



Ceruloplasmin Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1059

(Version 1.3C)

Detection and Quantification of Ceruloplasmin(CP)Activity inSerum,
Plasma, Other biological fluidsSamples.

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

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I. INTRODUCTION

Ceruloplasmin is a copper containing protein found primarily in the blood. It carries approximately 70% of the total copper present in the blood. Ceruloplasmin exhibits a weak oxidase activity, which is a more accurate method of assessing ceruloplasmin in serum than immunodiffusion or other non-enzymatic assays. Normal Ceruloplasmin levels are generally 1-4 μM (15-60 mg/dl), equivalent to approximately 50-150 mU/ml. Elevated amounts of serum ceruloplasmin are found in pregnancy, cancer, rheumatoid arthritis and in several mental conditions such as Alzheimer's, schizophrenia and OCD. Abnormally low amounts are found in Wilson's and Menkes' Diseases and in several other rare conditions.

Ceruloplasmin Colorimetric Microplate Assay Kit provides a simple and direct procedure for measuring ceruloplasmin content in a variety of samples. The assay is initiated with the enzymatic catalysis of the PPD by CP. The enzyme catalysed reaction products can be measured at a colorimetric readout at 540 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Reaction Buffer	10 ml x 1	4 °C
Substrate	Powderx 1	4 °C, keep in dark
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 540 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Substrate: Add 10 ml distilled water to dissolve before use. Store at -20 °C for 1-2 weeks after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1. For serum or plasma samples

Detect directly.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample*	Blank
Sample	10 μ l	--
Distilled water	--	10 μ l
Reaction Buffer	90 μ l	90 μ l
Substrate	100 μ l	100 μ l
Mix, put it into the oven at 37 °C for 10 minutes, measured at 540 nm and record the absorbance.		

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

Unit Definition: one unit is defined as the enzyme that will oxidize 1 μmol of Substrate per minute.

1. According to the volume of serum or plasma

$$\begin{aligned}\text{CP (U/ml)} &= (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\epsilon \times d) \times V_{\text{Total}} / V_{\text{Sample}} / T \\ &= 0.352 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})\end{aligned}$$

ϵ : molar extinction coefficient, $9.46 \times 10^3 \text{ L/mol/cm}$

d : the optical path of 96-Well microplate, 0.6 cm

V_{Total} : the total volume of the enzymatic reaction, 0.2 ml

V_{Sample} : the volume of sample, 0.01 ml

T : the reaction time, 10 minutes