

Copper Reagent

2 Part Liquid

PRODUCT SUMMARY

Stability	:	Until Expiry at 18-25°C
Linear Range	:	3-85 µmol/L (19-540 µg/dL)
Specimen Type	:	Serum
Method	:	Endpoint
Reagent Preparation	:	Mix equal quantities of Reagent 1 and Reagent 2.

IVD

INTENDED USE

This reagent is intended for the in vitro quantitative determination of Copper in serum on automated clinical chemistry analyzers.

CLINICAL SIGNIFICANCE¹

Copper is an essential trace element found predominantly bound to the copper transport protein caeruloplasmin, while a very small proportion is complexed with albumin and other metalloproteins.

The most significant clinical application of copper determination is in the diagnosis of Wilson's disease. This is associated with a decrease in the synthesis of caeruloplasmin, which results in low serum copper levels.

A second disorder of copper metabolism is Menkes' syndrome or kinky hair syndrome, an X-linked genetic defect in copper absorption.

Low serum copper levels have also been observed in a number of hypoproteinemias, while increased levels are found in a number of acute and chronic diseases such as leukemia, hemochromatosis and biliary cirrhosis.

METHODOLOGY^{2,3}

Copper bound to caeruloplasmin is released by the reducing agent Guanidine hydrochloride in an acidic medium. 2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino) aniline (5-Br-PSAA) reacts with the free copper to form a stable colored complex. The intensity of this color is proportional to the copper concentration in the sample and is measured photometrically at 580 nm.

REAGENT COMPOSITION

Active Ingredients

Reagent 1:

	Concentration
Acetate buffer, pH 4.2	0.4 mol/L
Guanidine HCl	5 mol/L
Catalyst	
Oxidisable agent	

Reagent 2:

5-Br-PSAA, sodium salt	0.1 mmol/L
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WARNING: Avoid ingestion and contact with skin, mouth and eyes. The toxicity of this reagent has not been determined. Flush with plenty of water when disposing. For further information, consult the Copper Reagent Material Safety Data Sheet.

R22	Harmful if swallowed.
R36/38	Irritating to eyes and skin.
S23	Do not breathe vapour.

REAGENT PREPARATION

Prepare the working reagent by mixing equal quantities of Reagent 1 and Reagent 2. Refer to specific instrument applications, available upon request from the Technical support group.

STABILITY AND STORAGE

Prior to Use:

When stored between 18-25°C, the reagents are stable until the expiration date stated on the bottle and kit box labels.

Working Reagent:

Working reagent is stable for at least 5 days at 2-8°C.

Indications of Reagent Deterioration:

- Turbidity;
- Reagent absorbance > 0.2 AU at 580 nm; and/or
- Failure to recover control values within the assigned ranges.

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorized Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		Manufactured by
	Consult instructions for use	REAG 1	Reagent 1
REAG 1	Reagent 1	REAG 2	Reagent 2
	Xn - Harmful		

SPECIMEN COLLECTION AND HANDLING³

Serum: Use non-hemolyzed serum.

Storage: Serum copper samples are stable for at least 2 days at room temperature (18-25°C) or for 1 week at 2-8°C.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance between 550 and 590nm.
- If required, pipettes for accurately dispensing measured volumes.
- Analyser specific consumables, e.g. sample cups.
- Assayed Normal and Abnormal control material.
- A suitable aqueous Copper standard.

ASSAY PROCEDURE

The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	37°C
Primary Wavelength	580 nm (550 - 590nm)
Assay Type	End Point
Direction	Increase
Sample:Reagent ratio	1:10
e.g. Sample vol	0.1 mL
Combined Reagent vol	1.0 mL
Incubation Time	300 seconds
Reagent Blank Limits	Low 0.10 AU
(580nm, 1cm lightpath)	High 0.20 AU
Linearity	3 - 85 µmol/L (19 - 540 µg/dL)
Sensitivity	4.1 ΔmAbs per µmol/L
(550 nm, 1 cm lightpath)	0.7 ΔmAbs per µg/dL

CALCULATIONS

Results are calculated, usually automatically by the instrument, as follows:-

$$\text{Copper} = \frac{\text{Absorbance of Unknown}}{\text{Absorbance of Calibrator}} \times \text{Calibrator Value}$$

