Version 10-05/24

User's Manual

Human Ceruloplasmin ELISA Kit

REF **DEIASL034**

Σ **96T**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This human ceruloplasmin antigen assay is intended for the quantitative determination of total ceruloplasmin antigen in human plasma, serum, urine, milk, saliva and cell culture samples. For research use only.

General Description

Ceruloplasmin (aka Ferroxidase I) is a 132kDa 1,046 amino acid glycoprotein which carries 95% of serum copper by binding 6 cupric ions per molecule [1]. Levels are decreased in Wilson's Disease (hepatolenticular degeneration) and heritable aceruloplasminemia leading to iron accumulation in the liver or brain from impaired iron homeostasis [2].

Principles of Testing

Human ceruloplasmin will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, biotin labeled anti-human ceruloplasmin primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody is reacted with peroxidase conjugated streptavidin. Following an additional washing step, TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of human ceruloplasmin. Color development is proportional to the concentration of total ceruloplasmin in the samples.

Reagents And Materials Provided

1. 96-well antibody coated microtiter strip plate (removable wells 8x12) containing anti-human ceruloplasmin antibody, blocked and dried.
2. 10X Wash Buffer: 1 bottle of 50mL.
3. Human ceruloplasmin standard: 1 vial lyophilized standard.
4. Anti-human ceruloplasmin primary antibody: 1 vial lyophilized polyclonal antibody.
5. Horseradish peroxidase-conjugated streptavidin: 1 vial concentrated HRP labeled antibody.
6. TMB substrate solution: 1 bottle of 10mL solution.

Materials Required But Not Supplied

1. Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement.
2. Manifold dispenser/aspirator or automated microplate washer.
3. Microplate reader capable of measuring absorbance at 450 nm.
4. Pipettes and Pipette tips.
5. Deionized or distilled water.
6. Polypropylene tubes for dilution of standard.
7. Paper towels or laboratory wipes.

8. 1N H2SO4 or 1N HCl
9. Bovine Serum Albumin Fraction V (BSA)
10. Tris (hydroxymenthyl) aminomethane (Tris)
11. Sodium Chloride (NaCl)

Storage

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

Specimen Collection And Preparation

Collect plasma using EDTA, citrate or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Reagent Preparation

1. TBS buffer: 0.1M Tris, 0.15M NaCl, pH 7.4.
2. Blocking buffer (BB): 3% BSA (w/v) in TBS.
3. 1X Wash buffer: Dilute 50 mL of 10X wash buffer concentrate with 450mL of deionized water.

Assay Procedure

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard

Reconstitute standard by adding 1ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 1,000ng/mL standard solution.

Dilution table for preparation of human ceruloplasmin standard:

Ceruloplasmin concentration (ng/mL)	Dilutions
1,000	(from vial)
500	500µL (BB) + 500µL (1000ng/mL)
200	600µL (BB) + 400µL (500ng/mL)
100	500µL (BB) + 500µL (200ng/mL)
50	500µL (BB) + 500µL (100ng/mL)
20	600µL (BB) + 400µL (50ng/mL)
10	500µL (BB) + 500µL (20ng/mL)
5	500µL (BB) + 500µL (10ng/mL)
2	600µL (BB) + 400µL (5ng/mL)
1	500µL (BB) + 500µL (2ng/mL)
0	500µL (BB) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition

Remove microtiter plate from bag and add 100µL ceruloplasmin standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µL wash buffer. Remove

excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures total human ceruloplasmin in the 1-1,000 ng/mL range. If the unknown is thought to have high ceruloplasmin levels, dilutions may be made in blocking buffer. A 1:10,000-1:100,000 dilution for normal plasma or serum and 1:10-1:50 dilution for breast milk is suggested for best results. Saliva and urine samples should be applied directly to the plate for best results.

Primary Antibody Addition

Reconstitute primary antibody by adding 10mL of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100µL to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µL wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Streptavidin-HRP Addition

Briefly centrifuge vial before opening. Dilute 2.5µL of HRP conjugated streptavidin into 2.5mL blocking buffer to generate a 1:1,000 dilution. Add 0.4ml of 1:1,000 dilution to 9.6ml of blocking buffer to generate a 1:25,000 dilution. Add 100µl of the 1:25,000 dilution to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µL wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100µL TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µL of 1N H2SO4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix

thoroughly by gently shaking the plate.

Measurement

Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A450).

