Fluorescent Zinc and heavy metals Indicators

Zinc detection is needed in studies like ecology, toxicology , neurology, enzyme activity, metal ion transport through ion channels (see technical tip). Fluorescent probes for Zn^{2+} simply overcomes conventional methods (like spectrometry, chromatography) in many applications, including Zn^{2+} assay in complexe samples, Zn^{2+} concentration inside cells. Zn^{2+} interferes also with many other ions probes (i.e. Fluo-3 for Ca^{+}), requiring to block it (see TPEN) or making Zn^{2+} study necessary.

Interchim provides many fluorescent probes for Zn^{2+} measurements (including Zinquin, Zynpyr, TFLZn). Zinc concentrations in the 1–100 nM range can also be measured using fluorescent indicators nominally designed for Ca^{2+} and Mg^{2+} detections such as Fura-2 and Mag-Fura-2 (see above).

Technical tip

Zn^{2+} in cell biology

As many other metal ions, Zn^{2+} is involved in various biological processes, for both structure and function of proteins. For example, it is estimated that up to 1% of the human genome codes for zinc finger proteins. The intracellular concentration of free Zn^{2+} is extremely low in most of cells (<1 nM), most being bound to proteins (matrix metalloproteinases (MMP) or nucleic acids.

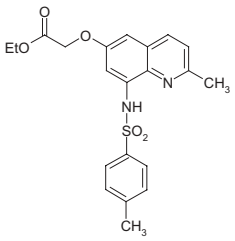
In the central nervous system, zinc has an additional role of neurosecretory product or cofactor. Then zinc is highly concentrated in the synaptic vesicles of a specific contingent of neurons called "zinc-containing" neurons.

These zinc containing neurons are a subset of glutamatergic neurons. The zinc in the vesicles of these neurons exceeds 1 mmol/L in concentration. Zinc-containing neurons are found almost exclusively in the forebrain, where in the mammals they have evolved into a complex and elaborate associational network that interconnects with most of cerebral cortices and limbic structures.

Zinquin

Zinquin, a derivative of quinoline, is an UV-excitable, blue fluorescent zinc indicator, analog of the widely used indicator TS-Q.

- Forms a complex with a zinc ion with two nitrogen atoms in the structure.
- Also makes fluorescent complex with cadmium ion, however, detectable amount of cadmium ions are not contained in normal living cells.
- Useful to study the role of intracellular zinc ions on apoptosis



abs em. **Zinquin EE**

$C_{21}H_{22}N_2O_5S$ MW : 414.48
Soluble in DMSO or ethanol
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see Zinquin free acid (FP-AM291A)
A membrane-permeable which is hydrolyzed into Zinquin free acid once entering cells.

Description	Cat.#	Qty
Zinquin EE	**FP-T3329A**	5 mg

abs em. **Zinquin free acid**

$C_{19}H_{18}N_2O_5S$ MW : 386.43
Soluble in DMSO
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$: 368/490 nm
Forms both 1:1 and 2:1 zinquin-zinc (II) complexes with respective stepwise K_d of 370 ± 60 nM and 85 ±16 nM in physiological media.

Description	Cat.#	Qty
Zinquin free acid	**FP-AM291A**	5 mg

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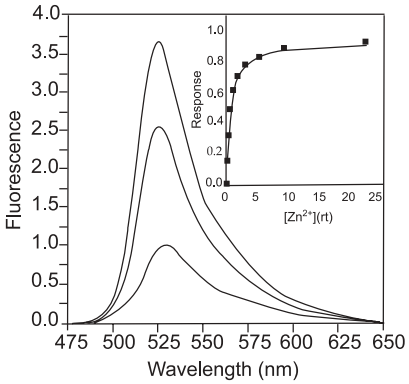


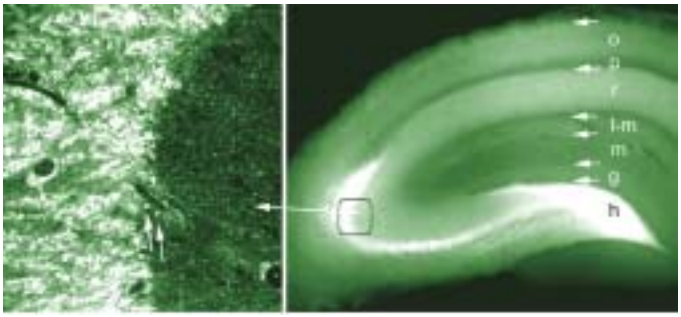
Zinpyr

Zinpyr stains zinc-injured neurons much better than the conventional TSQ dye.