Example:

Absorbance of calibrator	=	0.081
Absorbance of unknown	=	0.136
Value of calibrator	=	15.73 µmol/L (100 µg/dL)

$$\text{Copper} = \frac{0.136}{0.081} \times 15.73 = 26.4 \mu\text{mol/L}$$

$$\text{Copper} = \frac{0.136}{0.081} \times 100 = 168 \mu\text{g/dL}$$

NOTES

1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. Specimens with copper values above 85 µmol/L (540 µg/dL) should be diluted with isotonic saline and reassayed. Multiply results by the dilution factor.
3. The color reaction is stable for at least 10 minutes at 37°C.
4. Unit Conversion: µmol/L x 6.355 = µg/dL

CALIBRATION

Calibration is required. A suitable aqueous copper standard is recommended. For calibration frequency on automated instruments, refer to the instrument manufacturer's specifications. However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert. Recalibration is recommended at anytime if one of the following events occurs:-

- The Lot number of reagent changes
- Preventative maintenance is performed or a critical component is replaced
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-

- At least every eight hours
- When new bottles of reagent are used
- After preventative maintenance is performed or a critical component is replaced
- With every calibration

Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control. The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh standard, then repeat the test.
- If results are still out of control, perform a calibration with fresh reagent, then repeat the test.
- If results remain out of control contact Technical Services or your local distributor.

LIMITATIONS

1. Studies to determine the level of interference from iron and zinc were carried out. The following results were obtained:-
Iron: No interference from iron up to 100 µmol/L.
Zinc: No interference from zinc up to 100 µmol/L.
2. Avoid lipaemic, haemolysed and icteric samples.
3. Young DS⁴ has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES¹

Males 11.0 - 22.0 µmol/L (70 - 140 µg/dL)
Females 12.6 - 24.4 µmol/L (80 - 155 µg/dL)

The quoted values are representative of the expected range for this method and should serve as a guide only. It is recommended that each laboratory verify this range or derive a reference interval for the population that it serves.⁵

PERFORMANCE DATA

The following data was obtained using the Copper reagent on a well maintained automated clinical chemistry analyser. Users should establish product performance on their specific analyser used.

IMPRECISION

Imprecision was evaluated using two levels of commercial control and following the NCCLS EP5-T procedure.⁶

Within run:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (µmol/L / µg/dL)	16.25 / 103.27	31.50 / 200.00
SD (µmol/L / µg/dL)	0.46 / 2.92	0.56 / 3.56
CV (%)	2.83	1.78

Total:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (µmol/L / µg/dL)	16.25 / 103.27	31.50 / 200.00
SD (µmol/L / µg/dL)	0.80 / 5.08	1.03 / 6.55
CV (%)	4.92	3.27

ACCURACY

Comparison studies were also carried out using a similar commercially available Copper reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained:-

Number of sample pairs	50
Range of sample results	5 - 35 µmol/L (32 - 222 µg/dL)
Slope	0.95
Intercept	-0.43 µmol/L (2.7 µg/dL)
Correlation coefficient	0.997

Comparison studies were also carried out using AAS as a reference. The following statistics were obtained:-

Number of sample pairs	60
Range of sample results	3.5 - 35.0 µmol/L (22 - 222 µg/dL)
Slope	0.96
Intercept	1.15 µmol/L (7.3 µg/dL)
Correlation coefficient	0.97

LINEARITY

When run as recommended, the assay is linear between 3 and 85 µmol/L (19 and 540 µg/dL).


Linearity on various automated instruments may vary from this value. The user should consult the specific instrument application for the instrument specific linearity claim.

SENSITIVITY:

When run as recommended, the sensitivity of this assay is 4.1 ΔmAbs per µmol/L or approximately 0.7 ΔmAbs per µg/dL (1cm light path, 550 nm).

REFERENCES

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6. National Committee for Clinical Chemistry Standards. User evaluation of Precision Performance of Clinical Laboratory Devices NCCLS; 1984, NCCLS Publication EP5-T.

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REF	Reorder Information	
Catalogue No.	REAG 1	REAG 2
TR61001	1 x 50 mL	1 x 50 mL