Calculation

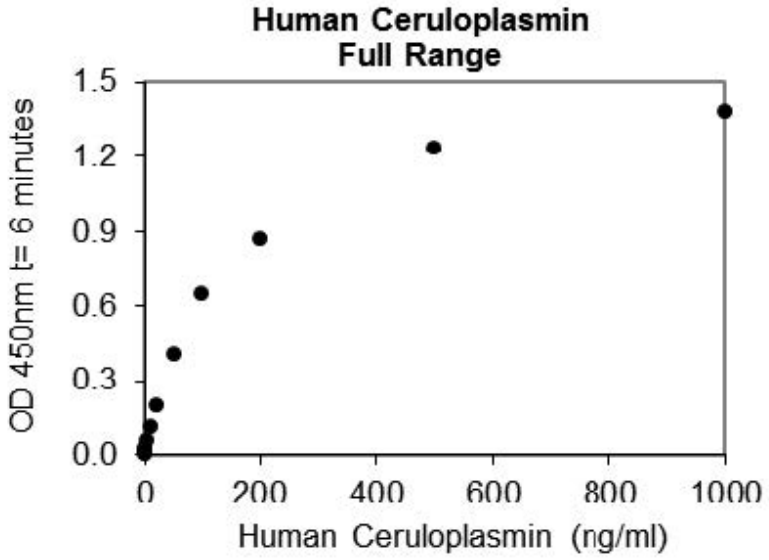
Plot A450 against the amount of ceruloplasmin in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of ceruloplasmin in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

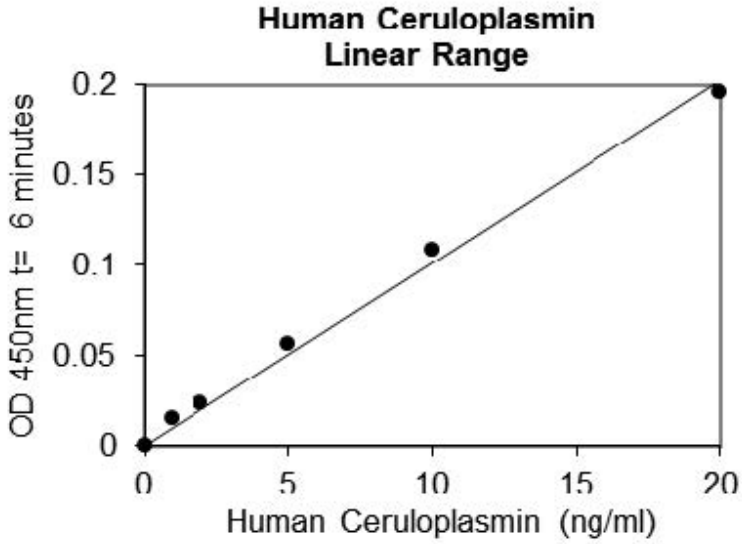
Interpretation Of Results

The concentration of ceruloplasmin in normal human plasma is 300µg/mL [3].

Typical Standard Curve

A typical standard curve (EXAMPLE ONLY):





Reference Values

Samples were evaluated for the presence of the antigen at varying dilutions.

Sample Type	Dilution	Mean (µg/mL)
EDTA Plasma	1:20,000	302
	1:40,000	311
Citrate Plasma	1:20,000	348
	1:40,000	328
Heparin Plasma	1:20,000	319
	1:40,000	394
Milk, centrifuged	1:100	1.76
	1:1,000	3.05
Urine, centrifuged	1:10	0.033
Saliva, centrifuged	1:16	0.046
	1:32	0.050

Sensitivity

The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD450: 0.052-0.061) and calculating the corresponding concentration. The MDD was 0.333 ng/mL.

Specificity

This assay recognizes natural human ceruloplasmin. Pooled normal plasma from mouse, rat, rabbit and sheep were assayed for cross-reactivity. No significant cross-reactivity was observed.

Linearity

To assess the linearity of the assay, human plasma samples containing high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay.

Sample	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % of Expected	102	103	105	104
Range	96-106%	96-107%	94-111%	93-114%

Recovery

These studies are currently in progress. Please contact us for more information.

Reproducibility

Intra-assay Precision: Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/mL)	5.79	11.25	16.26
Standard Deviation	0.256	0.216	0.712
CV (%)	4.42	1.92	4.38

Inter-assay Precision: These studies are currently in progress. Please contact us for more information.

Precautions

1. FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
2. Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
3. Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
4. Keep plate covered except when adding reagents, washing, or reading.
5. DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
6. DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

References

1. Takahashi N, et al.: Proc Natl Acad Sci USA. 1984, 81:390-394.
2. Hellman NE and Gitlin JD: Annu Rev Nutr. 2002, 22:439-458.
3. Hirschman SZ, et al.: Ann NY Acad Sci. 1961, 94:960-969.