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Catalog #CZN-001

MC Reagent Biochemical Assay Series

COSMO BIO CO., LTD.
Inspiration for Life Science

Zinc Assay Kit

5 Br-PAPS Chromogenic method

Biochemical Significance and Test Summary

Zinc is a cofactor of more than 200 kinds of metalloenzymes, and is also a trace element concerned in synthetic of ribonucleic acid or protein. It is widely known that acute zinc deficiency during the growth stage of the mammalian, results in a severe impairment of the skin or hair, and may lead to arrested development. Zinc is essential to reproduction of cell and its relevant supply is necessary for healthy growth.
This product is a direct colorimetric assay kit without deproteinization of the sample. At alkaline pH, in a buffered media, zinc reacts with the specific complex, forms a stable colored complex. The color intensity is proportional to the amount of zinc present in the sample.

1. Kit contents (100 tests)

R-1	Buffer	1 x 23 mL	Ready to use
R-2	Chelate color	1 x 0.5 mL	Ready to use
STD	200 µg/dL Zn Standard	1 x 1.2 mL	Ready to use

*Storage conditions: Store at 2-8°C. **Don't freeze**.
*Expiration: 1 year. After the bottles are opened, the kit should be used within one month.
*Measuring range: 4.0-1,000 µg/dL

2. Materials required but not provided

(1) Distilled water
(2) Micropipettors and pipette tips
(3) Clear flat-bottom 96-well plate
(4) Microplate reader with 560 nm capability

3. Assay preparation

(1) Bring all reagents to room temperature before use.
(2) Prepare working reagent: Mix 230 µL of R-1 and 5 µL of R-2 for 1 test.
(e.g.) Preparation for 50 tests
R-1: 230 µL x 50 tests = 11.5 mL
R-2: 5 µL x 50 tests = 250 µL
Mix 11.5 mL of R-1 and 250 µL of R-2 in a vessel.

4. Sample preparation

Serum/Plasma: Insoluble substances in serum and plasma samples should be removed by filtration or centrifugation. EDTA-plasma cannot be used.
Urine (24 hour pooled urine)/Biological fluid: Add 6M HCl to the sample and adjust pH 2.0-3.0 (e.g. 5-10 µL of 6 M HCl/1 mL of lysate). Centrifuge at 6,000 rpm for 15 minutes. Collect the supernatant and use it for assay.
Tissue: Add 5% TCA solution, vortex for 1 minute and incubate for 30 minutes at 4-8°C. Centrifuge at 6,000 rpm for 15 minutes. Collect the supernatant and use it for assay.

Note: Sample pH should be between pH 2 and pH 8.

5. Assay protocol

(1) Add 12 µL of Distilled water(Blank)/STD(Standards)/Samples into each well.
(2) Add 230 µL of Working Reagent to each well and incubate for 5 minutes at room temperature.
(3) Read the absorbance at 560 nm (550-580 nm) and 700 nm (reference wavelength). ----- OD

6. Calculations

$\Delta OD_{Standard} = OD_{Standard} - OD_{Blank}$, $\Delta OD_{Sample} = OD_{Sample} - OD_{Blank}$

Zinc (μg/dL) = $\Delta OD_{Sample} / \Delta OD_{Standard}$ x 200
Zinc (μM) = $\Delta OD_{Sample} / \Delta OD_{Standard}$ x 30.6

(Assay example)

	OD (560 nm)	OD (700 nm)	OD	Δ OD	Zinc (μg/dL)
DW (Blank)	0.066	0.026	0.040	-	-
Standard	0.196	0.027	0.169	0.129	-
Sample	0.113	0.028	0.085	0.045	69.8

(a) Measurement at 560 nm and 700 nm (reference wavelength):

OD = OD (560 nm) - OD (700 nm)

$\Delta OD_{Standard}$ = (0.196 - 0.027) - (0.066 - 0.026) = 0.129
ΔOD_{Sample} = (0.113 - 0.028) - (0.066 - 0.026) = 0.045

$Zinc_{Sample}$ (μg/dL) = $\Delta OD_{Sample} / \Delta OD_{Standard}$ x 200 = (0.045/0.129) x 200 = 69.8 (μg/dL)
$Zinc_{Sample}$ (μM) = $\Delta OD_{Sample} / \Delta OD_{Standard}$ x 30.6 = (0.045/0.129) x 30.6 = 10.7 (μM)

(b) Measurement at 560 nm:

$\Delta OD_{Standard}$ = 0.196 - 0.066 = 0.130
ΔOD_{Sample} = 0.113 - 0.028 - 0.066 = 0.047

$Zinc_{Sample}$ (μg/dL) = $\Delta OD_{Sample} / \Delta OD_{Standard}$ x 200 = (0.047/0.130) x 200 = 72.3 (μg/dL)
$Zinc_{Sample}$ (μM) = $\Delta OD_{Sample} / \Delta OD_{Standard}$ x 30.6 = (0.047/0.130) x 30.6 = 11.0 (μM)

7. Interferences

EDTA inhibits zinc to chromogenic system. The test is not affected by presence of Bilirubin-F and Bilirubin-C up to 40 mg/dL and chyle up to 500 FTU.

8. Quality Control

Use of control sera is recommended to monitor the quality of assay results.

9. Reference

Makino. T, Saito. M, Horiguchi. D, and Kina. K : A highly sensitive calorimetric determination of serum zinc using water-soluble pyridylazo dye. *Clinical Chimica Acta*, 120, p127-135 (1982).

10. Technical support & troubleshooting

(1) Unstableness of incubation temperature may result in unstable results.
(2) Use disposable test tube and glassware washed with 1M HNO_3 or 1M HCl, and rinse with distilled water.
(3) Accuracy to the microliter is important to obtain good results. Ensure maximum precision when pipetting.
(4) Temperature for the chromogenic reaction may affect the optical density. It may be necessary to adjust the reaction time depending on the room temperature.
(5) High concentration of proteins or lipid in cell lysate or in tissue extract may affect the observed value. Please remove them by ultrafiltration or centrifugation.
(6) Species of zinc-porphyrins cannot be analyzed using this assay kit.

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