Copper Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6751

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 .

1. 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 measu red at a colorimetric readout at 605 nm.

2. Kit Components

Component	Volume	Storage
96-Well Microplate	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	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, keep in dark
Technical Manual	1 Manual	-

3. 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

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Perform 2 -fold serial dilutions of the top standard solution using distilled water to 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 /0.97 μmol/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.

5. Sample Preparation

1. For liquid samples Pipette 120 μL liquid sample into 120 μL Assay Buffer 1 and mix by vertexing , and p ut it in ice bath for 10-15 minutes. Centrifuged tubes for 10 minutes at 12,000rpm and transfer 150 μL clear supernatant into a new centrifuge tube , add 46 μL Assay Buffer 2 to mix thoroughly.

2. For cell a 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 into 120 μL Assay Buffer 1 and mix by vertexing , and put it in ice bath for 10-15 minutes. Centrifuged tubes for 10 minutes at 12,000rpm and transfer 150 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 be avoided in sample preparation.

6. Assay Procedure

Add following reagents in to 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:

*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.

7. Calculation

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of

the standard curve

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

Calculate the initial concentration according to sample preparation procedure .

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

2.1 According to the volume of sample

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

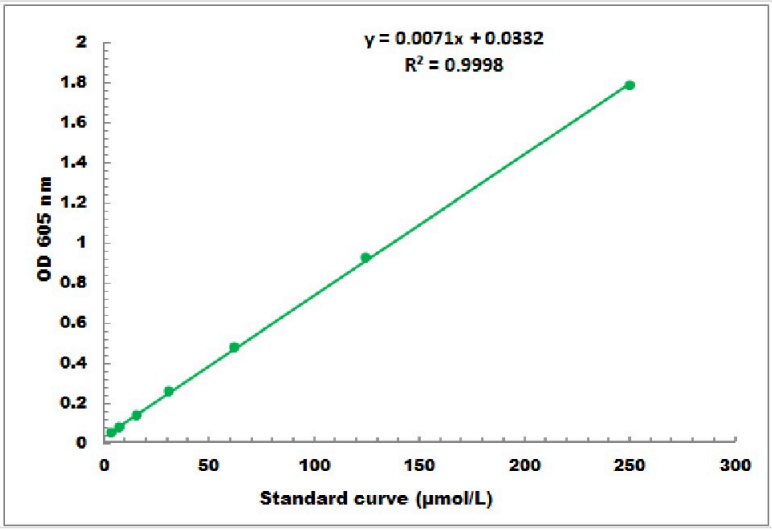
2.2 According to the quantity of cells or bacteria

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

Item	Definition
Slope	the absorbance slope of standard curve
$C_{Standard}$	the standard concentration, mg/ml
$V_{Standard}$	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, $n = \frac{V_{Assay\ 1} + V}{V} \times \frac{V_{Assay\ 2} + V_{Supernatant}}{V_{Supernatant}}$
$V_{Assay\ 1}$	the volume of Assay Buffer 1, μl
V	the volume of sample in sample preparation, μl
$V_{Assay\ 2}$	the volume of Assay Buffer 2, μl
Vsupernatant	the volume of supernatant, μl

8. 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