ABS_Bio™ Zinc Colorimetric Detection Kit

(Cat# K333-200; 200 assays; store kit at 4°C)

Introduction

Zinc is a mineral essential to both normal health and to optimal cellular function. As a cofactor for an excess of seventy enzymes, zinc plays an important role in many cellular processes. Zinc is also a component of specific DNA binding proteins, known as zinc finger proteins, commonly referred to as zinc fingers. Zinc fingers are vital for nutrient/gene interactions.

The ABS_Bio™ Zinc Colorimetric Detection Kit provides a simple, sensitive, one-step colorimetric assay to detect zinc in various samples. In this assay, Zinc bands to a chromogen that forms a colored complex. The intensity of the color, measured at 560 nm (550-570 nm), is directly proportional to the amount of zinc in the sample. The kit is supplied with sufficient reagents for 200 tests in 96-well plate assay and has a linear detection range of 0.11-25 µM (0.7-160 µg/dL). It could easily be modified for use in a 384-well or a high-throughput assay.

Kit Components (200 tests)

Assay Buffer: 45 mL Reagent: 0.8 mL Zinc Standard (125 µM): 1.5 mL EDTA (0.1 M): 1 mL

Storage and Handling: Store kit at 4°C. Shelf Life: 6 months after receipt. Warm up Reagents to room temperature before use.

Protocol

1. Sample preparation

Serum, Plasma, other bodily fluids, or cell culture supernatant can be measured directly by a series of dilutions (2-fold) of the sample to ensure the readings are within the standard curve range. Homogenize cell (2 x 10^6) or tissue (20 mg) samples in 200 µL cold PBS. Centrifuge to collect the supernatant. Metal chelators (e.g. EDTA, EGTA) should be avoided during sample preparation. It is recommended with all sample types to assay immediately or aliquot and store at -80°C. Transfer 50 µL of the sample into the 96-well clear flat bottom plate in duplicate (one as sample, one as sample blank need to add 2 µL EDTA solution).

2. Standard Preparation

Transfer 50 µL of 125 µM zinc standard into 200 µL dH_2O to generate a 25 µM zinc standard, then use the following table to generate 25, 15, 7.5, 2.5 and 0 µM zinc standards.

25 µM Zinc std(µL)	dH_2O(µL)	final zinc concentration µM
100	0	25
60	40	15
30	70	7.5
10	90	2.5
0	100	0.0

Transfer 50 µL of appropriate standards into the 96-well plate in duplicate. The blank control containing dH_2O only.

3. Reaction

Prepare enough working reagent by mixing 200 µL assay buffer and 4 µL reagent for each reaction.

Note: If sample has color, sample need run sample blank control (sample + 2 µL EDTA solution), this will only detect sample background.

Transfer 200 µL prepared working reagent into each reaction well. Tap plate to mix well. Incubate 10 min. at room temperature, protected from light.

4. Measurement

Read the optical density at 560 nm (550-570 nm).

5. Calculation

Average the duplicate OD560 nm reading for the standard. Subtract the average OD of the blank from the average OD of the standards and plot the result (ΔOD) versus the zinc concentration of the standards. Determine the slope and calculate the zinc concentration of the samples.

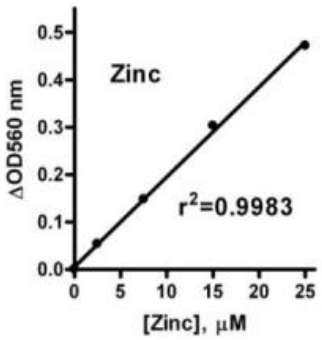
[Zinc]= (OD_{sample}-OD_{blank})/Slope x *n* (µM)

OD_{sample} and OD_{blank} are the optical densities of the sample and dH_2O. *n* is the sample dilution factor.

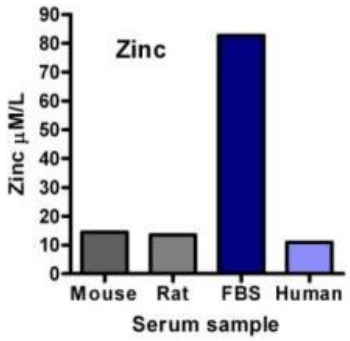
Note: if has sample blank, the sample blank reading must be subtracted from sample readings.

Conversions: 1 µM zinc equals 6.5 µg/dL.

Typical Standard Curve



Zinc standard in 96 wells-plate assay.



Serum sample in 96 wells-plate assay.

Sensitivity and Limit of Detection

The Limit of Detection was determined as 0.11 µM, with a linear detection range of up to 25 µM in the 96-well plate colorimetric assay. Sensitivity was determined as 0.05 µM. Samples with values above 25 µM should be diluted with dH_2O or assay buffer, re-assayed, and multiplied by the dilution factor.

Interferences

EDTA inhibits zinc to chromogenic system, should be avoided during sample preparation. The test is not affected by presence of bilirubin up to 40 mg/dL, hemoglobin up to 0.2 g/dL.

References

Makino, T. et al. 1982. Clinica Chimica Acta, 120:127-135
Makino, T. 1999, Clinica Chimica Acta, 282:65-76
Eliassion, R. 1987, international Journal of andrology, 10:435-440

Related Products:

Triglyceride Detection Kit (#K316-100)
ALP Fluorimetric Detection Kit (#K110-200)
NAD/NADH Detection Kit (#K120-100)
Creatinine Detection Kit (#K148-200)
Glucose Detection Kit (#K188-100)
Glutathione Detection Kit (#K140-100)
Glutathione Peroxidase Detection Kit (#K143-100)
Glutathione Reductase Detection Kit (#K146-100)
HRP Fluorimetric Detection Kit (#K210-100)
Xanthine Colorimetric Detection Kit (#K133-100)
ATP Detection Kit (#K135-100)
Acetate Detection Kit (#K308-100)
Ammonia Detection Kit (#K103-200)
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