CATACHEM

COPPER REAGENT KIT
V655-16
For Veterinary Use Only.

Contents	Product No.	Package
Copper Reagent Kit	**V655-16**	
Copper Reagent	V655-01	3 x 13 mL
Catacal Calibrator	C1200-10	1 x 5 mL

CALIBRATOR INFORMATION

Lot: XXXXXXXX Concentration: XXX ug/dL Exp: XXXX-XX-XX

Instructions for Use:

1. Open vial carefully to avoid any loss of lyophilized material.
2. Use a Class A volumetric pipette to add exactly 5 mL of distilled or deionized water (at 15-25°C) to the vial.
3. Replace stopper and let stand out of bright light for 30 minutes before use, swirling gently several times to ensure contents are dissolved.
4. Prior to use, mix the contents gently by inversion. Do not shake as the formation of foam should be avoided.
5. Proceed with sampling as directed by the instrument application.
6. Store unused material refrigerated at 2-8°C, tightly stoppered.
7. Be sure to thoroughly mix contents prior to each use by gently swirling.

Storage and Stability:

Catachem Catacal™, when unopened and stored at 2-8°C, is stable until the expiration date stated on the vial label. Prolonged exposure to light should be avoided. When opened and reconstituted, Catachem Catacal™ is stable for 7 days at 2-8°C. Reconstituted Catachem Catacal™ may also be frozen once, for up to 28 days at -20°C. Gently mix well after thawing, avoiding the formation of foam.

REAGENT PREPARATION

The Catachem Copper reagent is packaged ready for use.
Mix gently but thoroughly by inversion before use.

REAGENT STORAGE AND STABILITY

Store the unopened reagent kit at 2-8°C. When stored as directed, the unopened reagent is stable until the expiration date stated on the label.

NOT FOR USE IN UNPROFESSIONAL SETTINGS

FOR TECHNICAL ASSISTANCE:
Email: catachem@catacheminc.com
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MANUAL/AUTOMATED APPLICATION

Intended Use
For **In Vitro Diagnostic** use in the automated, quantitative determination of copper.

Clinical Significance [1,2]
Copper is one of the essential trace elements, stored mainly in the liver and released from the liver to maintain blood concentrations essential to normal physiological function. The most significant application of copper determination is in the diagnosis of hepatolenticular degeneration (Wilson/s disease). The concentration of copper is generally decreased because of low levels of the ferroxidase enzyme seruloplasmin. Increased serum copper levels (Hypercupremia) are found in a number of acute and chronic diseases, such as malignancies, leukemia and others.

Method Principle [3]
Under acid conditions protein unraveling agents free the copper ions. The dissociated copper ions then engage in a subsequent reaction with 4-(3,5-dibromo-2-pyridylazo)-N-ethyl-N-sulfopropylaniline (DiBr-PAESA) to produce a colored chelate complex with maximum absorption at 580nm. The intensity of the color thus produced is directly proportional to the Copper ions in the serum sample. The reaction scheme below depicts the steps that occur in this assay method.

$$Cu^{++} + 2\text{DiBr-Paesa} \longrightarrow Cu^{++}\text{DiBr-Paesa Complex}$$

REAGENTS

Copper Color Reagent
Each liter contains:

Buffer	
DiBr-PAESA	0.025 mmol
Stabilizer and nonreactive ingredients	

Precautions
Handle these reagents using good laboratory practice. **DO NOT PIPETTE REAGENT BY MOUTH.** Avoid contact with skin and eyes. If contact occurs, wash affected area with plenty of cold water. Clean spills immediately.

Reagent Storage And Stability
Store the Copper Reagent at 2-8°C. When stored as directed the reagent is stable until expiration date stated on the label. The Catachem Copper Reagents have been tested to reflect shipping conditions and is stable for the lifespan of the product if frozen up to 5 times or reaching temperatures up to 40°C for up to one week.

Working Reagent Preparation
The Copper reagent is packaged ready for use. No preparation is required. Once opened the Working Copper Reagent is stable until the expiration date indicated on the label when stored capped at 2-8°C.

Reagent Indications of Deterioration
- Turbidity
- Quality control values out of assigned ranges

If these reagent characteristics are observed call Catachem's technical representative.

