* FOR RESEARCH USE ONLY
* STORE AT 4℃ UPON ARRIVAL

Instruction manual

Zinc Assay kit LS

(5-Br-PAPS Chromogenic method)

Description

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.

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.

Kit contents

50 tests （Catalog # : ZN01ME ）

R-A Buffer 🔴	12 mL×1
R-R Chelate color (5-Br-PAPS) 🟡	0.27 mL×1
STD Zinc Standard 200 µg/dL 🟢	1.65 mL×1

(Catalog # : ZN02ME)=(Catalog # : ZN01ME) ×2

Note

A) Unstableness of incubation temperature may result in unstable results.

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

C) Accuracy in pipetting volume for samples and reagents may affect the quality of assay. Please note that samples, standards and Working Reagent must be poured accurately µL level.

D) Temperature for chromogen reaction may affect optical density. Please try to extend or shorten chromogen reaction time depending on room temperature.

E) In the cell lysate or the tissue extract use as specimen, high concentration of proteins or lipid, may affect observed value. Please remove its by ultrafiltration or centrifugation.

F) Species of zinc-porphyrins cannot be measured in this assay kit.

Operation

1. Sample preparation

◇Serum or Plasma

Insoluble substances in serum and plasma samples should be removed by filtration or centrifugation. EDTA-plasma cannot be used.

◇Tissue extract, Lysate, Other samples.

Urine (24 hour pooled urine), or other biological fluid:

Add 6M HCl to the sample and adjust pH 2.0-3.0 (e.g. 5-10µL 6M HCl/ 1mL of lysate.). Centrifuge at 6,000 rpm for 15 min. Collect the supernatant and use it for assay.

Tissue:

Add 3% TCA solution, vortex 1 min. and incubate at 4-8°C for 30 min. Centrifuge at 6,000 rpm for 15 min. Collect the supernatant and use it for assay.

* Sample pH should be between pH2 to pH8.

2. Assay preparation

(1)Bring all reagents to room temperature before use.

(2)Prepare enough working Reagent (WR).

	1 test	Example: 50 tests
R-A Buffer 🔴	230 (µL)	11.5 (mL)
R-R Chelate color 🟡	5 (µL)	250 (µL)

* WR is stored at 2-8 °C and use within one month after prepared.

3. Assay procedure.

Procedure using microplate reader.

(1 assay sample 242μL)

○Assay

(1) Add 12 μL of Distilled water (Blank) / STD (Standard)/ sample into each well.

(2) Add 230 μL of Working Reagent (WR) to each well and incubate at room temperature for 5 min.

(3) Read the absorbance at 560 nm (main) and 700nm(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	12	-	-
	STD	-	12	-
	Assay sample	-	-	12
2	WR	230	230	230
↓				
Mix and incubate for 5 minutes at room temperature Read the absorbance at 560 nm (main) and 700nm(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 200

Zinc (μM) = $\Delta OD_S/\Delta OD_{Std}$ X 30.6

(Assay example)

	OD (560nm)	OD (700nm)	OD	ΔOD	Zinc (μg/dL)
Blank	0.062	0.030	0.032	-	-
Standard	0.206	0.031	0.175	0.143	-
Sample	0.117	0.033	0.084	0.052	72.7

***Observed 560 nm with 700 nm**

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

ΔOD_{Std} = (0.206 – 0.031) – (0.062 – 0.030) = 0.143

ΔOD_S = (0.117 – 0.033) – (0.062 – 0.030) = 0.052

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

= 0.052/ 0.143 x 200 = 72.7 (μg/dL)

$Zinc_{Sample}$ (μM) = $\Delta OD_S/\Delta OD_{Std}$ x 30.6

= 0.052/ 0.143 x 30.6 = 11.1 (μM)

***Observed 560 nm only**

[OD = OD(560nm)]

ΔOD_{Std} = 0.206 – 0.062 = 0.144

ΔOD_S = 0.117 – 0.062 = 0.055

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

= 0.055/ 0.144 x 200 = 76.4 (μg/dL)

$Zinc_{Sample}$ (μM) = $\Delta OD_S/\Delta OD_{Std}$ x 30.6

= 0.055/ 0.144 x 30.6 = 11.7 (μM)

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

Performance

Measuring range 4.0 - 1,000 μg/dL

Imprecision Imprecision was evaluated using commercially available quality control serum.

Within run

	Mean μg/dL	S.D	C.V %
Level 1	68.96	3.05	4.4
Level 2	109.71	2.45	2.2

Interferences No interference by the note of substances were observed.
Conjugated bilirubin and unconjugated bilirubin 15 mg/dL
Triglyceride 500 mg/dL

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

Metallogenics Co.,Ltd. Sales Dept.
1-14-13, Fujimi, Chuo-ku, Chiba-shi, Chiba 260-0015 Japan
TEL : +81-43-227-6767
FAX : +81-43-227-6768
e-mail : sales@ak-j.com
URL: http://metallogenics.co.jp/