Zinc Assay Kit (Colorimetric)

LS-K288-250 (250 Tests) • **See Storage Conditions Below**

LSBio
LifeSpan BioSciences, Inc.

Introduction

Zinc is an essential trace element and plays many key roles in metabolism. It is required for the activity of more than 300 enzymes, the structure of many proteins, and control of genetic expression. Zinc status affects basic processes of cell division, growth, differentiation, development, performance and aging through its requirement for synthesis and repair of DNA, RNA and protein. The common causes of zinc deficiency are low dietary intakes and low bioavailability. Clinical signs of zinc deficiency include acrodermatitis, low immunity, diarrhea, poor healing, stunting, hypogonadism, fetal growth failure, teratology and abortion. Zinc deficiency has now been recognized to be associated with many diseases such as malabsorption syndrome, chronic liver disease, chronic renal disease, sickle cell disease, diabetes, malignancy, and other chronic illnesses. Simple, direct and automation-ready procedures for measuring zinc concentration in biological samples are highly desirable in Research and Drug Discovery. LSBio's zinc assay kit is designed to measure zinc directly in biological samples without any pretreatment. The present method utilizes a chromogen that forms a colored complex specifically with zinc. The intensity of the color, measured at 425 nm, is directly proportional to the zinc concentration in the sample.

Key Features

- Sensitive and accurate. Uses 50 µL samples. Linear detection range 0.12 µM (0.78 µg/dL) to 10 µM (65 µg/dL) zinc in 96-well assay format.
- Simple and high-throughput. The procedure involves addition of a single working reagent and incubation for 30 min. Can be readily automated as a high-throughput assay for thousands of samples per day.
- Improved reagent stability and versatility. The optimized formulation has greatly enhanced reagent and signal stability. Cuvette or 96-well plate assay formats possible.
- Low interference in biological samples. No pretreatments are needed.

Applications

- Direct Assays: zinc in serum, plasma (no EDTA), urine, saliva etc.
- Drug Discovery/Pharmacology: effects of drugs on zinc metabolism.
- Environment: zinc determination in waste water, soil etc.

Components

Component	K288-250
	250 Tests
Reagent A	50 mL
Reagent B	1 mL
Reagent C	1 mL
EDTA (100 mM)	1 mL
Zinc Standard (50 µM)	1 mL

Materials Not Supplied

Pipetting devices and accessories. Clear bottom 96-well plates (e.g. Corning Costar) and plate reader, or cuvettes and spectrophotometer for measuring OD 425nm.

Storage

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The kit is shipped at room temperature. Store Reagents B and C at -20°C and other components at 4°C.

Assay Procedure

Sample preparation: serum and plasma samples should be clear and free of turbidity or precipitates. If present, precipitates should be removed by filtration or centrifugation on a table centrifuge. Prior to assay, dilute serum or plasma samples 5-fold (n = 5) in deionized water.

Reagent preparation: equilibrate all reagents to room temperature. Vortex Reagents B and C before assay. Prepare enough Working Reagent: for each assay well, mix 200 µL Reagent A, 4 µL Reagent B and 4 µL reagent C.

Procedure using 96-well plate

1. Prepare standards in deionized water. Transfer 50 µL of the Zn^{2+} standards into wells of a clear flat-bottom 96-well plate.

No	STD + H_2O	Vol (µL)	Zn^{2+} (µM)
1	20µL + 80µL	100	10.0
2	15µL + 85µL	100	7.5
3	10µL + 90µL	100	5.0
4	5µL + 95µL	100	2.5
5	0µL + 100µL	100	0

   Transfer 50 µL Sample and Sample Blank (50 µL sample + 2 µL EDTA) into wells of a clear flat-bottom 96-well plate. Add 200 µL working reagent to all wells and tap plate lightly to mix.

2. Incubate 30 min at room temperature and read optical density at 425 nm (range 420 - 426 nm).

Procedure using cuvette

Transfer 200 µL standards, sample and sample blank (200 µL Sample + 8 µL EDTA) to appropriately labeled tubes. Add 800 µL working reagent and tap lightly to mix. Incubate 30 min and read optical density at 425 nm.

General Considerations

Because the shift in the peak wavelength (from 413 nm to 425 nm) is very small, the color change is not visually evident. Physiological concentrations of other metal ions do not interfere. Zn^{2+} chelators (e.g. EDTA, EGTA) should be avoided during sample preparation.

Calculations

Subtract blank OD (water, #5) from the standard OD values and plot the DOD against Zn^{2+} standard concentrations. Calculate DOD for the Sample (= ODSAMPLE - ODSAMPLE BLANK). Determine the Sample Zn^{2+} concentration from the standard curve by non-linear regression fitting with a single-site saturation binding function (DOD = a×[Zn2+]/(b+[Zn^{2+}]). If the Zn^{2+} concentration is higher than 10 µM, dilute sample in deionized water. Repeat the assay and multiply the results by the dilution factor. **Conversions:** 1 µM zinc equals 6.5 µg/dL or 0.065 ppm (65 ppb).

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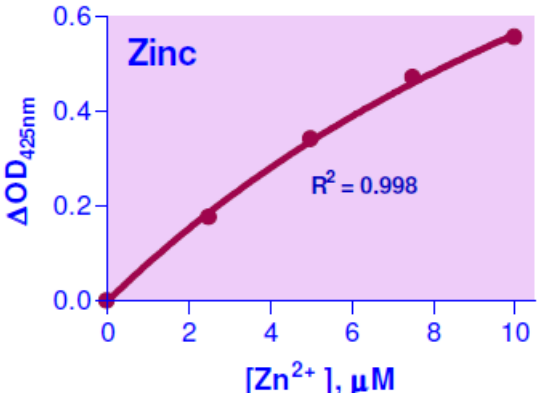
Sample Data

Samples were assayed in duplicate using the 96-well protocol. The zinc concentration (µg/dL) was 45.2 ± 4.4 for a human serum, 49.3 ± 3.2 for rat serum, 35.8 ± 1.4 for rat plasma, 113 ± 2 for Invitrogen fetal bovine serum, 30.8 ± 1.5 for human urine, 0.99 ± 0.31 for a sea water sample, <0.8 for a soil extract and a human saliva sample.



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