

EZMeta™ Ceruloplasmin Colorimetric Activity Kit

Cat #: D-AKC1550

Size: 96T

Storage: Stored at 4°C for 12 months, protected from light

Product Information

Applicable samples: Serum, Plasma and Urine

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 has the activity of oxidase and is an important antioxidant in the extracellular fluid. EZMeta™ Ceruloplasmin Colorimetric Activity Kit is specially developed for the detection of ceruloplasmin. The operation is simple and convenient, and the detection is more sensitive and accurate. Ceruloplasmin catalyzes the formation of colored substances from o-dianisidine dihydrochloride, with a characteristic absorption peak at 460 nm. According the enzyme activity definition, the ceruloplasmin activity in the sample can be calculated.

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
	96 T	
Assay Buffer	20 mL	4°C
Substrate	1	4°C, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 460 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Incubator
- Deionized water

Reagent Preparation

Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Substrate: Preparing before use, add 5 mL deionized water to fully dissolve. This reagents can be kept at 4°C for a week.

Please aliquot the unused reagents and store at -20°C, protected from light to avoid freezing and thawing.

Sample Preparation

Serum and urine and other liquid samples: Tested directly.

Assay Procedure

1. Preheated the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 460 nm, visible spectrophotometer was returned to zero with deionized water.
2. Add the following reagents respectively into each 96-well plate or microglass cuvette.

Reagent	Test Well (μL)
Sample	10
Assay Buffer	140
Substrate	50

Mix well, reading the values A_1 at 460 nm immediately, then incubate 10 min in 30°C, and read the values A_2 at 460 nm again. Finally, calculate $\Delta A = A_2 - A_1$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 1-2 samples. If ΔA_{Test} is greater than 1.0, the sample can be appropriately diluted with deionized water, the calculated result multiplied by the

dilution factor.

Data Analysis

A. 96-well plates calculation formula as below

Unit definition: one unit is defined as the enzyme that will oxidize 1 nmol of substrate per min in 1 mL sample at 30°C.

$$C_p \text{ (U/mL)} = [\Delta A \times V_{\text{Total}} \div (\epsilon \times d)] \div V_{\text{Sample}} \div T = 417 \times \Delta A$$

Where: ϵ : Substrate molar extinction coefficient, 9.6×10^3 mL/nmol/cm; d: the optical path of 96-well plate, 0.5 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 min.

B. Microglass cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Typical Data

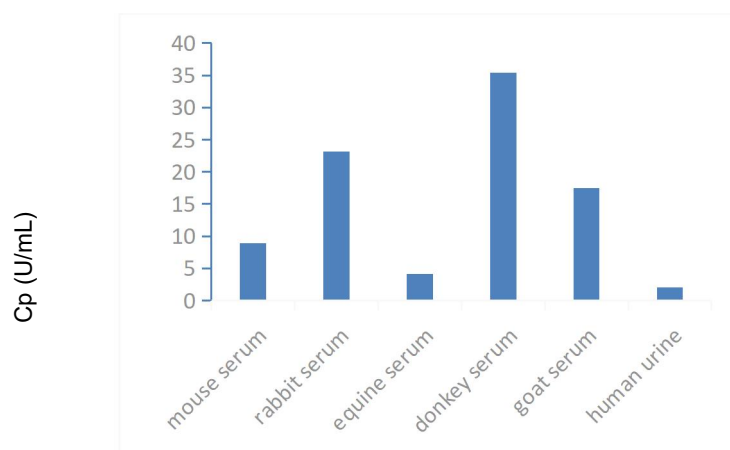


Figure 1. Ceruloplasmin activity in mouse serum, rabbit serum, equine serum, donkey serum, goat serum, and human urine respectively. Assays were performed following kit protocol.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.