Product Manual

Human Ceruloplasmin ELISA Kit

Catalog Number

PRB-5041 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Ceruloplasmin (Cp) is a ferroxidase enzyme encoded by the CP gene in humans. Cp is the main copper-transporting protein in the blood, and also functions in iron metabolism. Cp is made in the liver and has 6 atoms of copper bound to it. Cp carries over 95% of the total copper in human plasma and has a copper-dependent oxidase activity. The enzyme causes oxidation of Fe2+ (ferrous iron) into Fe3+ (ferric iron), and as a result helps transport iron (in addition to transferrin) in the plasma. Low levels of Cp have been observed in patients with hepatic disease. Low Cp levels may be an indication of Aceruloplasminemia, copper deficiency, Wilson disease, Menkes disease, or an overdose of Vitamin C. High levels of Cp may be an indication of pregnancy, copper toxicity / zinc deficiency, lymphoma, angina, schizophrenia, acute and chronic inflammation, rheumatoid arthritis, Alzheimer's disease, or obsessive-compulsive disorder.

Cell Biolabs' Human Ceruloplasmin ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of human Cp in plasma, serum, urine, cell or tissue lysate samples. The kit has a detection sensitivity limit of 2 ng/mL human Cp. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. PRB-5033: Human Alpha 2 Macroglobulin ELISA Kit
2. PRB-5039: Human Haptoglobin ELISA Kit
3. PRB-5044: Human Alpha 1 Acid Glycoprotein ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Human Cp Antibody Coated Plate (Part No. 50411B): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-Cp Antibody (1000X) (Part No. 50412C): One 10 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Human Cp Standard (Part No. 50413D): One 50 µL vial of 12.5 µg/mL Human Ceruloplasmin.

Materials Not Supplied

1. Plasma, serum, cell or tissue lysate
2. PBS containing 0.1% BSA

3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the Human Cp Standard at -80ºC to avoid multiple freeze/thaw cycles. Store the Biotinylated Anti-Cp Antibody at -20ºC. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-Cp Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute Biotinylated Anti-Cp Antibody or Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Human Cp Standard

Prepare a dilution series of human Cp standards in the concentration range of 0 to 125 ng/mL into Assay Diluent (Table 1).

Standard Tubes	12.5 µg/mL Human Cp Standard (µL)	Assay Diluent (µL)	Human Cp (ng/mL)
1	8	792	125
2	400 of Tube #1	400	62.5
3	400 of Tube #2	400	31.25
4	400 of Tube #3	400	15.62
5	400 of Tube #4	400	7.81
6	400 of Tube #5	400	3.91
7	400 of Tube #6	400	1.95
8	0	400	0

Table 1. Preparation of Human Cp Standards

Preparation of Samples

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

- Plasma: Collect blood with an anticoagulant such as heparin, citrate or EDTA and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Remove the plasma and assay immediately or store samples at -80ºC for up to three months. Normal plasma samples require about 5,000 to 10,000 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA.
- Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant

without disturbing the white buffy layer. Assay immediately or store samples at -80ºC for up to three months. Normal serum samples require about 5,000 to 10,000 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA.

- Urine: Harvest urine and centrifuge for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.
- Other Biological Fluids: Centrifuge samples for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.
- Cell or Tissue Lysate: Sonicate or homogenize sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.

Assay Protocol

1. Add 100 µL of human Cp unknown sample or standard to the Anti-Human Cp Antibody Coated Plate. Each human Cp unknown sample, standard and blank should be assayed in duplicate.
2. Incubate at room temperature for 1 hour on an orbital shaker.
3. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
4. Add 100 µL of the diluted Biotinylated Anti-Cp Antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.
5. Wash the strip wells 3 times according to step 3 above.
6. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash the strip wells 3 times according to step 3 above. Proceed immediately to the next step.
8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).

10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical results with the Human Ceruloplasmin ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