Specimen Collection And Stability
Clear non-hemolyzed sera are the specimens of choice. Serum should be separated immediately from the clot and analyzed promptly or stored at 2-8°C. Copper in serum is stable 7 days at room temperature, 10 days refrigerated at 2-8°C and for many months frozen at -20°C. [2]

Procedure
These instructions are outlined for performing the Catachem Copper assay using an automated procedure.

Materials Provided
Catachem Copper Reagent

Materials Required But Not Provided
- Automated analyzer
- Catachem Quality control material with assigned Copper values

Calibration
Catachem Catacal Calibrator, which contains a known Copper value, is recommended.

Calibration Schedule
Calibrate the Catachem Copper method each time the procedure is performed.

Quality Control
To monitor the quality performance of the procedure used, Catachem Catatrol Level I and Catatrol Level II with assigned Copper values should be included in the assay procedure as follows:

- Each time the Copper procedure is performed
- Replacement of reagent lot numbers
- Analyzer service/ maintenance

Directions For Use
The Catachem Copper method requires a single Working Reagent that comes ready for use.

Procedure
Program the CataChemwell-T analyzer for Catachem's Copper method as follows:

COPPER CATACHEMWELL-T APPLICATION SPECIFIC TEST PARAMETERS

Assay Name	**Copper**	**Units**	**µg/dL**
Type	**Chemistry**	**No. of Decimals**	**1**
Temp.	**37°C**	**Normal Range**	**70-155**

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Mode	One by One	Linearity	0-500
Assay Steps:		Reaction Type	+
Reagent 1 Vol µl	250	Standards:	
Incubation Sec.	120	#1	*
Read:		#2	**
Primary WL	580 nm	#3	
Secondary WL	630 nm	#4	
Sample Vol. µl	15		
Incubation Sec.	180	Blank Used	No
Read:			
Primary WL	580 nm	Controls:	
Secondary WL	630 nm	#1	***
		#2	***
Clean Probe		#3	
Rinse Probe		#4	

*** Use Deionized Water or Saline**
**** Enter value from Catachem Calibrator Package insert**
*****Enter value from Catachem** Copper **Control I and II Package Insert.**

Procedure Limitations
Samples with copper values greater than 500 µg/dL should be diluted 1:2 with distilled or deionized water and reassayed. Multiply results obtained by 2 to adjust for the sample dilution.

Normal Values
Adult Men	70-140 µg/dL
Adult Women	80-155 µg/dL

The values given here are only to be used as a guideline. It is recommended that each laboratory establish the normal range for the species under test and for the area in which it is located.

Interfering Substances
Certain substances and certain drugs are also known to influence the Copper values. A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young et al [5].

Method Performance Characteristics
Sensitivity: TBD
Linearity: In this procedure there is no significant nonlinearity over the range of 5-500 ug/dL; (78.5 umol/L)

Precision: Precision data was obtained using three levels of protein based controls and following the NCCLS EP5-T2 procedure [3]. The following results were observed.

Precision
Copper	Within-Run Precision		Total Precision	
Mean	SD	CV	SD	CV
µg/dL	µg/dL	%	µg/dL	%
50.58	2.97	5.88	3.43	6.78
97.60	2.34	2.40	3.25	3.33
165.44	2.50	1.52	2.96	1.79

ACCURACY
Correlation studies were carried out between this automated **Copper** method (Y) and a reference Atomic Absorption method (X). Serum samples were assayed and the results compared by the least squares regression. The following statistics were observed:

N	= 30
Range	= 37.5 – 184.9 µg/dL
Mean Y	= 105.0
Mean X	= 104.9
Y	= 0.971x + 0.32
Sy.x	= 0.995
r	= 0.9847

Bibliography

1. Fundamentals of Clinical Chemistry. Edited by Norbert Tietz 2nd. Ed. Philadelphia: W.B. Saunders; 1976.
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3. Akira Abe, Sumiko Yamashita, Aldo Noma. Sensitive, Direct Colorimetric assay for copper in serum. Clin Chem. 35/4 552-554 (1989)
4. Evaluation of Precision Performance of Clinical Chemistry Devices. Second Edition. NCCLS Document EP5-T2. Vol. 12, No. 4
5. Young D.S., Pestamer L.C., Gibberman V. Effects of drugs on clinical laboratory tests. Clin. Chem. 21, No. 5, 1975

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