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User's Manual and Instructions

Copper Assay Kit (Z5030024)

Quantitative Colorimetric Copper Determination at 359nm

DESCRIPTION

Copper is an essential trace element. Copper-containing enzymes play important roles in iron and catecholamine metabolism, free radical scavenging, and in the synthesis of hemoglobin, elastin and collagen. Copper is mainly present in caeruloplasmin in the liver. Low levels of copper have been associated with mental retardation, depigmentation, anaemia, hypotonia and scorbutic changes in bone. Levels of copper are key diagnostic indicator of diseases such as Wilson's disease, microcytic hypochromic anaemia and bone disease due to reduced collagen synthesis.

Simple, direct and automation-ready procedures for measuring copper concentrations find wide applications in research, drug discovery and environmental monitoring. BioChain's copper assay kit is designed to measure copper with no or minimal sample treatment. The improved method utilizes a chromogen that forms a colored complex specifically with copper ions. The intensity of the color, measured at 359nm, is directly proportional to copper concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples.

KEY FEATURES

Sensitive and accurate. Linear detection range 7 μg/dL (1.0 μM) to 300 μg/dL (47 μM) copper in 96-well plate assay.
Simple and high-throughput. The simple procedure can be readily automated as a high-throughput assay in 96-well plates for thousands of samples per day.
Improved reagent stability and versatility. The optimized formulation has greatly enhanced reagent and signal stability. Cuvet or 96-well plate assay.

APPLICATIONS

Direct Assays: biological, environmental, food and beverage samples.
Drug Discovery/Pharmacology: effects of drugs on Cu metabolism.

KIT CONTENTS (250 tests in 96-well plates)

Reagent A: 10 mL Reagent B: 1.5 mL Reagent C: 40 mL
Copper Standard: 1 mL 1.5 mg/dL Cu^{2+}

Storage conditions. The kit is shipped at room temperature. Store all reagents at 4 °C. Shelf life: 12 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Note: metal chelators (e.g. EDTA) interfere with this assay and should be avoided in sample preparation.

Procedure using 96-well plate:

1. Standards: transfer 100 μL dH_2O into one Eppendorf tube labeled "Blank". Into another tube labeled "Standard", mix 20 μL 1.5 mg/dL Standard and 80 μL dH_2O (final 300 μg/dL Cu^{2+}).

   Samples: transfer 100 μL samples into separate tubes.

   Add 35 μL Reagent A (trichloroacetic acid) to each tube and mix by vortexing. If samples contain protein (e.g. serum/plasma), precipitates form. Centrifuge tubes for 2 min at 14,000 rpm and use clear supernatant for assay. For samples that do not contain protein, the mixture remains clear and centrifugation is not necessary.

   Transfer 100 μL Blank, Standard and Sample into separate wells of a clear flat-bottom 96-well plate.

2. For each assay well, prepare Working Reagent by mixing 5 μL Reagent B and 150 μL Reagent C. Transfer 150 μL Working Reagent to each well and tap plate to mix thoroughly.

3. Incubate 5 min at room temperature and read optical density at 356-362nm (peak absorbance at 359nm).

Note: if sample OD values are higher than the OD value for the 300μg/dL Standard, dilute sample in dH_2O and repeat assay. Multiply the results by the dilution factor.

Procedure using cuvette:

Prepare standards and samples as for 96-well assay procedure.
1. Transfer 400 μL Standards and Samples into separate cuvets.
2. Add 600 μL Working Reagent. Mix by pipetting.
3. Incubate 5 min at room temperature and read optical density at 356-362nm (peak absorbance at 359nm).

CALCULATION

The copper concentration of Sample is calculated as

$$= \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{STANDARD} - OD_{BLANK}} \times 300 \ (\mu g/dL)$$

OD_{SAMPLE}, OD_{BLANK} and $OD_{STANDARD}$ are optical density values of the Sample, Blank and the 300 μg/dL Standard, respectively.
Conversions: 100 μg/dL Cu equals 15.5 μM, 0.0001% or 1 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

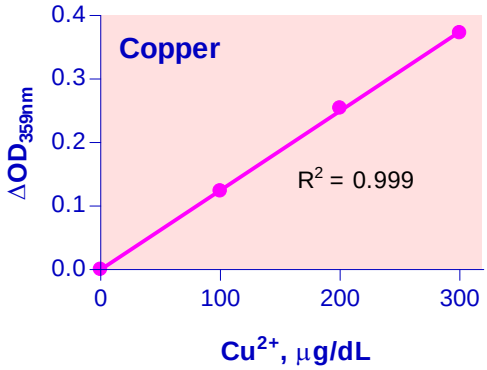
Pipeting devices and accessories. For 96-well plate assays: clear flat-bottom 96-well plates and plate reader. For cuvet assays: spectrophotometer and cuvets for measuring OD at 356-362nm.

GENERAL CONDSIDERATIONS

For scarce samples (e.g. mice serum or plasma), mix sample with dH_2O to a total of 100 μL, e.g. 50 μL serum + 50 μL dH_2O. Multiply the results by the dilution factor (2 fold).

EXAMPLES

Human serum, rat plasma, rat serum, and bovine serum were assayed in duplicate using the 96-well plate assay protocol. The copper concentrations were 97 ± 1, 104 ± 1, 101 ± 2, 78 ± 1 μg/dL, respectively.



Standard Curve in 96-well plate assay

LITERATURE

1. Stuerenburg HJ, Eggers C (2000). Early detection of non-compliance in Wilson's disease by consecutive copper determination in cerebrospinal fluid. J Neurol Neurosurg Psychiatry 69: 701-702.

2. Liska SK, Kerkay J, Pearson KH (1985). Determination of zinc and copper in urine using Zeeman effect flame atomic absorption spectroscopy. Clin Chim Acta. 151:231-236.

3. Tessman RK, Lakritz J, Tyler JW, Casteel SW, Williams JE, Dew RK. (2001). Sensitivity and specificity of serum copper determination for detection of copper deficiency in feeder calves. J Am Vet Med Assoc. 218:756-760.