Instruction manual

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

Copper Assay kit LS

(3,5-DiBr-PAESA Chromogenic method)

Description

Physiological function of protein holding copper as a cofactor is a regulation of in-vivo redox status. Many of copper enzymes react directly with oxygen. 95% of copper in plasma is bonded with alpha-2-globulin, ceruloplasmin and oxidase of ferroxidase activity. Deficiency of copper causes cardiopathy, osteoporosis, osteoarthritis, Menkes syndrome, and Wilson's disease. It is widely known that copper deficiency lowers the anti-oxidant function in vivo. On the contrary, excessive dosage or consumption of copper is poisonous to the health.

This product is a direct colorimetric assay kit without deproteinization of the sample. Dissociated copper from the ceruloplasmin-copper complex by weakly acid buffer and reduced by means of reducing ascorbic acid (:Cu2+→Cu+). Cu+ ions give a blue colored complex with 3, 5-DiBr-PAESA (as chromogen). The color intensity is proportional to the amount of copper present in the sample.

Kit contents

100 tests （Catalog # : CU03ME ）

R-A	Buffer ●	24 mL×1
R-R	Chelate color (3,5-DiBr-PAESA) ●	0.5 mL×1
STD	Copper Standard 200 μg/dL ●	1.2 mL×1

(Catalog # : CU04ME)=(Catalog # : CU03ME) ×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) Heme-containing copper 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 5% 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 ●	240 (μL)	12 (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 252µL)

○Assay

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

(2) Add 240 µL of Working Reagent (WR) to each well and incubate at room temperature for 10 min.

(3) Read the absorbance at 580 nm (main) and 750nm(sub). --> OD

* Select the filter: 570-590 nm at 580nm, 700-800 nm at 750 nm.

		Assay Sample		
Add \ (µL)		Blank OD_{Bl}	Standard OD_{Std}	Sample OD_S
1	Distilled water	12	-	-
	STD	-	12	-
	Assay sample	-	-	12
2	WR	240	240	240

↓

Mix and incubate for 10 minutes at room temperature
Read the absorbance at 580 nm (main) and 750nm(sub).
(Possible ranges of wavelength for select the filter
: 570-590 nm at 580nm, 700-800 nm at 750 nm.)

○Calculations

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

Copper (µg/dL) = $\Delta OD_S/\Delta OD_{Std}$ X 200

Copper (µM) = $\Delta OD_S/\Delta OD_{Std}$ X 31.5

(Assay example)

	OD (580nm)	OD (750nm)	OD	ΔOD	Copper (µg/dL)
Blank	0.069	0.028	0.041	-	-
Standard	0.160	0.054	0.106	0.065	-
Sample	0.106	0.038	0.068	0.027	83.1

***Observed 580 nm with 750 nm**

[OD = OD(580nm) - OD(750nm)]

ΔOD_{Std} = (0.160 - 0.054) – (0.069 - 0.028) = 0.065

ΔOD_S = (0.106 - 0.038) – (0.069 - 0.028) = 0.027

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

= 0.027/ 0.065 x 200 = 83.1 (µg/dL)

$Copper_{Sample}$ (µM) = $\Delta OD_S/\Delta OD_{Std}$ x 31.5

= 0.026/ 0.064 x 31.5 = 13.1 (µM)

***Observed 580 nm only**

[OD = OD(580nm)]

ΔOD_{Std} = 0.160 – 0.069 = 0.091

ΔOD_S = 0.106 – 0.069 = 0.037

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

= 0.037/ 0.091 x 200 = 81.3 (µg/dL)

$Copper_{Sample}$ (µM) = $\Delta OD_S/\Delta OD_{Std}$ x 31.5

= 0.037/ 0.091 x 31.5= 12.8 (µM)

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

Performance

Measuring range 3.0 - 400 µg/dL

Imprecision Imprecision was evaluated using commercially available quality control serum.

Within run

	Mean µg/dL	S.D	C.V %
Level 1	76.8	2.22	2.89
Level 2	178.6	5.35	2.99

Interferences No interference by the note of substances were observed.
Conjugated bilirubin and unconjugated bilirubin 40 mg/dL
Hemoglobin 0.1 g/dL Chyle 500 FTU

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.) Abe. A, Saito. Yamashita. S, Noma. A: Sensitive, Direct Colonmetric Assay for Copper in Serum. *Clinical Chem,* 35(4), p552-554 (1989).

2.) Sakamoto. A, Terui. Y, Yamamoto. T, Kasahara. T, Nakamura. M, Tomitori. H, Yamamoto. K, Ishihama. A, Michael. A. J, Igarashi. K, Kashiwagi. K: Enhanced biofilm formation and/or cell viability by polyamines through stimulation of response regulators UvrY and CpxR in the two-component signal transducing systems, and ribosome recycling factor, *Int J Biochem Cell Biol*. 44(11), p1877-86 (2012).

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