- Excitation / Emission wavelengths in the visible range (~500 nm)
- K_d for Zinc : < 1nM ; QY : ~ 0.9
- Lipophilic ; stains bouton zinc
- Threefold increase in fluorescence with zinc binding
- High specificity for zinc

References :
ZP4, an improved neuronal Zn sensor of the Zinpyr family ; Burdette Sc et al J Am Chem Soc. 2003 Feb 19;125(7):1778-87.
Identification of neuronal cells suffering zinc toxicity by using a novel fluorescent zinc sensor ; PNAS, 2003; under review.
Fluorescent sensors for $Zn^{(2+)}$ based on a fluorescein platform: synthesis, properties and intracellular distribution ; Burdette Sc et al ; J Am Chem Soc. 2001 Aug 15;123(32):7831-41.
Rapid translocation of zinc from presynaptic terminals into postsynaptic hippocampal neurons after physiological stimulation system; Yang Li et al ; J Neurophysiol. vol. 86, nov.2001"
Induction of Mossy Fiber3CA3 Long-Term Potentiation Requires Translocation of Synaptically Released Zn^{2+} ; Yang Li et al ; J. Neurosci., October 15, 2001, 21(20):8015–8025
Evidence that synaptically released zinc contributes to neuronal injury after TBI ; Brain Research 852 2000 268–273"

Technical tip



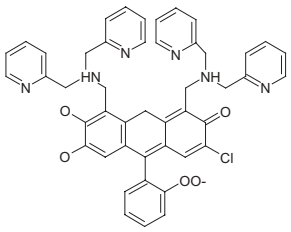
Zinpyr-1 stained hippocampal slice showing zinc containing axons.
The image on the left shows the border between stratum lucidum and radiatum when seen under a confocal microscope (Courtesy : Stefano L. Sensi, UC, Irvine).

Why use Zinpyr ?

- No need to use UV light
- Very stable at room temperature
- Low maintenance
- Pretty pictures

That's so easy to stain brain sections!

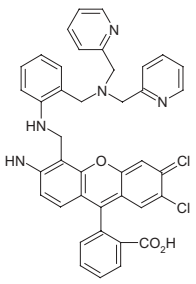
- Make up a 1 mM stock solution of Zpy-1 in DMSO
- From this stock, dilute a working solution in 0.9% saline to reach a final concentration of 10-20 µM
- Cover the brain section with the above working solution for 2-3 mins
- View under a microscope and image directly.



abs em. **Zinpyr-1 (Zpy1)**

$C_{46}H_{36}Cl_2N_6O_5$ MW : 823.74
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$: 515/525 nm ; EC : 79.5 x 10^3 M^{-1} cm^{-1}
K_d : 0.7 nM
This is a lipophilic zinc sensing fluorophore. It can be used to stain mossy fiber zinc. Fluorescence emission response of Zinpyr-1 to buffered Zn^{2+} solutions.

Description	Cat.#	Qty
Zinpyr-1 (Zpy1)	**FP-AY6881**	5 mg



abs em. **Zinpyr-4 (Zpy4)**

$C_{40}H_{31}ClN_4O_5$ MW : 683.17
Store at 4°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$: 515/525 nm
This is a congener of ZP-1, but is lipophobic, thus usefull for near membrane measurements. This stain can be used to stain zinc positive cells, that are seen in conditions such as trauma, hemorrhage and ischemia. Since it does not stain the mossy fibers, it provides a very good signal noise ratio.

Description	Cat.#	Qty
Zinpyr-4 (Zpy4)	**FP-AY6891**	1 mg

TFLZn and other Zn Indicators

TFLZn is a water soluble analog of TSQ9 that has been well documented regarding to its specificity over other ions (Budde 1997) , and applications in imaging of zinc in synaptic terminals of live hippocampal mossy fibers slices.

Reference : Imaging Free Zinc in Synaptic Terminals in Live Hippocampal Slices. Budde T, Minta A, White JA, Kay AR ; Neuroscience 1997 79:2 347-58.

abs em. TFLZn, AM

N-(6-methoxy-8-quinoyly)-p-acetoxymethylbenzoylsulphonamide
$C_{21}H_{20}N_2O_7S$ MW : 444.47
Soluble in DMSO or EtOH
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see TFLZn K salt (FP-AM936A)
Hydrolysis gives TFLZn product, that becomes fluorescent upon binding to Zn^{2+}.

