QuantiChrom™ Copper Assay Kit (DICU-250)

Quantitative Colorimetric Copper Determination at 354nm

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. BioAssay Systems' copper assay kit is designed to measure copper directly in serum or plasma without any pretreatment. The improved method utilizes a chromogen that forms a purple colored complex specifically with copper ions. The intensity of the color, measured at 350-360nm, 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 8 µg/dL (1.2 µM) to 300 µg/dL (47 µM) copper in 96-well plate assay.
Simple and high-throughput. The procedure involves addition of a single working reagent and incubation for 5 min. 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.
Low interference in biological samples. No pretreatments are needed. Assays can be directly performed on serum and plasma samples. Other metals do not interfere in this assay.

APPLICATIONS:

Direct Assays: Cu in biological samples (e.g. serum and plasma).
Drug Discovery/Pharmacology: effects of drugs on Cu metabolism.
Environmental/Food: Cu in soil, mineralized samples, beverages etc.

KIT CONTENTS (250 tests in 96-well plates)

Reagent A: 35 mL Reagent B: 12 mL Reagent C: 6 mL
Copper Standard: 1 mL 1.5 mg/dL Cu^{2+}

Storage conditions. Store all reagents at 4 °C. Shelf life: 12 months.

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

Reagent Preparation:

Prepare enough Working Reagent by mixing 6 volumes of Reagent A, 2 volumes Reagent B and 1 volume Reagent C. Fresh reconstitution is recommended. Equilibrate to room temperature before assay.

Procedure using 96-well plate:

1. Standards. Prepare 400 µL 300 µg/dL Premix by mixing 80 µL 1.5 mg/dL standard and 320 µL distilled water. Dilute standard as follows.

No	Premix + H_2O	Vol (µL)	Cu (µg/dL)
1	100µL + 0µL	100	300
2	80µL + 20µL	100	240
3	60µL + 40µL	100	180
4	40µL + 60µL	100	120
5	30µL + 70µL	100	90
6	20µL + 80µL	100	60
7	10µL + 90µL	100	30
8	0µL + 100µL	100	0

2. Transfer 50 µL diluted standard and samples to wells of a clear bottom plate. As a sample "Blank", add 200 µL water to the same sample. Add 200 µL Working Reagent to all other wells and mix.
3. Incubate 5 min at room temperature and read optical density at 350-360nm (peak absorbance at 354nm). Signal is stable for > 60 min.

Procedure using cuvette:

1. Transfer 250 µL diluted standards and samples to appropriately labeled tubes.
2. Add 1000 µL double distilled H_2O to sample "Blank" tube and 1000 µL Working Reagent to all other tubes. Mix by vortexing.
3. Incubate 5 min at room temperature, transfer to cuvets and read optical density at 350-360nm (peak absorbance at 354nm).

CALCULATION

Subtract blank OD (water, #8) from the standard OD values and plot the OD against standard concentrations. Determine the slope using linear regression fitting. The copper concentration of Sample is calculated as

$$= \frac{OD_{SAMPLE} - OD_{BLANK}}{Slope} \quad (\mu g/dL)$$

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

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices and accessories.

Procedure using 96-well plate:

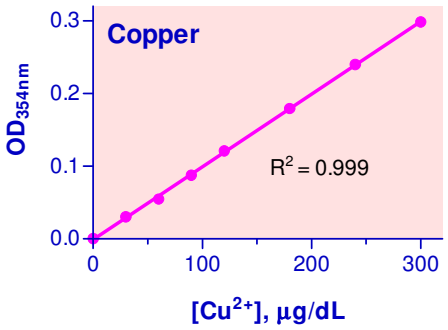
Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

Procedure using cuvette:

Spectrophotometer and cuvets for measuring OD at 350-360nm.

EXAMPLES:

Rat plasma, rat serum, goat serum and fetal bovine serum (Invitrogen) were assayed using the 96-well plate assay protocol. The copper concentrations were 103 ± 1 (n = 4), 106 ± 1 (n = 4), 152 ± 2, 114 ± 1 µg/dL (n = 4), respectively. CV < 2%.



Calibration curve in 96-well plate

LITERATURE

1. Stuerenburg HJ, Eggers C (200). 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.