Ceruloplasmin Assay Kit

Catalog No.: abx298825

Size: 100 Assays

Storage: Store all kit components in the dark at 4°C for up to 6 months.

Application: For quantitative detection of Ceruloplasmin activity in serum, plasma, other biological fluids.

Introduction: Ceruloplasmin (CP) is a ferroxidase enzyme that serves as the primary copper-carrying protein in the blood. Transporting over 95% of the copper present in human plasma, abnormal levels of CP have been implicated in a number of diseases, including Wilson's disease and Menke's disease. In healthy individuals, CP levels are generally in the range of 1-4 µM.

Ceruloplasmin exhibits weak oxidase activity. The concentration of the reaction product is directly proportional to the enzyme activity, which can be measured by measuring the absorbance at 540 nm.

Kit components

1. 96 well microplate
2. Reaction Buffer: 10 ml
3. Substrate: 1 vial
4. Plate Sealer: 3

Materials Required But Not Provided

1. Microplate reader (540 nm)
2. Microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Convection oven

For Reference Only

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Solution**
  Add 10 ml of Distilled water into the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use.

2. Sample

- **Serum and Plasma samples**
  Serum and plasma samples can be used directly.

- **Other biological fluids**
  Other biological fluids can be used directly. Where the sample concentration is unknown, we recommend conducting a pretest to determine the optimal dilution ratio.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected activity is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured activity within the detection range of the kit.

1. Set the sample and blank wells on the 96 well microplate and record their positions. We recommend setting up each sample and blank in duplicate.
2. Add 10 µl of sample to the sample wells.
3. Add 10 µl of Distilled Water to the blank wells.
4. Add 90 µl of Reaction Buffer to all wells.
5. Add 100 µl of Substrate Solution to all wells.
6. Tap the plate gently to mix, then put the plate in a convection oven for 10 minutes at 37°C.
7. Immediately record absorbance at 540 nm.

C. Calculations

One unit of Ceruloplasmin activity is defined as the amount of enzyme required to oxidize 1 µmol of substrate per minute.

CP activity per ml of sample:

$$\mathbf{Ceruloplasmin\ (U/ml)} = \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}})}{(\boldsymbol{\varepsilon} \times \mathbf{d})} \times \frac{\mathbf{V_{Total}}}{\mathbf{V_{Sample}} \times \mathbf{T}} = \mathbf{0.352} \times (\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}})$$

where:

$\mathbf{OD_{Sample}}$	Absorbance of the sample well
$\mathbf{OD_{Blank}}$	Absorbance of the blank well
$\boldsymbol{\varepsilon}$	Molar absorption coefficient (9.46 ml/mol/cm)
$\mathbf{d}$	Microplate optical path length (0.6 cm)
$\mathbf{V_{Total}}$	Volume of the reaction (0.2 ml)
$\mathbf{V_{Sample}}$	Volume of the sample (0.01 ml)
$\mathbf{T}$	Reaction time (10 minutes)

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