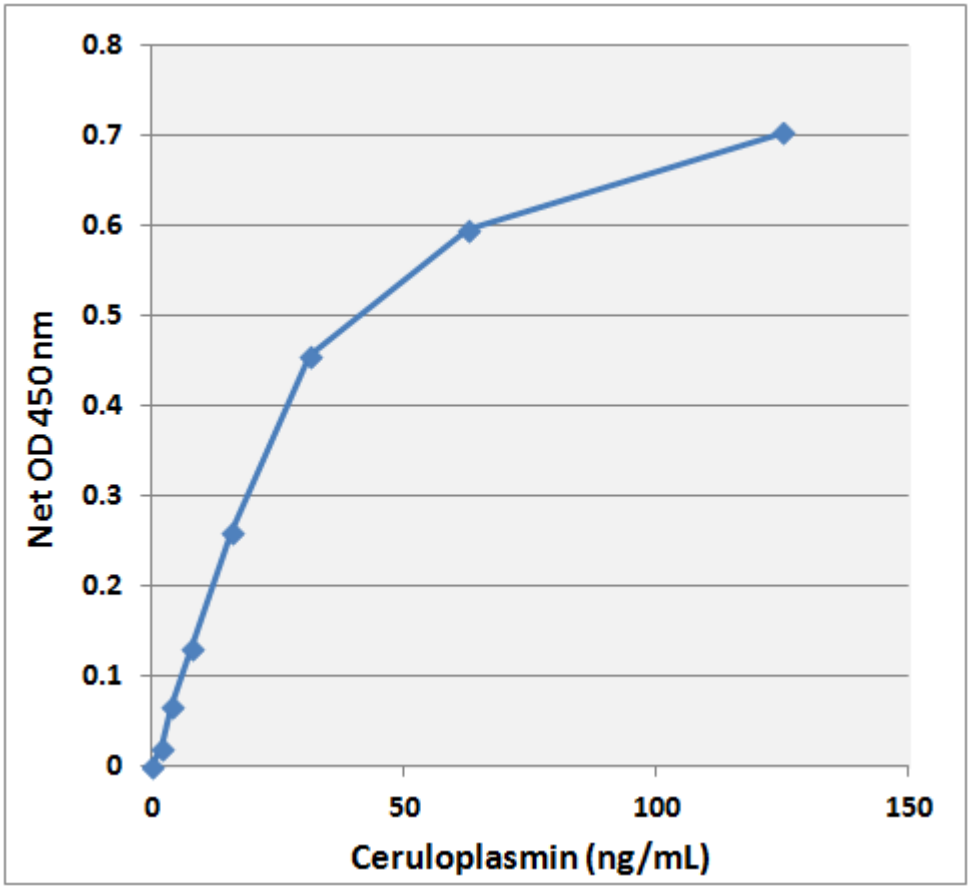

Figure 1: Human Ceruloplasmin ELISA Standard Curve.

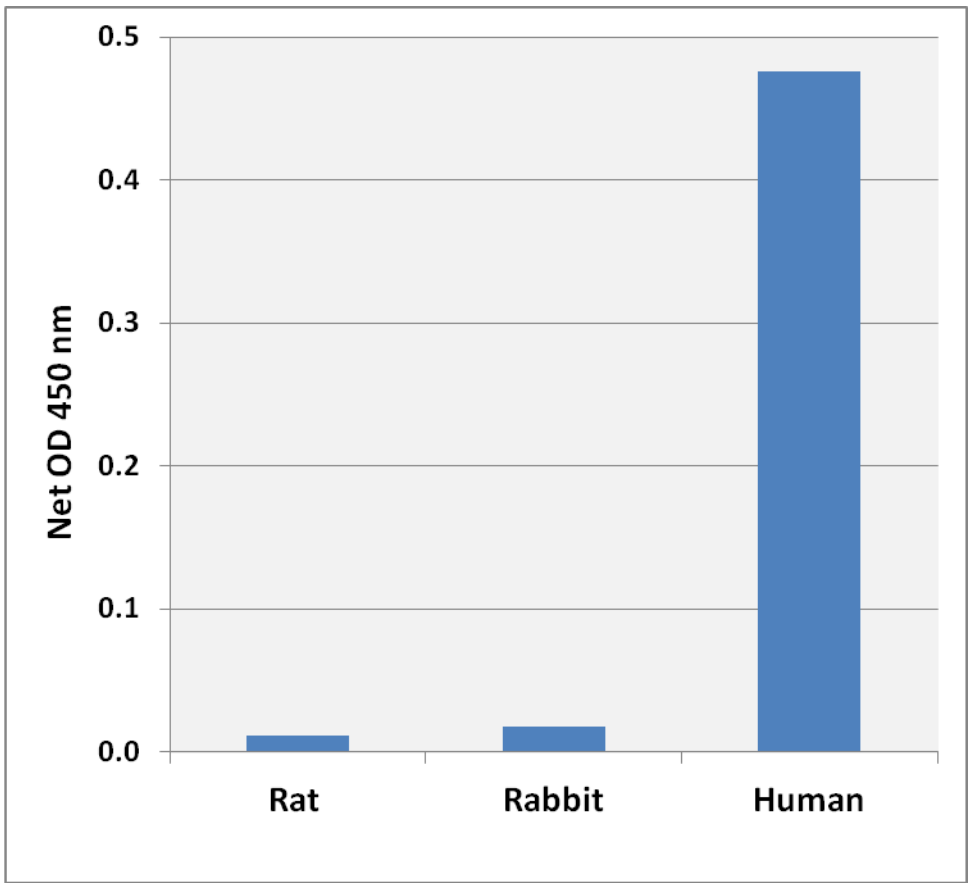

Figure 2: Detection of Ceruloplasmin in Plasma. Each plasma sample was diluted 6400 fold according to the protocol above and then tested using the Human Ceruloplasmin ELISA Kit.

References

1. Takahashi N, Ortel TL, Putnam FW (1984). *PNAS USA*. **81**: 390–4
2. Koschinsky ML, Funk WD, van Oost BA, MacGillivray RT (1986). *PNAS USA*. **83**: 5086–90
3. Royle NJ, Irwin DM, Koschinsky ML, MacGillivray RT, Hamerton JL (1987). *Som. Cell Mol. Gen*. **13**: 285–92.
4. Hellman NE, Gitlin JD (2002). *Ann. Rev. Nutr*. **22**: 439–58.
5. Song D, Dunaief JL (2013). *Front. Aging Neurosci*. **5**: 24
6. Novikova I, Zlotnikova M. (2011). *Biomed. Pap. Med. Fac. Univ. Palacky. Olomouc Czech Repub*. **155**:361-366.

Warranty

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