Metal Fluor™ Zn-520, Potassium Salt

Catalog number: 21262
Unit size: 1 mg

Component	Storage	Amount (Cat No. 21262)
Metal Fluor™ Zn-520, Potassium Salt	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)

OVERVIEW

Metal Fluor™ Zn-520 is designed for detection of higher zinc ion concentrations that are present in synaptic vesicles and released in response to electrical stimulation or excitotoxic agonists (0.05-50 µM) with minimal interfering calcium sensitivity. Metal Fluor™ Zn-520 has shown great fluorescence enhancement upon binding zinc ion (>250 folds). This cell-impermeant zinc ion indicator is useful for detecting extracellular zinc ion.

AT A GLANCE

Protocol Summary

1. Test samples (50 µL) or Zn^{2+} Standard
2. Add Zinc Probe Reagent (50 µL)
3. Incubate at room temperature for 5 - 10 minutes
4. Read fluorescence at Ex/Em= 485/525 nm

Important Note

Thaw the dye at room temperature before starting the experiment.

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	515 nm
Emission	525 nm
Excitation	485 nm
Recommended plate	Solid black

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

Zinc standard stock solution (1 mM)

Add 10 µL of 100 mM $ZnCl_2$ Standard into 990 µL HEPES buffer to get 1 mM $ZnCl_2$ standard solution.

Metal Fluor™ Zn-520

Make 1-4 mM stock solution is water.

Note: Store at -20°C in single-use aliquots.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/21262

ZnCl2 standard

Add 100 µL of 1 mM Zinc standard solution to 900 µL HEPES buffer to get 100 µM ZnCl2 standard solution (Zn7). Then take the 100 µM ZnCl2 standard solution to perform 1:3 serial dilutions to get serially diluted ZnCl2 standards (Zn6 - Zn1).

PREPARATION OF WORKING SOLUTION

Metal Fluor™ Zn-520 working solution

Make a 10-50 µM working solution from the Metal Fluor™ Zn-520 stock solution.

Note: Working concentration may be optimized empirically.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of $ZnCl_2$ standards and test samples in a solid black 96-well microplate. Zn = Zinc standard (Zn1 - Zn7, 0.1 to 100 µM); BL = blank control; TS = test sample.

BL	BL	TS	TS
Zn1	Zn1	...	...
Zn2	Zn2	...	...
Zn3	Zn3		
Zn4	Zn4		
Zn5	Zn5		
Zn6	Zn6		
Zn7	Zn7		

Table 2. Reagent compotition for each well.

Well	Volume	Reagent
Zn1 - Zn7	50 µL	Serial dilution (0.1 to 100 µM)
BL	50 µL	HEPES buffer
TS	50 µL	Sample

1. Dilute the test sample to a 5 - 100 µM range with HEPES buffer.
2. Prepare $ZnCl_2$ standards (Zn), blank controls (BL), and test samples (TS) according to the layout provided in Table 1 and Table 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
3. Add 50 µL of Zinc working solution into each well of $ZnCl_2$ standard, blank control, and test samples to make the total $ZnCl_2$ assay volume of 100 µL/well. For a 384-well plate, add 25 µL of HEPES buffer into each well instead, for a total volume of 50 µL/well.
4. Incubate the reaction for 5 - 10 minutes at room temperature, protected from light.
5. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 485/525 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the baseline corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Zinc Chloride samples. We recommend using the Online Linear Regression Calculator which can

be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

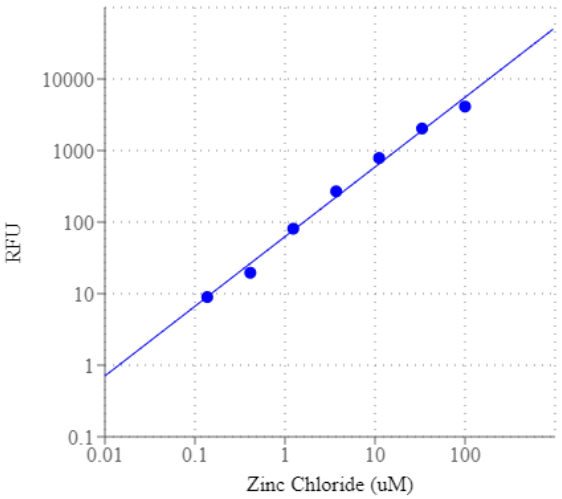

Figure 1. Zinc Chloride dose response was measured on a 96-well black plate with the Metal Fluor™ Zn-520.

DISCLAIMER

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