Amplite™ Fluorimetric Zinc Ion Quantitation Kit

Catalog number: 19000
Unit size: 200 Tests

Component	Storage	Amount
Component A: Metal Fluor™ Zn 520	Freeze (<-15 °C), Minimize light exposure	1 vial (50 µL)
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (15 mL)
Component C: ZnCl2 Standard	Freeze (<-15 °C), Minimize light exposure	1 vial (100 mM,100 µL)

OVERVIEW

Zinc is an essential trace mineral element that plays an important role in a number of biological processes. It is an essential factor required for many enzymes, protein structures, and control of genetic expression. Zinc status also affects basic processes of cell division, growth, differentiation, development, and aging. Clinical signs of zinc deficiency include acrodermatitis, low immunity, diarrhea, poor healing, stunting, hypogonadism, fetal growth failure, teratology and abortion. Simple, direct and automation-ready procedures for measuring are highly desirable in research and drug discovery. AAT Bioquest's Amplite™ Fluorimetric Zinc Quantitation Kit provides a simple method for detecting zinc concentration in biological samples using our proprietary Metaltel; Zn 520, in which Zinc binds to the probe with enhanced fluorescence. The Zinc probe exhibits a large increase in fluorescence in response to Zn2+ (greater than 200~300 folds). It has high Zn2+-specificity with little responses to other metals, including Ca2+, Mg2+, Mn2+, and Cu2+. The assay can be used with biological samples such as serum, plasma, and urine with detection sensitivity at 0.2 µM (13 ng/mL).

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 Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	485 nm
Emission:	525 nm
Cutoff:	515 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.

1. Zinc standard solution (1 mM):
Add 10 µL of 100 mM $ZnCl_2$ Standard (Component C) into 990 µL Assay Buffer (Component B) to get 1 mM $ZnCl_2$ standard solution.

PREPARATION OF STANDARD SOLUTION

$ZnCl_2$ standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/19000

Add 100 µL of 1 mM Zinc standard solution to 900 µL Assay Buffer (Component B) to get 100 µM $ZnCl_2$ standard solution (Zn7). Then take the 100 µM $ZnCl_2$ standard solution to perform 1:3 serial dilutions to get serially diluted $ZnCl_2$ standards (Zn6 - Zn1).

PREPARATION OF WORKING SOLUTION

Add 25 µL of Metal Fluor™ Zn 520 (Component A) into 5 mL Assay Buffer (Component B) to make Zn working solution.

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	Assay Buffer (Component B)
TS	50 µL	sample

1. Dilute the test sample to a 5 - 100 µM range with Assay Buffer (Component B).
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 Zinc assay 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 base-

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line 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

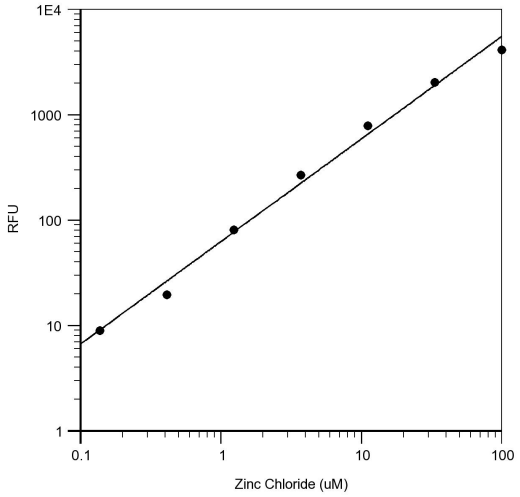

Figure 1. Zinc Chloride dose response was measured on a 96-well black plate with the Amplite™ Fluorimetric Zinc Ion Quantitation Kit.

DISCLAIMER