QuantiChrom™ Zinc Assay Kit (DIZN2-250)

Quantitative Colorimetric Zinc Determination at 425nm

DESCRIPTION

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. 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.

Simple, direct and automation-ready procedures for measuring zinc concentration in biological samples are highly desirable in Research and Drug Discovery. BioAssay Systems' 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. Detection range 0.05 µM (0.32 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 pre-treatments 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 wastewater, soil etc.

KIT CONTENTS (for 100 samples in 96-well assay)

Reagent A: 50 mL **Reagent B: 60 mg**
EDTA: 1 mL 100 mM **Zinc standard: 1 mL 50 µM**

Storage conditions. The kit is shipped at room temperature. Store kit components at 4°C. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

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 before use. Each assay well requires 0.2mg Reagent B in 200µL Reagent A. Prepare fresh Working Reagent (WR), by dissolving Reagent B in Reagent A to a final concentration of 1 mg/mL. Vortex for at least 30 seconds to ensure complete dissolution of the powder. Unused WR may be stored at –20°C for up to 3 days. Reagent B should be tightly sealed to prevent moisture exposure.

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	12µL + 88µL	100	6.0
3	6µL + 94µL	100	3.0
4	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 WR 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 WR 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.

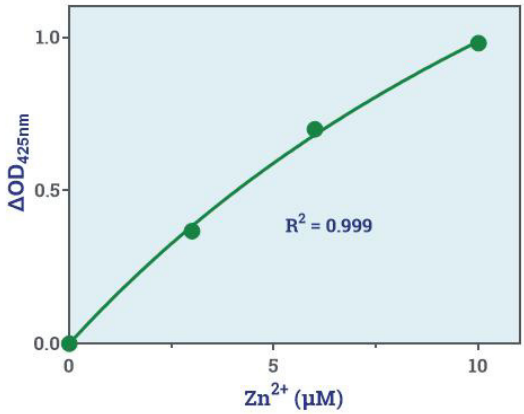
CALCULATION

Subtract blank OD (water, #4) from the standard OD values and plot the ΔOD against Zn^{2+} standard concentrations. Calculate ΔOD for the Sample (= OD_{SAMPLE} - $OD_{SAMPLE\ BLANK}$). Determine the Sample Zn^{2+} concentration from the standard curve by non-linear regression fitting with a single-site saturation binding function ($\Delta OD = a\times[Zn^{2+}]/(b+[Zn^{2+}])$). 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).

MATERIALS REQUIRED, BUT NOT PROVIDED

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



Standard Curve in 96-well plate assay

LITERATURE

1. Prasad. AS (2003) Zinc deficiency. BMJ 326:409-410.

2. Neal DE Jr, Kaack MB, Fussell EN, Roberts JA. (1993) Changes in seminal fluid zinc during experimental prostatitis. Urol Res. 21(1): 71-74.

3. Fuentes J, Miro J, Riera J. (1982) Simple colorimetric method for seminal plasma zinc assay. Andrologia. 14(4): 322-327.