Copper Microplate Assay Kit

User Manual

Catalog # FTA0140

(Version 1.2B)

Detection and Quantification of Copper (Cu^{2+}) Content in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

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.

This assay kit utilizes a chromogen that forms a colored complex specifically with copper ions. The reaction products can be measured at a colorimetric read out at 605 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 2	4 °C
Reaction Buffer	4 ml x 1	4 °C
Masking Reagent	Powder x 1	4 °C
Dye Reagent	Powder x 1	4 °C, keep in dark
Dye Reagent Diluent	1 ml x 1	4 °C
Standard (1 mmol/L)	1 ml x 1	4 °C
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Note:

Masking Reagent: add 1 ml Distilled water to dissolve before use.

Dye Reagent: add 1 ml Dye Reagent Diluent to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 605 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Centrifuge
6. Timer

IV. SAMPLE PREPARATION

1. For liquid samples

Pipet 0.5 ml of sample into a small test tube, add 0.5 ml of Assay buffer, and mix by vortexing. If samples contain protein, precipitates form. Centrifuge tubes for 5 min at 12,000 rpm and use clear supernatant for assay. The supernatant should be water clean; if not, it is recentrifuged.

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

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	140 µl	--	--
Standard	--	140 µl	--
Distilled water	--	--	140 µl
Reaction Buffer	25 µl	25 µl	25 µl
Masking Reagent	10 µl	10 µl	10 µl
Dye Reagent	25 µl	25 µl	25 µl
Mix, incubate at room temperature for 15 minutes, record absorbance measured at 605 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

VI. CALCULATION

1. According to the liquid sample

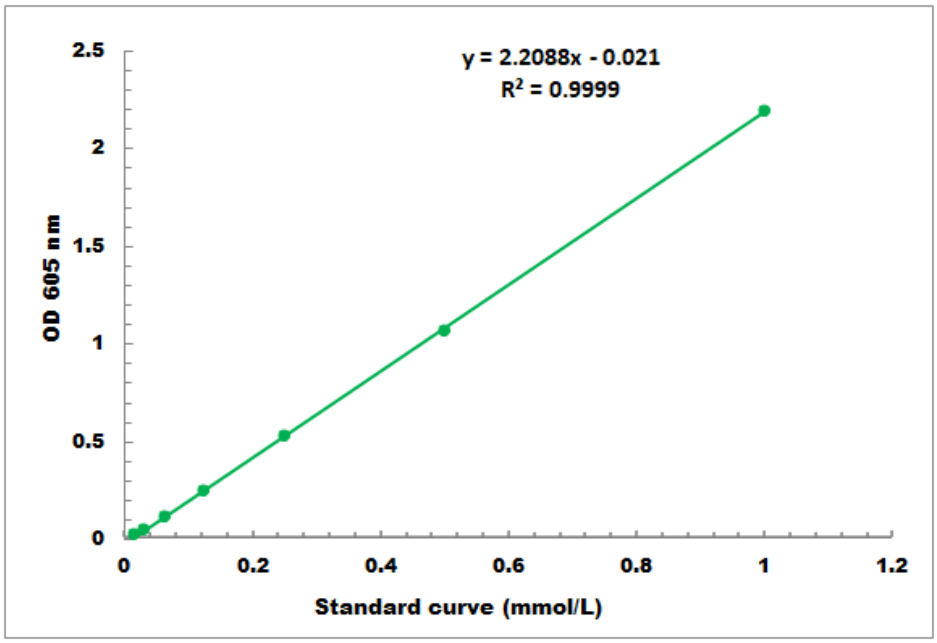
Cu^{2+} (mmol/L) = $C_{Standard}$ × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) × 2

=2 × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank})

$C_{Standard}$: the concentration of Standard, 1 mmol/L.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.01 mmol/L - 1 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES