Copper

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1154

(Version 1.11E)

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 complexspecifically with copper ions.The reaction productscan be measured at a colorimetric readout at 605 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer 1	12 ml x 1	4 °C
Assay Buffer 2	5 ml x 1	4 °C
Reaction Buffer	4 ml x 1	4 °C
Masking Reagent	Powderx 1	4 °C
Dye Reagent	Powderx 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, keep in dark
Technical Manual	1 Manual	

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
7. Ice bath

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 250/125/62.5/31.2/15.6/ 7.8/3.9/1.95/0.97μmol/L.

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.

V. SAMPLE PREPARATION

1.For liquid samples

Pipette 120 µl liquid sample into 120 µl Assay Buffer 1 and mix by vertexing, and put it in ice bath for 10-15 minutes. Centrifuged tubes for 10minutesat 12,000rpm and transfer150 µlclear supernatant into a new centrifuge tube, add 46 µl Assay Buffer 2 to mix thoroughly.

2.For cell samples

Collect cell into centrifuge tube, discard the supernatant after centrifugation, add lysis buffer (not provide) according to the lysis buffer manufacturer's instructions. Then pipette 120 µl lytic sample into120 µl Assay Buffer 1 and mix by vertexing, and put it in ice bath for 10-15 minutes. Centrifuged tubes for 10minutes at 12,000rpm and transfer150 µl clear supernatant into a new centrifuge tube, add 46 µl Assay Buffer 2 to mix thoroughly.

3.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mldistilled water on ice.Then pipette 120µl homogeneous sample into120 µl Assay Buffer 1 and mix by vertexing, and put it in ice bath for 10-15 minutes. Centrifuged tubes for 10minutes at 12,000rpm and transfer150 µl clear supernatant into a new centrifuge tube, add 46 µl Assay Buffer 2 to mix thoroughly.

Note:

1. Metal chelators (e.g. EDTA) interfere with this assay and should beavoided in sample preparation.

VI. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent*	Sample**	Standard	Blank
Sample	140 µl	--	--
Standard	--	140 µl	--
Distilled water	--	--	140 µl
Reaction Buffer	40µl	40µl	40µl
Masking Reagent	10 µl	10 µl	10 µl
Dye Reagent	10 µl	10 µl	10 µl
Mix,incubateat room temperature for15 minutes, record absorbance measured at 605 nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(\mu mol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}\times n(\mu mol/L)$$

2.2According to the quantity of cells or bacteria

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times N\times V_{Sample}}\times n(\mu mol/10^4)$$

2.3According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times W\times V_{Sample}}\times n\ \ (\mu mol/g)$$

Slope: the absorbance slope of standard curve

$C_{Standard}$: the standard concentration, μmol/L

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

N: the quantity of cell or bacteria, 10^4

n: the dilution factor, in the recommend sample preparation

$$n=\frac{V_{Assay\ 1}+V}{V}\times\frac{V_{Assay\ 2}+V_{Supernatant}}{V_{Supernatant}}$$

V: the volume of sample in sample preparation,μl

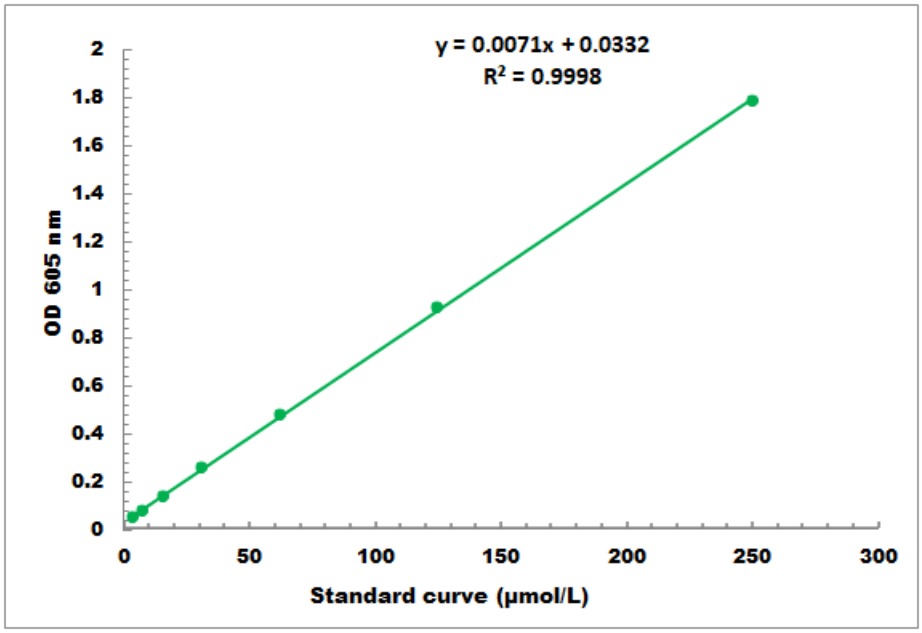
$V_{Assay\ 1}$: the volume of Assay Buffer 1, μl

$V_{Assay\ 2}$: the volume of Assay Buffer 2, μl

$V_{supernatant}$: the volume of supernatant, µl

VIII. 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