

PRINCIPLE:

At pH 4.70, in a buffered media, Copper is released from ceruloplasmine complex. Ascorbic acid reduces to cuprous state which reacts with the specific complexant 3-5 Di Br-PAESA, and forms a stable coloured complex. The colour intensity is proportional to the amount of copper present in the sample.

SAMPLE:

Do not use haemolyzed serum or plasma.
Use only heparin salts as anticoagulants.

REAGENTS:

1. Reagent 1
Buffer pH 4.7, additives
2. Reagent 2
Complexant 3,5 Di Br-PAESA
3. Reagent 3
Ascorbic acid
4. Standard
Copper standard
Standard concentration see on the vial label

Stored at +2 °C to +8 °C.

PREPARATION OF REAGENTS:

Reagent 1 and Reagent 3: add one small spoon of Reagent 3 into one vial of reagent 1, and mix.
Reagent is stable 15 days at 2 – 8 °C.

Working solution:

(only for analysis with monoreagent)
Mix 10 volumes of Reagent 1 + Reagent 3 with 0.5 volumes of Reagent 2.
Stability : 3 – 4 hours at room temperature or 3 weeks at – 20 °C.

PROCEDURE:

Analysis with monoreagent:

Wavelength: 582 nm (570 – 590 nm)
Cuvette: 1 cm light path
Temperature: 37 °C
Zero: reagent blank

Pipette into tubes	Blank	Standard	Sample
Distilled water	50 µl	-	-
Standard	-	50 µl	-
Sample	-	-	50 µl
Working reagent	1000 µl	1000 µl	1000 µl
Mix and stand for 4-5 min. at 37°C. The colour is stable for at least 1 hour.			

Calculation:

$$\frac{A_{\text{sample}}}{A_{\text{standard}}} \times 100 = \mu\text{gCu/dL of sample}$$

Analysis with chromogen starter:

Pipette into tubes	Reagent blank	Standard	Sample
Distilled water	50 µl	-	-
Standard	-	50 µl	-
Sample	-	-	50 µl
Reagent 1 and Reagent 3	1000 µl	1000 µl	1000 µl
Mix and read at 582 nm against Reagent blank, the absorbance of sample (A sample 1). Add:			
Reagent 2	50 µl	50 µl	50 µl
Mix and stand for 4-5 min. at 37°C and read at 582 nm against Reagent Blank the absorbance of Sample (A sample 2) and Standard (A standard). The colour is stable for at least 1 hour.			

Calculation:

$$\frac{A_{\text{sample2}} - A_{\text{sample1}}}{A_{\text{standard}}} \times 100 = \mu\text{g/dL} \times 0.157 = \mu\text{mol/L}$$

EXPECTED VALUES:

Women:	12.6-24.4 µmol/L (80 – 155 µg/dL)
Man:	11.0-22.0 µmol/L (70 – 140 µg/dL)

LINEARITY:

up to 78.6 µmol/L (500 µg/dL)

QUALITY CONTROL:

CONTRO-N	20 x 5 ml	Cat. No. 101-0083
CONTRO-P	20 x 5 ml	Cat. No. 101-0084

NOTES:

1. Use disposable test tubes and glassware washed with Hydrochloric acid 1N solution and distilled water.
2. A proportional variation of reaction volumes does not change the result.
3. If the Copper concentration in the serum > 78.65 µmol/L, dilute sample 1:2 with distilled water repeat the assay (result x 2).

REFERENCES:

1. Abe A. Yamashita S. et al Clin Chem. 552, 35 (1989)