Technical Bulletin

Copper Assay Kit

Catalogue number MAK127

Product 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 ceruloplasmin in the liver. Low levels of copper have been associated with mental retardation, depigmentation, anemia, hypotonia, and scorbutic changes in bone. Levels of copper are key diagnostic indicator of diseases such as Wilson's disease, microcytic hypochromic anemia, and bone disease due to reduced collagen synthesis.

The Copper Assay Kit is designed to measure copper concentrations in biological, environmental, food, and €beverage samples with minimal-to-no sample treatment. The method utilizes a chromogen that forms a colored complex specifically with copper ions. The intensity of the color, measured colorimetrically (359 nm), is directly proportional to copper concentration in the sample. The range of linear detection is 7 µg/dL (1.0 µM) to 300 µg/dL (47 µM).

Components

The kit is sufficient for 250 assays in 96 well plates.

Component	Volume
Reagent A Catalog Number MAK127A	10 mL
Reagent B Catalog Number MAK127B	1.5 mL
Reagent C Catalog Number MAK127C	40 mL
Copper Standard, 1.5 mg/dL Catalog Number MAK127D	1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening.

Storage/Stability

The kit is shipped ambient and storage at 2-8 °C, protected from light, is recommended.

Procedures

Use ultrapure water for the preparation of Standards. Metal chelators (For example EDTA) interfere with this assay and should be avoided in Sample preparation.

96 well Plate Assay Reaction Procedure

1. Standard for Colorimetric Detection – Add 20 μL of the 1.5 mg/dL Standard to 80 μL of ultrapure water in a tube to prepare a 300 μg/dL Standard solution. Add 100 μL of ultrapure water to another tube for use as a blank. Add 35 μL of Reagent A to each tube and mix by vortexing.
2. Sample Preparation – Aliquot 100 μL of each Sample into separate tubes. Add 35 μL of Reagent A to each tube and mix by vortexing.

   Note: If Samples contain protein (For example-serum/plasma), precipitates form. Centrifuge tubes for 2 minutes at 14,000 rpm and use clear supernatant for assay.
3. Transfer 100 μL of blank (water), prepared Standard, and Samples into separate wells of a clear flat bottom 96 well plate.
4. Set up the Master Reaction Mix according to the scheme in Table 1. 150 μL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Volume
Reagent B	5 μL
Reagent C	150 μL

5. Add 150 μL of the Master Reaction Mix to each well. Mix well using a horizontal shaker or by pipetting, and incubate the reaction at room temperature for 5 minutes. Protect the plate from light during the incubation.
6. Measure the absorbance of the blank (water), prepared Standard, and Samples at 5 minutes at 359 nm.

Cuvette Assay Reaction Procedure

1. Prepare Standard and Samples as for 96 well assay but scale up volumes 4-fold.
2. Transfer 400 μL of blank (water), prepared Standard, and Samples into separate cuvettes.
3. Set up the Master Reaction Mix according to the scheme in Table 2. 600 μL of the Master Reaction Mix is required for each reaction.

Table 2.
Master Reaction Mix

Reagent	Volume
Reagent B	20 μL
Reagent C	600 μL

4. Add 600 μL of the Master Reaction Mix to each cuvette. Mix well by pipetting, and incubate the reaction at room temperature for 5 minutes. Protect the plate from light during the incubation.
5. Measure the absorbance of the blank (water), prepared Standard, and Samples at 5 minutes at 359 nm.

Results

If sample absorbance values are higher than the absorbance value for the 300 μg/dL Standard, dilute sample in water and repeat assay. Multiply the results by the dilution factor.

A new standard must be set up each time the assay is run.

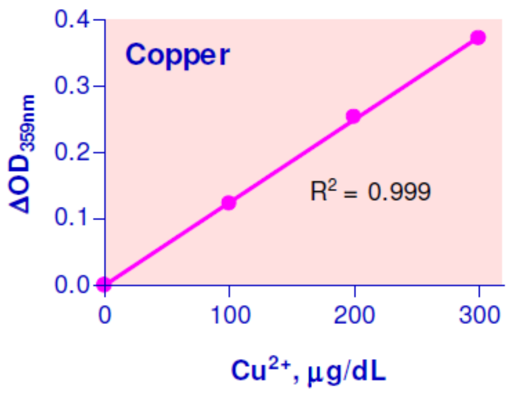
The copper concentration of a sample is calculated as:

$$\frac{A_{sample} - A_{blank}}{A_{standard} - A_{blank}} \times 300 = \mu g/dL\ (Cu^{2+})$$

Where:
A_{sample} = absorbance of sample
$A_{standard}$ = absorbance of standard
A_{blank} = absorbance of blank (water)

Conversion factors for Cu:

100 µg/dL = 15.5 µM or 1 ppm.



Standard Curve in 96-well plate assay

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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