

# COPPER MONOLiquid (DiBr-PAESA)

COLORIMETRIC DETERMINATION IN SERUM AND PLASMA, WITHOUT DEPROTEINIZATION  
 Only for in vitro diagnostic use

Kit: 2 x 15 ml

Cod. COP355

## SUMMARY

Copper is present in the body in a particular area: it is a trace element associated with a large number of metalloproteins. In the biological systems Copper is in the 2 valence states and permits to metalloproteins to play a central role in the oxidation-reduction reactions. Ceruloplasmin, ascorbate oxidase, tyrosinase, lysyl oxidase, superoxide dismutase, cytochrome c oxidase and so on, are a few of the most important copper-containing enzymes: they can bind and react directly with molecular oxygen.

Several pathologies and alteration of the physiological conditions are due to modifications or to the lost of copper-enzymes: from connective tissue defects (cardiac, skeletal and vascular) to failure in pigmentation, from ataxia in motor neurons to abnormal catecholamine conversions.

After these, Copper play an important role in the Iron metabolism. Low levels may interfere in the right iron absorption and severe copper deficiency gives elevated anemia: ceruloplasmin has a strong action on ferrous iron to give ferric iron, exactly before its binding to plasma transferrin.

## PRINCIPLE

Copper dissociated from proteins, in particular conditions of ionic strength gives with Di-Br-PAESA a stable colored complex, which intensity of color is proportional at the concentration of Copper in the sample.

## REAGENTS

Components of the kit:

Cod. COP355

**\*REAGENT 1** (liquid)

2 x 15 ml

Buffer pH 4.9 >0.05 mol/L

Di-Br-PAESA >0.001 mmol/L

Reducing agents

Stabilizers

**\*REAGENT 2** Standard (liquid)

1 x 5 ml

Copper 200 µg/dL

**STABILITY:** the reagents, at 15-25°C, are stable up to the expiry date shown on the package **if not contaminated during handling.**

## AUXILIARY REAGENTS for QUALITY CONTROL (Not supplied with the kit)

To grant the correct test performances we suggest to use following kits:

- PATHOLOGICAL CONTROL

Cod. CPU

- NORMAL CONTROL

Cod. CNU

## PREPARATION OF THE WORKING REAGENT

Ready to use.

Mix kindly and let the working reagent reach the working temperature before use. **Close immediately after handling. Incompetent handling will release us from any responsibility.**

## SAMPLE

• No haemolyzed serum or heparin plasma

## PROCEDURE

- Wavelength: 580 nm (570-600 nm)
- Pathlength: 1 cm
- Temperature: 37°C
- Method: end point
- Reaction: 5 minutes
- Linearity: up to 500 µg/dL
- Sample/Reagent: 1/20

**Let the reagents reach the working temperature before use.**

Pipette in vial or cuvette so labelled :

R/B: Reagent Blank; ST: Standard; S: Sample:

	R/B	ST	S
*Reagent 1	1000 µl	1000 µl	1000 µl
Distilled water	50 µl	----	----
*Reagent 2 Standard	----	50 µl	----
Sample	----	----	50 µl

Mix carefully. Read the absorbance of the standard (Ast) and sample (As) against reagent blank, after 5 minutes at 37°C. The color is stable for 30 minutes at room temperature.

## CALCULATION

( As / Ast) x 200 = µg/dL of Copper

( As / Ast) x 31.46 = µmol/L of Copper

## REFERENCE VALUES

Men 70 - 140 µg/dL (11.0 - 22.0 µmol/L)

Women 80 - 155 µg/dL (12.6 - 24.4 µmol/L)

Babies (birth-6mths) 20 - 70 µg/dL (3.15 - 11.0 µmol/L)

It is advisable that every laboratory determines its normal reference values.

## PERFORMANCE CHARACTERISTICS

These performance characteristics was determined using a spectrophotometer or analyzers typically found in clinical laboratories, under the stated assay conditions.

**Linearity:** The Copper concentration is determined between 10-500 µg/dL.

For concentrations ≥ 500 µg/dL dilute the sample 1:2 with saline sol., repeat the determination and multiply the result x 2.

**Sensitivity:** The minimum detectable is 10 µg/dL.

## Within-run Precision:

	Mean (µg/dL) ± 2s	CV %
Serum 1	128.6 ± 17.82	6.9
Serum 2	264.0 ± 21.3	4.0

## Run-to-run (Day-to-day) Precision:

	Mean (µg/dL) ± 2s	CV %
Serum 1	120.3 ± 21.6	9.0
Serum 2	254.8 ± 27.7	5.4

**Interferences:** See References point 2.

**Correlation:** A group of 20 samples was assayed by this procedure and using a similar commercially available Copper Reagent on Cobas MIRA. Comparison of the data gave following results:

Linear regression Y = 1.022X + 6.25  
 Correlation coefficient r = 0.9924 n = 20



## NOTE

1. A proportional variation of the reaction volumes does not change the result.
2. We suggest do not mix Reagents from different Production lots.
3. For concentration of Copper higher than 500 µg/dL dilute the sample 1:2 with saline solution, repeat the determination and multiply x2.
4. PAY ATTENTION!  
Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.
5. Very deep attention must be given to interfering substances: certain drugs and other substances are able to influence levels of Copper (see References 2.).
6. The reagent must be used only for the intended destinations, by expert people and in the due lab. conditions.
7. The clinical diagnosis cannot be done using the result of only one test, but have to be done integrating different lab. and clinical data.
8. To avoid interference or contaminations, use plastic material throwaway or very clean tubes washed with diluted HCl and distilled water.

## REFERENCES

1. Textbook of Clinical Chemistry, Ed. by N.W. Tietz, W.B. Saunders Co., Philadelphia (1999).
2. Young D.S., Effect of drugs on Clinical Lab. Test, 5<sup>th</sup> Ed. AACC Press (2000).
3. Abe A. et al., Clin. Chem., 35, 552 (1989).

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