Amplite™ Colorimetric Zinc Ion Quantitation Kit

Catalog number: 19001
Unit size: 200 Tests

Component	Storage	Amount
Component A: Zn-620™	Freeze (<-15 °C), Minimize light exposure	1 vial (50 µL)
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (20 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™ Colorimetric Zinc Quantitation Kit provides a simple method for detecting zinc concentration in biological samples using our proprietary Zin-620™, in which Zinc binds to the probe with the enhanced absorption around 620 nm. The Zinc probe exhibits a large increase in 620 nm absorption in response to Zn2+ (>100 folds). Our kit formulation has enhanced Zn2+-specificity with little responses to other metals, e.g., Ca2+ and Mg2+. The assay can be used with biological samples such as serum, plasma, and urine with detection sensitivity at 1 µM. Our Amplite™ Colorimetric Zinc Quantitation Kit (#19000) is even more sensitive, and can be used for detecting as low as 0.1 uM Zn ion.

AT A GLANCE

Protocol summary

1. Prepare Zn^{2+} Standards or test samples (50 µL)
2. Add Zinc working solution (50 µL)
3. Incubate at room temperature for 5 - 10 minutes
4. Read absorbance ratio of A_{610nm}/A_{470nm}

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

KEY PARAMETERS

Instrument:	Absorbance microplate reader
Absorbance:	610/470 nm
Recommended plate:	Clear bottom

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. ZnCl2 standard solution (1 mM):
Add 10 µL of 100 mM $ZnCl_2$ Standard solution (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/19001

Add 100 µL of 1 mM $ZnCl_2$ standard solution to 900 µL Assay Buffer (Component B) to get 100 µM $ZnCl_2$ standard solution (Zn1). Take 100 µM $ZnCl_2$ standard solution (Zn1) and perform 1:2 serial dilutions in Assay Buffer (Component B) to get serially diluted $ZnCl_2$ standards (Zn7 - Zn2).

PREPARATION OF WORKING SOLUTION

Add 25 µL of Zn-620™ (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 clear bottom 96-well microplate. Zn= Zinc Standards (Zn1 - Zn7, 100 to 1.56 µM), BL=Blank Control, TS=Test Samples.

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

Table 2. Reagent composition for each well.

Well	Volume	Reagent
Zn1 - Zn7	50 µL	Serial Dilutions (100 to 1.56 µM)
BL	50 µL	Assay Buffer
TS	50 µL	test sample

1. Dilite the test sample to 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 Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
3. Add 50 µL of Zn working solution to 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 Zn working solution 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 absorbance ratio increase with a absorbance plate reader at $A_{610nm}/A_{470nm.}$

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Abs 610/Abs 470) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain

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the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Zinc Ion 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

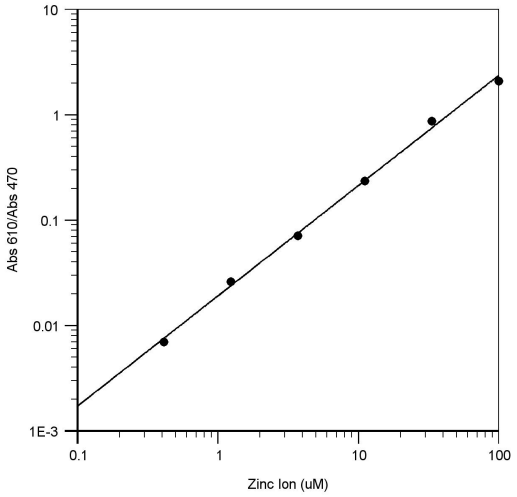

Figure 1. Zinc Chloride dose response was measured on a 96-well clear bottom plate with the Amplite™ Colorimetric Zinc Ion Quantitation Kit.

DISCLAIMER