Copper Microplate Assay Kit

User Manual

Catalog # CAK1154

(Version 1.9D)

Detection and Quantification of Copper (Cu) 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 4	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 (250 µmol/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 before use, heat at 70 °C to dissolve it.

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 tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly.

Note:

1. Metal chelators (e.g. EDTA) interfere with this assay and should be avoided in sample preparation.
2. If the samples contain more proteins, it may cause deposition in the assay. The samples can be boiled in water bath for 5 mins, centrifuged at 10000g for 10 minutes, then add the supernatant into the plate.

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	40 µl	40 µl	40 µl
Masking Reagent	10 µl	10 µl	10 µl
Dye Reagent	10 µl	10 µl	10 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the liquid sample

$$Cu\ (nmol/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 250 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

2. According to the weight of sample

$$Cu\ (nmol/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay})$$

$$= 250 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

$$Cu\ (nmol/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay})$$

$$= 250 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

W: the weight of sample, g;

$C_{Standard}$: the concentration of Standard, 250 μmol/L = 250 nmol/ml;

V_{Sample}: the volume of sample, 0.14 ml;

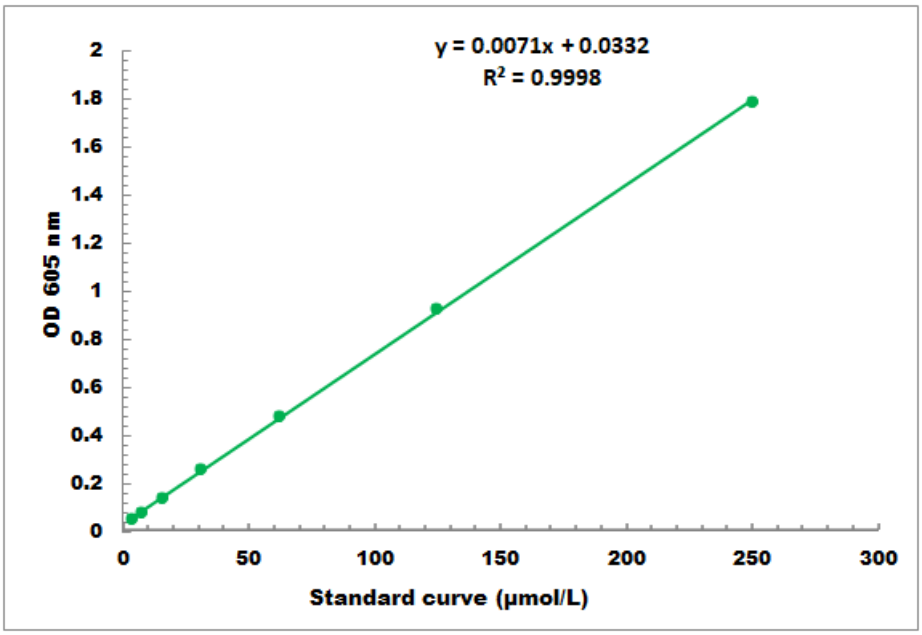
$V_{Standard}$: the volume of standard, 0.14 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

N: the quantity of cell or bacteria, $N \times 10^4$.

VII. TYPICAL DATA

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



Detection Range: 1 μmol/L - 250 μmol/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