Description	Cat.#	Qty
TFLZn, AM	**FP-AM694A**	5 mg

abs em. TFLZn, K salt

N-(6-methoxy-8-quinoyly)-p-carboxybenzoylsulphonamide
$C_{18}H_{15}N_2O_5SK$ MW : 410.50
Soluble in water
Store at 20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (Zn^{2+}) : 380/510 nm ; EC : 5 000 $M^{-1}cm^{-1}$; K_d : 20 µM

Description	Cat.#	Qty
TFLZn K salt	**FP-AM936A**	5 mg

abs em. TFLZn free acid

$C_{18}H_{16}N_2O_5S$ MW : 372.40
Soluble in basic buffer
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$ (hydrolyzed) : see TFLZn K salt (FP-AM936A)

Description	Cat.#	Qty
TFLZn free acid	**FP-AM937A**	5 mg

abs em. TSQ

N-(6-methoxy-8-quinolyl)-p-toluenesulfonamide
$C_{17}H_{16}N_2O_3S$ MW : 328.40
Soluble in EtOH
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$: 334/485 nm ; EC : 4 200 $M^{-1}cm^{-1}$

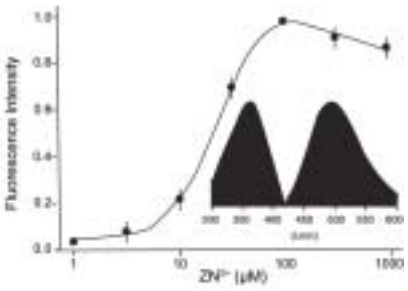
Description	Cat.#	Qty
TSQ	**FP-AM668A**	25 mg

References :
Zinc-specific N-(6-methoxy-8-quinolyl)-para-toluenesulfonamide as a selective nontoxic fluorescence stain for pancreatic islets. Jindal RM, et al ; Biotech Histochem 1993 68:4 196-205. A fluorescence method to determine picomole amounts of Zn(II) in biological systems ; Reyes JG, et al ; Biol Res 1994 27:1 49-56. Role of zinc during hamster sperm capacitation; Andrews JC, et al ; Biol Reprod 1994 51:6 1238-47. Ca^{2+} and Zn^{2+} dependence of DNA synthesis in untransformed and in Ha-ras(val-12)-expressing NIH 3T3 cells. Back CJ, et al Exp Cell Res 1993 208:1 303-10.

abs em. Dansylaminoethyl-cyclen

1-[2-(5-Dimethylamino-1-naphthalenesulfonamido)ethyl]-
1,4,7,10-tetraazacyclododecane, tetrahydrochloride, dihydrate
$C_{22}H_{40}C_{l4}N_6O_2S$, $2H_2O$ MW : 630.50
Soluble in water
$\lambda_{exc.}/\lambda_{em.}$ (Zn complex) : 323:528 nm
Dansylaminoethyl-cyclen is a water-soluble and cell membrane permeable fluorescent zinc indicator (Dr. E. Kimura). The fluorescence intensity of the complex with Zn^{2+} is 5 times higher than with free ligand. Most other cations do not form fluorescent complexes with this reagent. Cadmium ion, that forms a fluorescent complex but with a 10 times lower stability constant, does not affect zinc detection in most cases. Copper ion (Cu(II)), which forms a non-fluorescent complex, also does not affect zinc detection because is extremely rare as a free ion in normal living cells.

Description	Cat.#	Qty
Dansylaminoethyl-cyclen	**FP-T3122A**	5 mg



Zn^{2+} filtration et λmax of TFLZn.

See also Mg/Ca^{2+} indicators
Many Ca^{2+} indicators (E32-E55) and Zn^{2+} indicators (E56-E58) chelate also heavy metals like Cu^{2+}, Fe^{2+}, Co^{2+}, Mn^{2+}.

Several of these Zinc indicators may serve for the study of other heavy metals in cells. Please inquire. Other Metal indicators are presented in section C. Application to cells may need an extraction.

See also Calcein
Calcein (FP-46625) is strongly quenched by Fe^{3+}, Co^{2+}, Cu^{2+} and Mn^{2+} at physiological pH but not by Ca^{2+} or Mg^{2+} ions. So, calcein has been used for measuring Fe^{3+} (0.1-10µM) and protein bound Iron.