

Instruction manual

- * FOR RESEARCH USE ONLY
- * STORE AT 4°C 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 HNO₃ 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		
		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) = $\Delta OD_S / \Delta OD_{Std} \times 200$

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

(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)]

$\Delta OD_{Std} = (0.160 - 0.054) - (0.069 - 0.028) = 0.065$

$\Delta OD_S = (0.106 - 0.038) - (0.069 - 0.028) = 0.027$

Copper_{Sample} (µg/dL) = $\Delta OD_S / \Delta OD_{Std} \times 200$
= $0.027 / 0.065 \times 200 = 83.1$ (µg/dL)

Copper_{Sample} (µM) = $\Delta OD_S / \Delta OD_{Std} \times 31.5$
= $0.026 / 0.064 \times 31.5 = 13.1$ (µM)

***Observed 580 nm only**

[OD = OD(580nm)]

$\Delta OD_{Std} = 0.160 - 0.069 = 0.091$

$\Delta OD_S = 0.106 - 0.069 = 0.037$

Copper_{Sample} (µg/dL) = $\Delta OD_S / \Delta OD_{Std} \times 200$
= $0.037 / 0.091 \times 200 = 81.3$ (µg/dL)

Copper_{Sample} (µM) = $\Delta OD_S / \Delta OD_{Std} \times 31.5$
= $0.037 / 0.091 \times 31.5 = 12.8$ (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 Colorimetric 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).

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/>