Human Ceruloplasmin ELISA Kit
(Urine and Cell Culture Samples)

Introduction

Ceruloplasmin is an abundant α2-serum glycoprotein that contains 95% of the copper found in the plasma of vertebrate species (1). Ceruloplasmin is a cooper-binding protein that normally removes iron from cells by its ferroxidase activity. Ceruloplasmin concentration on average is 14.6 (±4.0) mg/dl (2)**.** Low levels of ceruloplasmin lead to the abnormal deposition of iron in cells, including those of the pancreas, liver, retina and the basal ganglia region of the brain. Some diseases associated with ceruloplasmin are Wilson's disease (3), Hemochromatosis (4), Menkes disease (5) and Aceroluplasminemia (1).

Principal of the Assay

The Human Ceruloplasmin ELISA kit is designed for detection of human urine, and cell culture supernatant. This assay employs a quantitative sandwich enzyme immunoassay technique that measures ceruloplasmin in 4 hours. A polyclonal antibody specific for ceruloplasmin has been pre-coated onto a microplate. Ceruloplasmin in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for ceruloplasmin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **Human Ceruloplasmin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human ceruloplasmin.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human Ceruloplasmin Standard:** Human Ceruloplasmin in a buffered protein base (320 ng, lyophilized).
- **Biotinylated Ceruloplasmin Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against human ceruloplasmin (80 µl).

- **MIX Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 µl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Store unopened kit at 2-8^{0}C up to expiration date.
- Opened MIX Diluent may be stored for up to 1 month at 2-8^{0}C. Store reconstituted reagents at -20^{0}C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation and Storage

- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20^{0}C or below. Avoid repeated freeze-thaw cycles
- **Urine:** Collect urine using sample pot. Centrifuge samples at 600 x g for 10 minutes and assay. Dilute samples 1:10 into MIX Diluent. Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8^{0}C.
- **Standard Curve:** Reconstitute the 320 ng of Ceruloplasmin Standard with 1 ml of MIX Diluent to generate a solution of 320 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the standard solution (320 ng/ml) 1:4 with MIX Diluent to produce 80, 20, 5, and 1.25 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20^{0}C.

Standard Point	Dilution	[Ceruloplasmin] (ng/ml)
P1	Standard (320 ng/ml)	320.00
P2	1 part P1 + 3 parts MIX Diluent	80.00
P3	1 part P2 + 3 parts MIX Diluent	20.00
P4	1 part P3 + 3 parts MIX Diluent	5.00
P5	1 part P4 + 3 parts MIX Diluent	1.25
P6	MIX Diluent	0.00

- **Biotinylated Ceruloplasmin Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent. Any remaining solution should be frozen at -20^{0}C.
- **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20^{0}C.

Assay Procedure

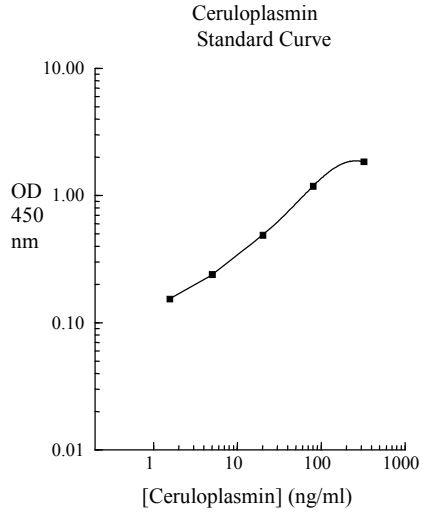
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30^{0}C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to complete remove liquid at each step.
- Add 50 µl of Biotinylated Ceruloplasmin Antibody to each well and incubate for one hour.
- Wash five times with 200 µl of Wash Buffer as above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash five times with 200 µl of Wash Buffer as above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the triplicate readings for each standard and sample.
  To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of Ceruloplasmin is typically 1 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.1% and 7.1% respectively.

Linearity

	Average Percentage of Expected Value
Sample Dilution	Urine
1:5	93%
1:10	105%
1:20	118 %

Recovery

Standard Added Value	10 – 200 ng/ml
Recovery %	84-119 %
Average Recovery %	101.5 %

Cross-Reactivity

Species	% Cross Reactivity
Beagle	< 0.5
Bovine	< 0.1
Monkey	< 30 (suggest dilution 1:100 for plasma/serum)
Mouse	< 3
Rat	< 0.5
Swine	< 1

- If cell culture supernatants contains 10% FBS, The minimum detectable dose of human ceruloplasmin will be 4 ng/ml.

References

(1) Harris, Z. L, et al. *Proc. Natl. Acad. Sci.* Vol. 92, pp. 2539-2543, March 1995
(2) Aliyazicioğlu, Y et al. *The Turkish Journal of Pediatics* 2007; 49: 52-54
(3) Czaja, M. J et al. *J. Clin. Invest.* Volume 80, October 1987, 1200-1204
(4) Kumar, A et al. *WJM, October 1995-Vol 163, No. 4*
(5) Moller, L.B. et al. *Am. J. Hum. Genet. 66:1211–1220, 2000*

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