Amplite™ Colorimetric Zinc Ion Quantitation Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 19001 (200 assays)	Keep in freezer Avoid exposure to light	Absorbance microplate readers

Introduction

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, and teratology. Simple, direct and automation-ready procedures for measuring zinc ion are highly desirable in research and drug discovery. AAT Bioquest's Amplite™ Colorimetric Zinc Quantitation Kit provides a robust 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. In zinc-free solution, the absorbance is at 480 nm, while when Zinc ions bind to the probe, it exhibits a large increase in 620 nm (>100 folds). The concentration of Zinc can be measured colorimetrically at absorbance ratio of A_{610nm}/A_{480nm}.The assay can be used with biological samples such as serum, plasma, and urine with detection sensitivity at 2 µM (130 ng/mL). AAT Bioquest's Amplite™ Fluorimetric Zinc Quantitation Kit (#19000) is even more sensitive, and can be used for detecting as low as 0.2 uM Zn ion.

Kit Components

Components	**Amount**
Component A: Zn-620™	1 vial (50 µL)
Component B: Assay Buffer	1 bottle (20 mL)
Component C: $ZnCl_2$ Standard	1 vial (100 mM, 100 µL)

Assay Protocol for Zn^{2+} Test Samples

Brief Summary

Test samples (50 µL) or Zn^{2+} Standard → Add Zinc Probe Reagent 50 µL → Incubate at room temperature for 5-10 minutes→ Read absorbance ratio of A_{610nm}/A_{480nm}

1. $ZnCl_2$ standards and test sample preparations:

1.1 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.

1.2 Add 100 µL of 1 mM Zinc Standard solution (Component C) to 990 µL Assay Buffer (Component B) to get100 µM $ZnCl_2$ standard solution.

1.3 Take 300 µL of 100 µM $ZnCl_2$ standard solution (from Step 1.2) to perform 1:2 serial dilutions to get 50, 25, 12.5, 6.25, 3.13, 1.56 and 0 µM serially diluted $ZnCl_2$ standards.

1.4 Dilute the test sample to 5-100 µM range with Assay Buffer (Component B).

1.5 Add 50 µL of $ZnCl_2$ standards, diluted sample and control into each well as shown in Table 1and 2.

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Table 1. Layout of $ZnCl_2$ standards and test samples in a clear bottom 96-well microplate

BL	BL	TS	TS	….	….						
Zn 1	Zn 1	….	….	….	….						
Zn 2	Zn 2										
Zn 3	Zn 3										
Zn 4	Zn 4										
Zn 5	Zn 5										
Zn 6	Zn 6										
Zn 7	Zn 7										

Note: Zn= Zinc Standards, BL=Blank Control, TS=Test Samples.

Table 2. Reagent composition for each well

Zinc Standard	**Blank Control**	**Test Sample**
Serial dilutions*: 50 µL	Assay Buffer: 50 µL	50 µL

**Note: Add the serially diluted $ZnCl_2$ standards from 100 µM to 1.56 µM into wells from Zn1 to Zn7 in duplicate.*

2. Run Zinc assay:

2.1 Add 25 µL of Zn-620™ (Component A) into 5 mL Assay Buffer (Component B) to make Zn assay mixture. Add 50 µL Zn assay mixture to each well of $ZnCl_2$ standard, blank control, and test samples (see Step 1.5, Table 1 and 2) to make the total $ZnCl_2$ assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of samples and 25 µL of assay reaction mixture into each well.

2.2 Incubate the reaction for 5-10 minutes at room temperature, protected from light.

2.3 Monitor the absorbance increase with a absorbance plate reader at 610 nm and 480 nm.

3. Zinc calibration curve:

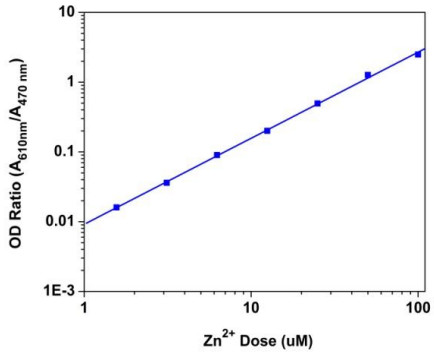

Figure 1. $ZnCl_2$ dose response was measured on a 96-well clear bottom plate with the Amplite™ Colorimetric Zinc Quantitation Kit. As low as ~ 2 µM Zn^{2+} can be detected with 5 minutes incubation time (n=3).

References

1. Knoell, DL et al (2009). Zinc deficiency increases organ damage and mortality in a murine model of polymicrobial sepsis. Crit Care Med 37(4):1380-8.
2. Padiglia, A et al (2010). Sensitivity to 6-n-propylthiouracil is associated with gustin (carbonic anhydrase VI) gene polymorphism, salivary zinc, and body mass index in humans. Am J Clin Nutr 92(3):539-45.
3. Kelly, E et al (2011). Redistribution of labile plasma zinc during mild surgical stress in the rat. Transl Res 157(3):139-49.