Instruction manual

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

Low level 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 copper binding protein or organic ligand by weakly acid buffer and reduced by means of reducing ascorbic acid (:$Cu^{2+} \rightarrow Cu^{+}$). 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 # : CU20ME ）

R-A	Buffer	🔴	14 mL×1
R-R	Chelate color (3,5-DiBr-PAESA)	🟡	0.5 mL×1
STD	Copper Standard 80 µg/dL	🟢	10 mL×1

(Catalog # : CU21ME)=(Catalog # : CU20ME) ×2

Note

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

B) Use disposable test tube and glassware washed with 1 M HNO_3 or 1 M 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) Copper-porphyrin complex cannot be measured in this assay kit.

Operation

Sample preparation

◇Urine

Urine sample can be used directly.

◇Tissue extract, Lysate, Other samples.

Tissue:

Add 0.1 M HCl, 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.

◇Other biological fluid:

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

* Sample pH should be between pH2 to pH8.
* Serum, plasma cannot be measured this kit. In case a serum and plasma copper quantified, recommend a Copper Assay kit LS (CU03ME/CU04ME, Metallo assay) use.

Assay preparation

A. Visual colorimetry (not use microplate reader)
→ **P.2**

B. UV/Vis spectrophotometry (96-well reader)
(1-pointend method, for colorless (clear) sample)
→ **P.3**

C. UV/Vis spectrophotometry (96-well reader)
(2-pointend method , for colored sample)
→ **P.4**

A. Visual colorimetry (not use microplate reader)

1. Assay preparation

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

(2) Prepare Copper standard solution.

Distilled water	75 (µL)
STD Copper standard ●	25 (µL)
Copper level (µg/dL)	20 (µg/dL)

(3)Prepare enough working reagent (WR).

			1 test	e.g) 100 tests
R-A	Buffer	●	140 (µL)	14 (mL)
R-R	Chelate color	●	5 (µL)	500 (µL)

*** Please use the WR immediately.**

2.Assay procedure.

(1 assay sample 240 µL)

○**Assay**

(1) Add 100 µL of Distilled water (Blank) / STD_L (Low level Copper Standard, Cu= 20 µg/dL)/ STD_H (High level Copper Standard, Cu= 80 µg/dL)/ sample into each well.

(2) Add 140 µL of Working Reagent (WR) to each well and incubate at room temperature for 10 min.

(3) Compare the color of sample with STD_L and STD_H.

* Adjust the copper standard solution as necessary.

Add \ (µL)		Assay Sample			
		Blank	STD_L	STD_H	Sample
1	Distilled water	100	-	-	-
	STD_L (Cu level 20 µg/dL)	-	100	-	-
	STD_H (Cu level 80 µg/dL)	-	-	100	-
	Assay sample	-	-	-	100
2	WR	140	140	140	140

↓

Mix and incubate for 10 minutes at room temperature.

Compare the color of sample with STD_L and STD_H.

Performance

Measuring range 10 - 80 µg/dL

Interferences No interference by the note of substances was observed.
Iron and Zinc 100 µg/dL

B. UV/Vis spectrophotometry (96-well reader)

(1-point end, for normal sample)

1. Assay preparation

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

(2) Prepare Copper standard solution.

Distilled water	75 (μL)
STD Copper standard ●	25 (μL)
Copper level (μg/dL)	20 (μg/dL)

(3)Prepare enough working reagent (WR).

			1 test	e.g) 100 tests
R-A	Buffer	●	140 (μL)	14 (mL)
R-R	Chelate color	●	5 (μL)	500 (μL)

*** Please use the WR immediately.**

2.Procedure using microplate reader.

(1 assay sample 240 μL)

○Assay

(1) Add 100 μL of Distilled water (Blank) / STD (Standard)/ sample into each well.

(2) Add 140 μL of Working Reagent (WR) to each well and incubate at room temperature for 10 min.

(3) Read the absorbance at 582 nm (main) and 750 nm (sub). --> OD

* Select the filter: 570-590 nm at 582 nm, 700-800 nm at 750 nm.

	(μL) Add	Assay Sample Blank OD_{Bl}	Standard OD_{Std}	Sample OD_S
1	Distilled water	100	-	-
	STD (Cu level 40μg/dL)	-	100	-
	Assay sample	-	-	100
2	WR	140	140	140

↓

Mix and incubate for 10 minutes at room temperature
Read the absorbance at 582 nm (main) and 750 nm (sub).
(Possible ranges of wavelength for select the filter
: 570-590 nm at 582 nm, 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 40

Copper (μM) = $\Delta OD_S / \Delta OD_{Std}$ X 6.30

(Assay example)

	OD (582nm)	OD (750nm)	OD	ΔOD	Copper (μg/dL)
Blank	0.080	0.038	0.042	-	-
Standard	0.208	0.050	0.158	0.116	-
Sample	0.092	0.038	0.054	0.012	4.1

***Observed 582 nm with 750 nm**

[OD = OD(582 nm) - OD(750 nm)]

ΔOD_{Std} = (0.208 - 0.050) – (0.080 - 0.038) = 0.116

ΔOD_S = (0.092 - 0.038) – (0.080 - 0.038) = 0.012

$Copper_{Sample}$ (μg/dL) = $\Delta OD_S / \Delta OD_{Std}$ x 40
= 0.012/ 0.116 x 40 = 4.1 (μg/dL)

