# Copper Assay Kit

Catalog Number KA1615

250 assays

Version: 03

Intended for research use only

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## Introduction

### Intended Use

Application

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

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.

### Background

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. 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 359 nm, is directly proportional to copper concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples.

## General Information

### Materials Supplied

List of component

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

### Materials Required but Not Supplied

Pipetting devices and accessories.

For 96-well plate assays:
Clear flat-bottom 96-well plates and plate reader.

For cuvette assays:
Spectrophotometer and cuvets for measuring OD at 356-362 nm.

### Storage Instruction

Store all reagents at 4°C. Shelf life: 12 months after receipt.

### Precautions for Use

✓ Precautions

Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents.

## Assay Protocol

### Assay Procedure

*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 dH2O (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 356-362 nm (peak absorbance at 359 nm).
   *Note: if sample OD values are higher than the OD value for the 300 µg/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 356-362 nm (peak absorbance at 359 nm).

## Data Analysis

### Calculation of Results

The copper concentration of Sample is calculated as

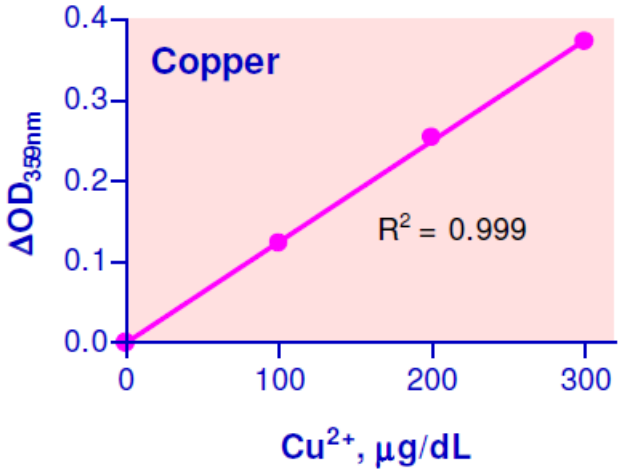
$$= \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{STANDARD} - OD_{BLANK}} \times 300 (ug/dL)$$

$OD_{SAMPLE}$, $OD_{BLANK}$ 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.

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).

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

## Resources

### References


1. Bartnikas TB (2012). Known and potential roles of transferrin in iron biology. Biometals 25 (4): 677-686.
2. Piret JP et al (2012). Differential toxicity of copper (II) oxide nanoparticles of similar hydrodynamic diameter on human differentiated intestinal Caco-2 cell monolayers is correlated in part to copper release and shape. Nanotoxicology 6 (7): 789-803.
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