Pariksha Biotech
A game changer in IVD

Urine Copper Estimation Kit

High-Q Urine Copper-ML

(Di Brom-PAESA)

HIGH®
Command on Quality

Intended Use:
Kit for the in vitro quantitative determination of Copper in Urine on automated clinical chemistry analyzers.

Clinical Significance:

Copper is an essential trace element found predominantly bound to the copper transport protein ceruloplasmin, while a very small proportion is complexed with albumin and other metallo proteins.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 ceruloplasmin, 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 hypo proteinemias, while increased levels are found in a number of acute and chronic diseases such as leukemia, hemo chromatosis and biliary cirrhosis.

Urine copper estimation is of great importance. The Copper Urine Test can be done on either a single random sample or a 24-hour specimen. Copper is a mineral which plays a role in a number of bodily processes. Copper is found in a number of foods such as nuts, chocolate, shellfish, grains, and liver. Healthy copper levels contribute to energy production and nervous system function. Most copper in the body is bound to the enzyme Ceruloplasmin. Copper deficiency is rare but can be caused by malabsorption disorders such as Celiac and Cystic Fibrosis. Wilson Disease, an inherited disorder, can cause the liver to store excess copper which can lead to toxicity. Copper exposure can also come from environmental pollution, industrial settings, or drinking water transported by aging copper pipes. Symptoms of copper toxicity include nausea, fatigue, uncontrolled shaking, loss of coordination, jaundice, and involuntary muscle contractions.

A copper urine test is usually ordered to aid in the diagnosis of Wilson Disease. It can also be ordered when a person is suspected of having copper toxicity or deficiency. Urine copper testing is typically done as a followup to irregular results on a Copper Blood Test.

Methodology:
Copper bound to ceruloplasmin is released by the reducing agent Guanidine hydrochloride in an acidic medium. Dibromo-PAESA (4-(3,5-dibromo-2-pyridylazo)-N-ethyl-N-sulfopropylaniline) 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 578 nm.

Reagent Composition	Active Ingredients	Concentration
	Acetate buffer, pH 4.2	0.4 mol/L
	Guanidine HCl	5 mol/L
	Catalyst	
	Oxidisable agent	
	Dibromo-PAESA	0.1 mmol/L

Reagent Preparation:
Reagent is available as Mono Reagent and does not need any reconstitution.
Reagent is stable till the expiry date when properly stored at 2-8°C

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.

Specimen collection and Handling
Urine:
Random Urine without preservative. Centrifuge the Urine before testing to remove all the precipitates and extraneous materials. Use the clean supernatant as a specimen

24 Hrs Urine collected in a container with or without preservative. Centrifuge the Urine before testing to remove all the precipitates and extraneous materials. Use the clean supernatant as a specimen

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

System Parameters

Assay Type	End Point
Temperature	37°C
Primary Wavelength	578 nm
Blank	Distilled Water Blank
Reagent vol	500 µL
Urine Sample vol	500 µL
Calibrator Concentration	On the Label (Lot Specific)
Incubation Time	5 Minutes
Units	µg/dL
Linearity	20

Procedure:

	Calibrator	Test
Urine Copper Reagent	**500 µL**	**500 µL**
Urine Copper Calibrator	**500 µl**	**-----**
Random Urine or 24 Hr Urine Sample	**-----**	**500 µl**

Mix well and incubate for 5 minutes at 37°C

Read absorbance of Calibrator (C) and Test (T) against Distilled Water Blank (B) at 578 nm

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

$$\text{Urine Copper} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Calibrator}} \times \text{Calibrator Value (Batch Specific \& On the label)}$$

Calculation of Urine Copper µg / 24 hrs: Procedure:

1. **Measure and record 24 hours urine volume in liters before testing..**
2. **Determine the Urine Copper concentration in µg/dL using High-Q Urine Copper Reagent Kit**
3. **Convert the Urine Copper Concentration from µg/dL to µg/L by multiplying with factor "10".**
4. **Multiply the Urine Copper Concentration (µg/L) with 24 hrs. Urine Volume.**

Calculation of Urine Copper µg / 24 hrs:

Urine Copper Concentration (µg/dL) X 10 X volume of 24 hrs urine collected in litres.

Example:

24 hours urine volume = 2.5 L

Urine Copper Conc. Determined by High-Q Urine Copper Kit = 3.0 µg/dL

Total Copper excreted in urine/24 hours based on the above formula =3.0 x 10 x 2.5 = 75 µg/24 Hrs

Above example falls in abnormal range (Pathological and indicates disease).

Notes:
1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. Specimens with copper values above 20 µg/dL should be diluted with distilled water and re assayed. Multiply results by the dilution factor.
3. The color reaction is stable for at least 10 minutes at 37°C.

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Reference Range:

Random Urine	**Up to 2.5 µg/dL**
24 Hrs Urine	**Up to 60.0 µg/24 Hrs**

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.

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 analyzers 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 20and 600 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, 578 nm).

References:

1. Tietz NW (Ed). "Textbook of Clinical Chemistry" WB Saunders, 1986; 929-933.
2. Makino T., Clin Chim Acta, 1989; 185:7-16.

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.

Ordering information

Ref./Cat.	**Pack Size**	**Presentation**
P-COP(U) - 25	**25 ml**	**Mono Reagent with Calibrator**
P-COP(U) - 50	**50 ml**	

Product Features

- **Mono Reagent with Calibrator**
- **5 Minutes End Point Reaction**
- **Linearity Up to 20 µg/dL**
- **Measuring Wavelength 578 nms**
- **No cross reactivity with other metals**
- **Copper specific Dye (Di Brom-PAESA) is used**
- **Results correlate with Atomic Absorption Spectrometry**

Symbols used with IVD devices

	Date of manufacture		Manufactured by
IVD	In vitro diagnostic device		Keep away from sunlight
	Do not freeze		This way up
	Use by (yyyy-mm-dd or mm/yyyy)	R	Reagent
CAL	Calibrator Material	LOT	Batch code
2°C 8°C	Temperature limitation (store at)	CONTROL	Control
	Consult instructions for use		Keep dry Keep away from rain
REF	Catalog Number		

eIFU Indicator

Pariksha's world inside
SCAN TO EXPLORE MORE

Manufactured in India by :
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