$Copper_{Sample}$ (μM) = $\Delta OD_S / \Delta OD_{Std}$ x 6.30
= 0.012/ 0.116 x 6.30 = 0.65 (μM)

***Observed 582 nm only**

[OD = OD (582 nm)]

ΔOD_{Std} = 0.208 – 0.080 = 0.128

ΔOD_S = 0.092 – 0.080 = 0.012

$Copper_{Sample}$ (μg/dL) = $\Delta OD_S / \Delta OD_{Std}$ x 40
= 0.012/ 0.128 x 40 = 4.1 (μg/dL)

$Copper_{Sample}$ (μM) = $\Delta OD_S / \Delta OD_{Std}$ x 6.30
= 0.012/ 0.128 x 6.30 = 0.59 (μM)

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

Performance

Measuring range 2-80 μg/dL
(D.L = 1.0 μg/dL)

Imprecision Imprecision was evaluated using commercially available quality control urine.

Within run

	Mean (μg/dL)	S.D (μg/dL)	C.V (%)
Level 1	2.80	0.26	9.5
Level 2	2.46	0.23	9.5

Interferences No interference by the note of substances was observed.
Iron and Zinc 100 μg/dL

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C. UV/Vis spectrophotometry (96-well reader)
(2-point end, for colored sample)

1. Assay preparation

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

(2) Prepare Copper standard solution.

Distilled water	75 (μL)
STD Copper standard ●	25 (μL)
Copper level (μg/dL)	20 (μg/dL)

2.Procedure using microplate reader.
(1 assay sample 245 μL)

○**Assay**

(1) Add 100 μL of Distilled water (Blank) / STD (Standard)/ sample into each well.
(2) Add 140 μL of R-A buffer to each well and incubate at room temperature for 5 min.
(3) Read the absorbance at 582 nm (main) and 750 nm (sub). --> OD1
(4) Add 5 μL of R-R to each well.
(5) Read the absorbance at 582 nm (main) and 750 nm (sub). --> OD2
* Select the filter: 570-590 nm at 582 nm, 700-800 nm at 750 nm.

		Assay Sample		
Add (μL)		Blank OD_{Bl}	Standard OD_{Std}	Sample OD_S
1	Distilled water	100	-	-
	STD (Cu level 40 μg/dL)	-	100	-
	Assay sample	-	-	100
2	R-A buffer	140	140	140
↓ Mix and incubate for 5 minutes at room temperature. Read the absorbance at 582 nm (main) and 750 nm (sub).				
3	R-R Chelate color	5	5	5
↓ Mix and incubate for 5 minutes at room temperature. Read the absorbance at 582 nm (main) and 750 nm (sub).				

○**Calculations**

$\Delta OD_{Std} = (OD2_{Std} - OD1_{Std}) - (OD2_{Bl} - OD1_{Bl})$

$\Delta OD_S = (OD2_S - OD1_S) - (OD2_{Bl} - OD1_{Bl})$

Copper (μg/dL) $= \Delta OD_S/\Delta OD_{Std} \times 40$

Copper (μM) $= \Delta OD_S/\Delta OD_{Std} \times 6.30$

(Assay example)

	OD1 (582nm)	OD2 (582nm)	OD	ΔOD	Copper (μg/dL)
Blank	0.026	0.080	0.054	-	-
Standard	0.028	0.208	0.180	0.126	-
Sample	0.028	0.092	0.010	0.010	3.0

***Observed 582 nm**
[OD = OD2 (582 nm) – OD1 (582 nm)]

$\Delta OD_{Std} = (0.208 - 0.028) - (0.080 - 0.026) = 0.126$

$\Delta OD_S = (0.092 - 0.028) - (0.080 - 0.026) = 0.010$

$Copper_{Sample}$ (μg/dL) $= \Delta OD_S/\Delta OD_{Std} \times 40$
$= 0.010/0.126 \times 40 = 3.0$ (μg/dL)

$Copper_{Sample}$ (μM) $= \Delta OD_S/\Delta OD_{Std} \times 6.30$
$= 0.010/0.126 \times 6.30 = 0.50$ (μM)

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

Performance

Measuring range 2-80 μg/dL
(D.L = 1.0 μg/dL)

Imprecision Imprecision was evaluated using commercially available quality control urine.

Within run

	Mean (μg/dL)	S.D (μg/dL)	C.V (%)
Level 1	2.80	0.24	8.6
Level 2	2.21	0.03	1.4

Interferences No interference by the note of substances was observed.
Iron and Zinc 100 μg/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.) Wilson S. A. K., *Brain, 34, 295-309 (1912).*
2.) Osman M. A., *Patel R. B., Schuna A.,*
3.) Sundstrom W. R., Welling P.G., *Clin. Pharmacol. Ther., 33, 465-470 (1983).*
4.) Taira K., Takagawa K., Okawa M., Yoshida H., *Jpn. J. Pediatr. Med., 29, 139-144(1997).*
5.) A. Abe et al, *Clin. Chem., 35 (4), 552 (1989)*

Manufacturing-and-selling contractor

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