

Amplite® Colorimetric Zinc Ion Quantitation Kit

Catalog number: 19001
Unit size: 200 Tests

Component	Storage	Amount (Cat No. 19001)
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: ZnCl ₂ 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 Zn-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® Fluorimetric Zinc Ion Quantitation Kit (#19000) is even more sensitive, and can be used for detecting as low as 0.1 uM zinc ion.

AT A GLANCE

Protocol Summary

1. Prepare Zn²⁺ 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 Note

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

KEY PARAMETERS

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

ZnCl₂ standard solution (1 mM)

Add 10 µL of 100 mM ZnCl₂ Standard solution (Component C) into 990 µL Assay Buffer (Component B) to get 1 mM ZnCl₂ standard solution.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:

<https://www.aatbio.com/tools/serial-dilution/19001>

ZnCl₂ standard

Add 100 µL of 1 mM ZnCl₂ standard solution to 900 µL Assay Buffer (Component B) to get 100 µM ZnCl₂ standard solution (Zn1). Take 100 µM ZnCl₂ standard solution (Zn1) and perform 1:2 serial dilutions in Assay Buffer (Component B) to get serially diluted ZnCl₂ 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 ZnCl₂ 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. Dilute the test sample to 1.56 - 100 µM range with Assay Buffer (Component B).
2. Prepare ZnCl₂ 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 ZnCl₂ standard, blank control, and test samples to make the total ZnCl₂ 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 $A_{610\text{nm}}/A_{470\text{nm}}$.

EXAMPLE DATA ANALYSIS AND FIGURES

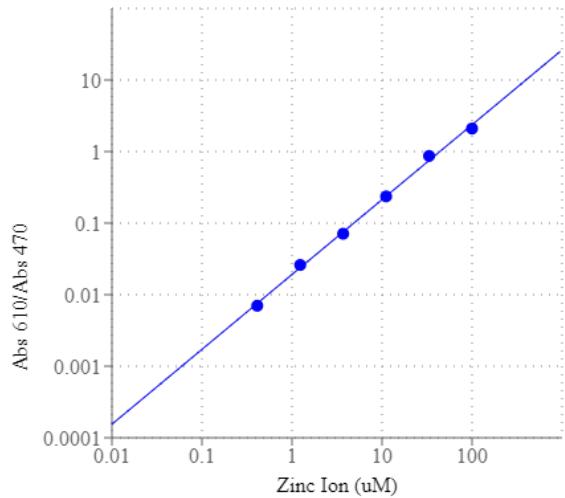


Figure 1. Zinc Chloride dose response was measured on a 96-well clear bottom plate with the Amplate® Colorimetric Zinc Ion Quantitation Kit.

DISCLAIMER

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