

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 ($\text{Cu}^{2+} \rightarrow \text{Cu}^+$). 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

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

$$\text{Copper } (\mu\text{g/dL}) = \Delta OD_{\text{Sample}} / \Delta OD_{\text{Standard}} \times 200$$

$$\text{Copper } (\mu\text{M}) = \Delta OD_{\text{Sample}} / \Delta OD_{\text{Standard}} \times 31.4$$

(Assay example)

	OD (580 nm)	OD (700 nm)	OD	Δ OD	Copper ($\mu\text{g/dL}$)
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):

$$OD = OD (580 \text{ nm}) - OD (700 \text{ nm})$$

$$\Delta OD_{\text{Standard}} = (0.155 - 0.053) - (0.067 - 0.028) = 0.063$$

$$\Delta OD_{\text{Sample}} = (0.097 - 0.037) - (0.067 - 0.028) = 0.021$$

$$\text{Copper}_{\text{Sample}} (\mu\text{g/dL}) = \Delta OD_{\text{Sample}} / \Delta OD_{\text{Standard}} \times 200 = (0.021 / 0.063) \times 200 = 66.7 (\mu\text{g/dL})$$

$$\text{Copper}_{\text{Sample}} (\mu\text{M}) = \Delta OD_{\text{Sample}} / \Delta OD_{\text{Standard}} \times 31.4 = (0.021 / 0.063) \times 31.4 = 10.5 (\mu\text{M})$$

(b) Measurement at 580 nm:

$$\Delta OD_{\text{Standard}} = 0.155 - 0.067 = 0.088$$

$$\Delta OD_{\text{Sample}} = 0.097 - 0.067 = 0.030$$

$$\text{Copper}_{\text{Sample}} (\mu\text{g/dL}) = \Delta OD_{\text{Sample}} / \Delta OD_{\text{Standard}} \times 200 = (0.030 / 0.088) \times 200 = 68.2 (\mu\text{g/dL})$$

$$\text{Copper}_{\text{Sample}} (\mu\text{M}) = \Delta OD_{\text{Sample}} / \Delta OD_{\text{Standard}} \times 31.4 = (0.030 / 0.088) \times 31.4 = 10.7 (\mu\text{M})$$

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 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).

10. Technical support & troubleshooting

- (1) Unstability 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.



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(Supported languages: Japanese and English)