

## Ceruloplasmin Colorimetric Microplate Assay Kit

Cat #: orb390765 (manual)

*For research use only. Not intended for diagnostic use.*

### Product Features

**Intend Use:** Detection and Quantification of Ceruloplasmin (CP) Activity in Serum, Plasma, Other biological fluids Samples.

**Detection Range:** 0.01 U - 1 U

**Sample Types:** Serum, Plasma, Other biological fluids Samples

### Assay Principle

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  $\mu\text{M}$  (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.

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.

### Kit Components

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

Note: Substrate: add 10 ml distilled water to dissolve before use.

### Materials Required but Not Supplied

1. Microplate reader to read absorbance at 540 nm

Explore. Bioreagents.

2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

### Sample Preparation

1. For serum or plasma samples Detect directly.

### Assay Procedure

Add following reagents into the microplate:

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

- 1) Reagents must be added step by step, cannot be mixed and added together.

### Calculation of Results

Unit Definition: one unit is defined as the enzyme that will oxidize 1  $\mu\text{mol}$  of Substrate per minute.

1. According to the volume of serum or plasma

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

$\epsilon$ : molar extinction coefficient,  $9.46 \times 10^3$  L/mol/cm

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

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

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

T: the reaction time, 10 minutes