# Ceruloplasmin Microplate Assay Kit

# User Manual

## Catalog # CAK1059

(Version 1.2C)

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

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

## 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.

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 | 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.

## 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. SAMPLE PREPARATION

1. For serum or plasma samples

Detect directly.

## V. ASSAY PROCEDURE

Add following reagents into the microplate:

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

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

## VI. 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

$CP\ (U/ml) = (OD_{Sample} - OD_{Blank}) / (\varepsilon \times d) \times V_{Total} / V_{Sample} / T$

$= 0.352 \times (OD_{Sample} - OD_{Blank})$

ε: molar extinction coefficient, $9.46 \times 10^3$ 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.

## VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

## VIII. NOTES