared, use DMSO stock solutions as soon as possible to avoid decomposition and a resulting loss of cell loading efficacy. Store stock solutions of AM esters frozen, desiccated, and **protected from light**. Because the integrity of AM esters is primarily dependent on minimizing their exposure to water, we recommend that you use high-quality anhydrous DMSO. **Avoid repeated freezing and thawing of DMSO stock solutions.**

Experimental Protocols

Cell Loading Guidelines

You may load the water-soluble salt forms of fluorescent indicators into cells by microinjection (see **Note** below) or by diffusion from a patch pipette.[13]

Note: Typical injection volume of 1–10 mM indicator solution is ~1% of the cell volume, giving a final intracellular indicator concentration of 10–100 µM.

The following loading protocols using cell-permeant AM esters are provided as an introductory guide only; refer to published procedures for details.[14-16]

1.1 Dilute an aliquot of DMSO stock solution (1–5 mM) to a final concentration of 1–5 µM in the buffered physiological medium of choice.

Addition of non-ionic detergent Pluronic® F-127 can assist in dispersing the non-polar AM ester in aqueous media. You can conveniently accomplish this by mixing the aliquot of AM ester stock solution in DMSO with an equal volume of 20% (w/v) Pluronic in DMSO (Cat. no. P3000MP) before diluting in loading medium, making the final Pluronic concentration about 0.02%. Invitrogen offers Pluronic® F-127 in 30 mL units of a sterile 10% (w/v) solution in water (Cat. no. P6866) and 2 g solid units (Cat. no. P6867).

1.2 Incubate the cells with the AM ester for 15 to 60 minutes at 20°C to 37°C.

You need to empirically determine the exact loading concentration, time, and temperature; in general it is desirable to use the minimum dye concentration required to yield fluorescence signals with adequate signal to noise levels. Subcellular compartmentalization, an inherent problem with the AM ester loading technique, is usually lessened by lowering the incubation temperature.[14,15]

1.3 Before you begin fluorescence measurements, wash cells in indicator-free medium to remove any dye that is nonspecifically associated with the cell surface, and then incubate for a further 30 minutes to allow complete de-esterification of intracellular AM esters.

Response Calibration

You can carry out response calibration by measuring the fluorescence intensity of the carboxylate salt form of the indicator in solutions with precisely known free Zn^{2+} concentrations. If the Zn^{2+} concentrations are unbuffered, the approximation $Zn^{2+}_{total} \sim Zn^{2+}_{free}$ is only valid if the indicator concentration is very low (<0.1 µM). You can prepare EGTA-buffered zinc calibration solutions using analogous calcium buffer methodology.[17,18] Zn^{2+} concentrations from 1 nM–10 µM have been obtained in this way for calibration of the indicator mag-fura-5.[5] Chelators with lower Zn^{2+} affinity such as acetamidoiminodiacetic acid (ADA; K_d (Zn^{2+}) = 1.27×10^{-7} M at pH 7, I = 0.1 and 20°C) provide more optimal buffering in the micromolar range than EGTA (K_d (Zn^{2+}) = 6.8×10^{-9} M at pH 7, I = 0.1 and 20°C).

For indicators such as FluoZin™-1 and Newport Green™ DCF, you may determine the K_d using the following equation, in which F denotes fluorescence intensity measured at a single wavelength:

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

In the above equation, the values of F are dependent on the concentration of indicator. It is important to recognize that the ion-binding and spectroscopic properties of fluorescent indicators can vary quite markedly in cellular environments. Consequently, *in situ* response calibrations of intracellular indicators often yield K_d values significantly different from *in vitro* determinations. Perform *in situ* calibrations of zinc indicators by exposing loaded cells to controlled Zn^{2+} concentrations in the presence of an ionophore. Pyrithione at a working concentration of 20 µM is the most widely used Zn^{2+} ionophore.[5,8,9] Alternatively, 4-bromo A-23187 (Cat. no. B1494) has been used in some cases.[6] The zero reference level (F_{min}) for intracellular Zn^{2+} calibrations is usually set by adding 50–100 µM TPEN (Cat. no. T-1210; $K_d(Zn^{2+}) = 2.6 \times 10^{-16}$ M).

References

1. Trends Pharmacol Sci 21, 395 (2000); **2.** Science 265, 1464 (1994); **3.** J Membrane Biol 123, 63 (1991); **4.** J Biol Chem 270, 2473 (1995); **5**. J Neurosci 17, 9554 (1997); **6.** J Neurochem 71, 2401 (1998); **7.** Nature 308, 734 (1984); **8.** Proc Natl Acad Sci 96, 2414 (1999); **9.** J Neurosci 19, RC31 (1999); **10.** Eur J Neurosci 12, 3813 (2000); **11.** J Physiol 528, 39 (2000); **12.** J Neurochem 75, 1878 (2000); **13.** *Imaging Neurons: A Laboratory Manual* R. Yuste, F. Lanni and A. Konnerth, Eds., pp 35.1–35.10, Cold Spring Harbor Laboratory Press (2000); **14.** Methods Enzymol 302, 341 (1999); **15.** Methods Enzymol 307, 441 (1999); **16.** *Cell Biology: A Laboratory Handbook, 2nd Edition*, J.E. Celis, Ed., Volume 3, pp 363–374, Academic Press (1998); **17.** Methods Cell Biol 40, 3 (1994); **18.** Methods Enzymol 172, 230 (1989); **19.** Cell Calcium 31, 245 (2002); **20.** Cell Calcium 34, 281 (2003).

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

Cat. no.	Product Name	Unit Size
B1494	4-bromo A-23187, free acid	1 mg
F24180	FluoZin™-1, tripotassium salt *cell impermeant*	500 µg
F24181	FluoZin™-1, AM *cell permeant*	50 µg
F24189	FluoZin™-2, AM *cell permeant*	50 µg
F24194	FluoZin™-3, tetrapotassium salt *cell impermeant*	500 µg
F24195	FluoZin™-3, AM *cell permeant*	100 µg
N24190	Newport Green™ PDX	1 mg
N24191	Newport Green™ PDX acetoxymethyl ether	1 mg
N7990	Newport Green™ DCF, dipotassium salt *cell impermeant*	1 mg
N7991	Newport Green™ DCF diacetate *cell permeant*	1 mg
P10020	PowerLoad™ concentrate, 100X	5 mL
P3000MP	Pluronic® F-127 *20% solution in DMSO*	1 mL
P6866	Pluronic® F-127 *10% solution in water* *0.2 µm filtered*	30 mL
P6867	Pluronic® F-127 *low UV absorbance*	2 g
R36350	RhodZin™-3, dipotassium salt *cell impermeant*	500 µg
R36351	RhodZin™-3, AM *cell permeant*	50 µg
T1210	tetrakis-(2-pyridylmethyl)ethylenediamine (TPEN)	100 mg

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