IM-CCU400-140718E

FOR RESEARCH USE ONLY

Catalog #CCU-400

MC Reagent Biochemical Assay Series

Copper Assay Kit

Biochemical Significance and Test Summary

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 bound with ceruloplasmin (ferroxidase). 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. Dissociated copper from the ceruloplasmin-copper complex 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.

1. Kit contents (100 tests)

R-1	Buffer (with reductant)	1 x 24 mL	Ready to use
R-2	Chelate color	1 x 0.5 mL	Ready to use
STD	200 µg/dL Cu Standard	1 x 1.2 mL	Ready to use

*Storage conditions: Store at 2-8°C. **Don't freeze**.
*Expiration: 1 year. After the vials are opened, the kit should be used within one month.
*Measuring range: 3-400 µg/dL

2. Materials required but not provided

(1) Distilled water
(2) Micropipettors and pipette tips
(3) Clear flat-bottom 96-well plate
(4) Microplate reader with 580 nm capability

3. Assay preparation

(1) Bring all reagents to room temperature before use.
(2) Prepare enough working reagent: Mix 240 µL of R-1 and 5 µL of R-2 for 1-test.
(e.g.) Preparation for 50 tests
R-1: 230 µL x 50 tests = 12.0 mL
R-2: 5 µL x 50 tests = 250 µL
Mix 12.0 mL of R-1 and 250 µL of R-2 in a vessel.

4. Sample preparation

Serum/Plasma: Insoluble substances in serum and plasma samples should be removed by filtration or centrifugation. EDTA-plasma cannot be used.
Urine (24 hour pooled urine)/Biological fluid: Add 6M HCl to the sample and adjust pH 2.0-3.0 (e.g. 5-10µL of 6M HCl/1mL of lysate). Centrifuge at 6,000 rpm for 15 minutes. Collect the supernatant and use it for assay.
Tissue: Add 5% TCA solution, vortex for 1 minute and incubate for 30 minutes at 4-8°C. Centrifuge at 6,000 rpm for 15 minutes. Collect the supernatant and use it for assay.

Note: Sample pH should be between pH 2 and pH 8.

5. Assay protocol

(1) Add 12 µL of Distilled water (Blank)/STD (Standard)/Sample into each well.
(2) Add 240 µL of Working Reagent to each well and incubate for 10 minutes at room temperature.
(3) Read the absorbance at 580 nm (570-590 nm) and 700 nm (reference wavelength). ----- OD

6. Calculations

$\Delta OD_{Standard} = OD_{Standard} - OD_{Blank}$, $\Delta OD_{Sample} = OD_{Sample} - OD_{Blank}$

Copper (μg/dL) = $\Delta OD_{Sample} / \Delta OD_{Standard} \times 200$
Copper (μM) $= \Delta OD_{Sample} / \Delta OD_{Standard} \times 31.4$

(Assay example)

	OD (580 nm)	OD (700 nm)	OD	Δ OD	Copper (μg/dL)
DW (Blank)	0.067	0.028	0.039	-	-
Standard	0.155	0.053	0.102	0.063	-
Sample	0.097	0.037	0.060	0.021	66.7

(a) Measurement at 580 nm and 700 nm (reference wavelength):

OD = OD (580 nm) – OD (700 nm)

$\Delta OD_{Standard} = (0.155 - 0.053) – (0.067 - 0.028) = 0.063$
$\Delta OD_{Sample} = (0.097 - 0.037) – (0.067 - 0.028) = 0.021$

$Copper_{Sample}$ (μg/dL) = $\Delta OD_{Sample}/\Delta OD_{Standard} \times 200 = (0.021/ 0.063) \times 200 = 66.7$ (μg/dL)
$Copper_{Sample}$ (μM) = $\Delta OD_{Sample}/\Delta OD_{Standard} \times 31.4 = (0.021/ 0.063) \times 31.4 = 10.5$ (μM)

(b) Measurement at 580 nm:

$\Delta OD_{Standard} = 0.155 - 0.067 = 0.088$
$\Delta OD_{Sample} = 0.097 - 0.067 = 0.030$

$Copper_{Sample}$ (μg/dL) = $\Delta OD_{Sample}/\Delta OD_{Standard} \times 200 = (0.030/ 0.088) \times 200 = 68.2$ (μg/dL)
$Copper_{Sample}$ (μM) = $\Delta OD_{Sample}/\Delta OD_{Standard} \times 31.4 = (0.030/ 0.088) \times 31.4 = 10.7$ (μM)

7. Interferences

EDTA inhibits copper to chromogenic system. The test is not affected by presence of bilirubin-F and bilirubin-C up to 40 mg/dL.

8. Quality Control

Use of control sera is recommended to monitor the quality of assay results.

9. 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).

10. Technical support & troubleshooting

(1) Unstableness of incubation temperature may result in unstable results.
(2) Use disposable test tube and glassware washed with 1M HNO3 or 1M HCl, and rinse with distilled water.
(3) Accuracy to the microliter is important to obtain good results. Ensure maximum precision when pipetting.
(4) Temperature for the chromogenic reaction may affect the optical density. It may be necessary to adjust the reaction time depending on the room temperature.
(5) High concentration of proteins or lipid in cell lysate or in tissue extract may affect the observed value. Please remove them by ultrafiltration or centrifugation.
(6) Species of copper-porphyrins cannot be analyzed using this assay kit.