



COPPER

Colorimetric determination of Copper in serum and plasma



ORDER INFORMATION

REF	Kit size
GD0890 00	2x25 + 2x25 ml
KL0890 00	2x20 + 2x20 ml

INDICATION

Copper is a co-factor of different enzymes such as cytochrome oxidase, tyrosinase, uricase. It is involved in iron metabolism, promoting its intestinal uptake, and its mobility from storage tissue.

A decrease level of copper is correlated to a decreased level of proteins in the serum, therefore in case of inadequate nutrition or not correct uptake (celiac disease, sprue), loss of protein through faeces or urine (nephritic syndrome), or in Wilson disease. An increase of copper level occurs in pregnancy, acute or chronic infections, surgery, myocardium infarction, iperthyroidism, and haematological diseases.

METHOD PRINCIPLE

Copper (Cu^{++}) reacts with the chromogen Di-Br-PAESA at room temperature yielding a coloured complex which intensity is proportional to the Copper concentration present in the sample.

The method does not require serum deproteinisation either sample blank.

COMPOSITION

REAGENT A:

Acetate buffer, pH 4.9 100 mmol/l
Reducing agents and preservatives

Reagent A at temperature $< 18\text{ }^{\circ}\text{C}$ forms a particulate. In this case dissolve it warming the reagent at around $25\text{ }^{\circ}\text{C}$ for 5 minutes.

REAGENT B:

3,5 Di-Br-PAESA 0.02 g/l
Preservatives

STANDARD:

Sulphate Copper 1x5 ml
200 $\mu\text{g}/\text{dl}$ as Cu^{++} ion

PREPARATION OF REAGENTS

Bireagent procedure:

The reagents are liquids ready to use.

Monoreagent procedure:

Mix 1 part of Reagent A and 1 part of Reagent B to obtain the working reagent (ex. 10 ml of RA + 10 ml of RB).

Storage and stability

Store at $2-8\text{ }^{\circ}\text{C}$. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature

Working reagent is stable for 20 days if stored at $2-8\text{ }^{\circ}\text{C}$.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- NaCl solution 9 g/l

SAMPLES

Serum, plasma with heparin, do not use chelating agents as anticoagulant or haemolysed samples.

Stable 8 days at $2-8\text{ }^{\circ}\text{C}$.

Specimen collection / Preanalytical factors

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use commercial Quality Control sera with known Copper concentration. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Allow the reagents to reach working temperature before using.

Bireagent procedure

Pipette into disposable or well clean cuvettes:

	Blank	Standard	Sample
Standard	-	100 μl	-
Sample	-	-	100 μl
Distilled H_2O	100 μl	-	-
Reagent A	750 μl	750 μl	750 μl
Mix and incubate 5 minutes at room temperature ($20-25\text{ }^{\circ}\text{C}$). Then add:			
Reagent B	750 μl	750 μl	750 μl
Mix and incubate for 10 minutes at room temperature ($20-25\text{ }^{\circ}\text{C}$). Read the absorbance (A) of standard and samples at 580 (570-590) nm against Blank. Colour is stable for 30 minutes.			

Monoreagent procedure

Pipette into disposable or well clean cuvettes:

	Blank	Standard	Sample
Standard	-	100 μl	-
Sample	-	-	100 μl
Distilled H_2O	100 μl	-	-
Working reagent	1500 μl	1500 μl	1500 μl
Mix and incubate for 10 minutes at room temperature ($20-25\text{ }^{\circ}\text{C}$). Read the absorbance (A) of standard and samples at 580 (570-590) nm against Blank. Colour is stable for 30 minutes.			

Note:

- Reaction volumes may be proportionally changed.
- It is possible to read the absorbance at 600 nm. In this case the values will be 30% lower than the ones obtained at the 570-590 nm range.

CALCULATION OF RESULTS

Utilize the following formula:

$$\text{Copper, } \mu\text{g/dl} = \frac{\text{A sample}}{\text{A standard}} \times 200$$

REFERENCE VALUES

Men:	80 ÷ 140 $\mu\text{g/dl}$
Women:	80 ÷ 155 $\mu\text{g/dl}$
Newborn:	12 ÷ 67 $\mu\text{g/dl}$
Children up to 10 years:	30 ÷ 150 $\mu\text{g/dl}$

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision

Within-run and between-run coefficients of variation have been calculated on replicates of three samples at different Copper concentrations. The obtained results are reported in the following tables:

Within-run				
Sample	n	Mean ($\mu\text{g/dl}$)	SD	%CV
Serum # 1	10	69	2.55	3.7
Serum # 2	10	189	4.54	2.4
Serum # 3	10	438	14.45	3.3

Between-run				
Sample	n	Mean ($\mu\text{g/dl}$)	SD	%CV
Serum # 1	10	68	3.01	4.4
Serum # 2	10	187	5.94	3.2
Serum # 3	10	417	11.91	2.9

Linearity

The assay is linear up to 500 $\mu\text{g/dl}$.

Sensitivity

Test sensitivity, in terms of detection limit, is 5 $\mu\text{g/dl}$.

Correlation

A study based comparing this method with atomic absorption method gave the following results:

$$y = 1.028x + 1.698 \mu\text{g/dl} \quad r = 0.936$$

Interferences

Highly lipemic sera may interfere in the assay; it is recommended to centrifuge or filter (with membranes of 0.2 μm) the sample.

Do not use haemolysed serum since haemoglobin interferes.

Bilirubin does not interfere up to 20 mg/dl.

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/ECC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of the laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

BIBLIOGRAPHY

- PASQUINELLI F., Diagnostica e Tecniche di Laboratorio, (pag.:1099-1102) Rossini Ed. (1984)
- AKITA ABE, SUMICO YIAMASHITA, Clin. Chem. 35(4): 197, 552-554 (1989)
- CIUTI R., GALLI A., Giorn. It. Chim. Clin. 12 (2): 91-100 (1987)
- CIUTI R., GALLI A., Giorn. It. Chim. Clin. 12 (2): 101-111 (1987)
- NCCLS Document, "Procedures for the collection of arterial blood specimens", Appr. Std., 3rd Ed. (1999).
- EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC