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* STORE AT 4℃ UPON ARRIVAL

Instruction manual

Low Level Zinc（Zn）Assay Kit LS

（5-Br-PAPS Chromogenic method）

Description

This product is a direct colorimetric assay kit that does not require sample deproteinization.

At alkaline pH in a buffered medium, zinc reacts with a specific reagent to form a stable-colored complex.

The color intensity is proportional to the amount of zinc present in the sample.

Zinc is a cofactor for over 200 types of metalloenzymes and also a trace element involved in the synthesis of ribonucleic acids and proteins.

It is widely known that acute zinc deficiency during the growth stage of mammals results in severe impairment of the skin and hair, and may lead to stunted development.

Zinc is essential for cell reproduction, and an adequate supply is necessary for healthy growth.

In recent years, zinc has attracted increasing attention as a trace element in medical and nutritional research.

Kit contents

100 tests　（Catalog # : ZN10ME）

R-R　Chelate color（5-Br-PAPS）●	20 mL×1
STD　Zinc Standard　50 μg/dL ●	4 mL×1

（Catalog # : ZN11ME）=（Catalog # : ZN10ME）×2

Note

A) Instability in incubation temperature may result in unstable results.

B) Use disposable test tubes and glassware washed with 1 M HNO_3 solution and distilled water.

C) Accuracy in pipetting the volumes for samples and reagents may affect the quality of assay. Please note that samples, standards and Working Reagent must be dispensed accurately at the microliter level.

D) The temperature during the chromogen reaction may affect optical density.

E) In cell lysates or tissue extracts used as specimens, high concentrations of proteins or lipids may affect the measured values. Please remove them by ultrafiltration or centrifugation.

F) Zinc tightly bonds to macromolecules, such as porphyrins or strong chelators, is not measurable under these assay conditions. (ex. zinc-porphyrins)

G) Samples containing chelating agents (such as EDTA or citrate) are not suitable for this assay.

H) Serum, plasma, and urine samples are not compatible with this method. For such samples, please use the Zinc Assay Kit LS (ZN01ME/ZN02ME).

Operation

1. Sample preparation

◇Tissue extract, Lysate, Saliva, Other samples.

Add 6 M HCl to the sample to adjust the pH to 2.0–3.0 (e.g., 5–10 μL of 6 M HCl per 1 mL of lysate).

Centrifuge at 6,000 rpm for 15 minutes.

Collect the supernatant and use it for the assay.

Tissue:

Add 0.01 M HCl or HNO_3 solution, vortex for 1 minute, and incubate at 4–8°C for 30 minutes.

Centrifuge at 6,000 rpm for 15 minutes.

Collect the supernatant and use it for the assay.

※ Adjust the sample pH to 2.0–8.0 before proceeding with the assay.

2. Assay preparation

Bring all reagents to room temperature before use.

3. Assay procedure.

Procedure using microplate reader.

(Volume per assay sample: 240µL)

○Assay

(1) Add 40 µL of Distilled water (Blank) / STD (Standard)/ sample into each well.

(2) Add 200 µL of Chelate color (R-R) to each well and incubate at room temperature for 10 min.

(3) Read the absorbance at 560 nm (main) and 700 nm(sub). --> OD

(Sensitivity: 550nm max, 570nm 60%, 580nm 20% or less)

Add (µL)		Assay Sample Blank OD_{Bl}	Standard OD_{Std}	Sample OD_S
1	Distilled water	40	-	-
	STD	-	40	-
	Assay sample	-	-	40
2	Chelate color(R-R)	200	200	200
↓				
Mix and incubate for 10 minutes at room temperature Read the absorbance at 560 nm (main) and 700 nm(sub).				

○Calculations

$\Delta OD_{Std} = OD_{Std} - OD_{Bl}$

$\Delta OD_S = OD_S - OD_{Bl}$

Zinc (µg/dL) = $\Delta OD_S / \Delta OD_{Std}$ X 50

Zinc (µM) = $\Delta OD_S / \Delta OD_{Std}$ X 7.65

(Assay example)

	OD (560nm)	ΔOD	Zinc (µg/dL)
Blank	0.068	-	-
Standard	0.177	0.108	-
Sample	0.152	0.084	38.5

[OD = OD(560 nm)]

ΔOD_{Std} = 0.177 – 0.068 = 0.108

ΔOD_S = 0.152 – 0.084 = 0.084

$Zinc_{Sample}$ (µg/dL) = $\Delta OD_S / \Delta OD_{Std}$ x 200

= 0.084 / 0.108 x 50 = 38.5 (µg/dL)

$Zinc_{Sample}$ (µM) = $\Delta OD_S / \Delta OD_{Std}$ x 7.65

= 0.084/ 0.108 x 7.65 = 5.95 (µM)

*In diluted sample of seminal fluid, multiply the result by dilution-factor.

Performance

Measuring range: 1.0 - 60 µg/dL

Imprecision: Imprecision was evaluated using commercially available quality control.

Within run

	Mean µg/dL	S.D	C.V %
Sample	39.33	0.94	2.39

Expiration date and preservation conditions

Storage conditions: Store at 2-8°C. Don't freeze.

Expiration: 1 year from the date of manufacture. After the bottles are opened, the kit should be used in 1 month.

Reference

(1) 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).

(2) Joshua. C, Jia. H, Hirokazu. H, Besim. Ben-N, Bruce. M, Makoto. O: The Therapeutic Effect on Bone Mineral Formation from Biomimetic Zinc Containing Tricalcium Phosphate (ZnTCP) in Zinc-Deficient Osteoporotic Mice. *PLoS One,* 8(8) (2013)

Manufacturing-and-selling contractor

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*Metallogenics™ is the name of reagent kit from Cellspect Co., Ltd.