

COPPER

[Summary and Explanation]

Copper is a very important trace element for human health due to its key roles in biological processes such as energy and iron metabolism, defense against antioxidants, synthesis of neuropeptides, and immune system functions. In case of copper deficiency, significant symptoms such as neutropenia, anemia and abnormalities in bone structure are observed, while hepatocerebral and neurodegenerative findings are seen in toxicity.

[Principle of Assay]

Copper found in the samples change the red-orange color of DiBr-PAESA to violet under acidic conditions. The change of absorbance at 572 nm is proportional to total copper concentration in the sample. The assay can be calibrated with copper sulfate dissolved in deionized water.

[Reagent Composition]

Reagent 1: Buffer Solution
Surfactants

Reagent 2: Chromogen Solution
DiBr-PAESA
Surfactants
Preservatives

[Intended Use]

This product can be used for quantitative *in vitro* determination of total copper from human serum, plasma, urine and semen.

[Stability and Preparation of Reagents]

R1 Buffer

Contents are ready to use, no preparation is required. Stable up to the expiry date when stored at 2-8°C after opened.

R2 Chromogen solution

Contents are ready to use, no preparation is required. Stable up to the expiry date when stored at 2-8°C after opened.

CAL and QC Standard and levels

Contents are ready to use, no preparation is required. Stable up to the expiry date when stored at 2-8°C after opened. Any copper calibrator is applicable, however, Rel Assay calibrator and controls are recommended.

[Performance Characteristics]

Low linearity: 3 µg/dl

High linearity: 600 µg/dl

If the results obtained were greater than linearity limit, dilute the sample 1/10 with deionized water and multiply the result by 10.

[Accuracy]

Results obtained with Rel Assay Copper Measurement Kit and atomic absorption spectrometry, the gold standard method, are correlated.

The results obtained using 173 samples were the following:

Correlation coefficient (r): 0.97.

Regression equation: $0,97x + 2,41$

The results of the performance characteristics depend on the analyzer used.

[Precision]

Intra-assay CV was determined with 30 replicates of 2 controls. The %CV result was 1.85 and inter-assay %CV result was 2.74.

[Normal range]

Adult Serum: 70-160 µg/dl

Newborn Serum: 20-70 µg/dl

Urine: 0-40 µg/24h

Pregnancy: 110-312 µg/dl

Each laboratory is recommended to establish their own reference values.

[Handling method]

1. This product is designed to be used in the spectrophotometer, plate reader or automated biochemistry analyzer.

2. Could be used in various automated biochemistry analyzers, an example of operating application is shown below;

Sample	R1	R2	M.wav	S.wav
17µl	160µl	25µl	572 nm	None

3. R2 is added to the mixture two minutes after R1 and sample are mixed. Measurement is done at the end of five minutes.

Make sure that cuvettes of the auto-analyzer or plate reader are thoroughly cleaned before the assay to obtain accurate and sensitive results. Use Reagent Grade 1 water for the assay.

[Components]

All reagents are ready to use

	Content	Amount
R1	Buffer	50 ml
R2	Chromogen	10 ml
Standard	508 µg/dl Zn ⁺⁺	4 ml
Level 1	32 µg/dl Zn ⁺⁺ (24-40)	4 ml
Level 2	320µg/dl Zn ⁺⁺ (255-385)	4 ml

[Storage and Validity Date]

Storage: The assay kit should be stored at 2-8°C.

Validity date: The validity of each reagent is indicated on the bottle and on the kit box.

(The kit is stable for up to expiry date when stored at 2-8°C after opened.)

[Sample]

Blood serum, plasma and urine could be used as sample.

Dilute semen samples with a ratio of 1/10 using 320 mOsm pH 7.4 phosphate buffer before evaluation.

Serum samples are stable up to 1 week stored at 4°C, 2 months at -20°C and 6 months at -80°C.

Collect samples in collection tubes produced for trace element testing. Do not use tubes containing separation gel.

[Procedure and Calculation]

Wavelength	572
Temperature	37 °C

Pipette into cuvette

	Samples	Standards (CAL, LEVELS, H ₂ O)
CAL, LEVELS, H ₂ O	-----	51
Sample	51	-----
Reagent 1	480	480
Reagent 2	75	75

Mix well

Mix, read the absorbance of sample, standards, control levels and water. Absorbance obtained from water is extracted from all samples, standard and control levels' absorbance. Extracted standard absorbance is accepted as 508 µg/dl. Calculate the factor and multiply the absorbance obtained from samples with factor to calculate the result. Level 1 is expected as 32±8 µg/dl and level 2 is expected as 320±66 µg/dl.

[Interferences]

No interferences were observed with bilirubin up to 18 mg/dL, hemoglobin up to 0.5 g/dL and triglycerides up to 1000 mg/dL.

EDTA interfere with the results.

Our studies have shown that there is no interference with other metal ions such as zinc, iron, cobalt, magnesium and aluminum.

A list of drugs and other interfering substances with copper determination has been reported by Young et. al (6).

[Recovery]

Serum pool was prepared and copper concentration was measured. Then copper concentrations were re-measured following addition of copper sulfate in ratios of 1/10 and 1/100 into the same serum pool. As a result, recovery was found in between %95 and %105 range.

[Safety precautions and warning]

1. For *in vitro* diagnostic use only.
2. Do not pipette by mouth.
3. Exercise the normal precautions required for handling laboratory reagents.
4. Wear disposable gloves while handling the kit reagents and wash hands thoroughly afterwards.
5. Do not use reagents beyond the expiry date.
6. The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.
7. Dispose cleaning liquid and also such used washing cloth or tissue paper with care, as they may also contain infectious agents.
8. Health and safety data sheets are available on request.

[References]

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