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Human Ceruloplasmin ELISA Kit

Catalog No: CKH015A **Size:** 1 x 96 wells
Catalog No: CKH015B **Size:** 5 x 96 wells

Intended use:

This Human Ceruloplasmin ELISA assay is for the quantitative determination of total ceruloplasmin in human plasma, serum, urine, milk, saliva, and cell culture supernatants. The kit has been formulated **For Research Use Only**.

Background:

Ceruloplasmin (aka Ferroxidase I) is a 132 kDa, 1,046 amino acid glycoprotein which carries 95% of serum copper by binding 6 cupric ions per molecule. 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.

Assay Principle:

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

Reagents Provided for 1 x 96 Wells:

Items	Quantity
A: Microtiter plate coated, blocked and dried with Anti-Human Ceruloplasmin	1 96-well coated microtiter strip plate (with 8 x 12-well removable strips)
B: Wash Buffer Concentrate (10x)	1 bottle of 50 ml
C: Human Ceruloplasmin Standard	1 vial (lyophilized)
D: Anti-Human Ceruloplasmin Primary pAb	1 vial (lyophilized)
E: HRP Conjugated Streptavidin	1 vial concentrated solution
F: TMB Substrate Solution*	1 bottle of 10 ml

***Hazard Information:**
Avoid skin and eye contact when using TMB substrate solution as it may be irritating to eyes, skin and respiratory system. Wear safety goggles and gloves.

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Storage and Stability:

All kit components must be stored at 4°C upon arrival. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary antibody may be stored at -80°C for later use. **DO NOT freeze/thaw the standards and primary antibody more than once.** All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.

Other Reagents and Supplies Required:

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1 N H_2SO_4 or 1 N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

Precautions:

- **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.
- Always pour substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- **DO NOT** smoke, drink or eat in areas where specimens or reagents are being handled.

Preparation of Reagents:

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

Sample Collection:

Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Assay Procedure:

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

1. Preparation of Standard

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

Dilution table for preparation of Human Ceruloplasmin Standard:

Human Ceruloplasmin Concentration (ng/ml)	Dilutions
1,000	(from Vial)
500	500 µl (BB) + 500 µl (1,000 ng/ml)
200	600 µl (BB) + 400 µl (500 ng/ml)
100	500 µl (BB) + 500 µl (200 ng/ml)
50	500 µl (BB) + 500 µl (100 ng/ml)
20	600 µl (BB) + 400 µl (50 ng/ml)
10	500 µl (BB) + 500 µl (20 ng/ml)
5	500 µl (BB) + 500 µl (10 ng/ml)
2	600 µl (BB) + 400 µl (5 ng/ml)
1	500 µl (BB) + 500 µl (2 ng/ml)
0	500 µl (BB) Zero point to determine background

NOTE: Dilutions for the standard curve and zero standard must be made and applied to the plate immediately.

2. Standard and Unknown Addition

Remove microtiter plate from bag and add 100 µl Ceruloplasmin Standards (in duplicate) and unknowns to wells. Carefully record the position of the standards and unknowns. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or Kimwipe.

NOTE: The assay measures Ceruloplasmin levels in the 1-1,000 ng/ml range. If the unknown is thought to have high Ceruloplasmin levels, dilutions must be made in blocking buffer or the OD readings will be out of range. A 1:10,000-1:50,000 dilution for normal human plasma or serum and a 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 the best results.

3. Primary Antibody Addition

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

4. Streptavidin-HRP Addition

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

5. Substrate Incubation

Add 100 µl 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 50 µl of 1 N H_2SO_4 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.

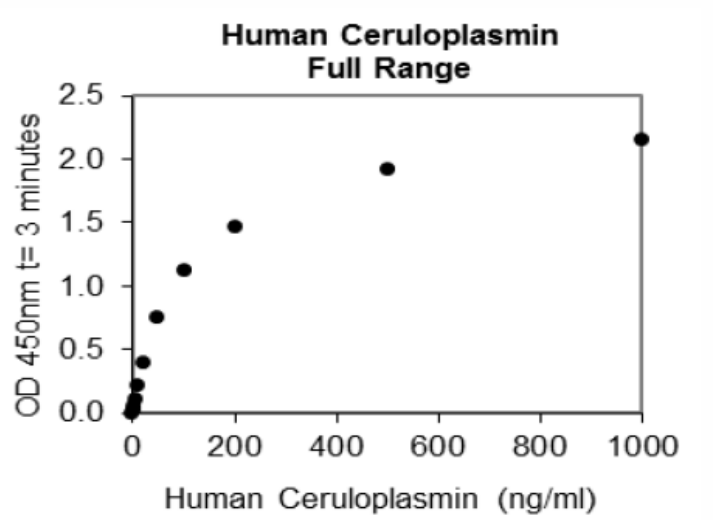
6. Measurement

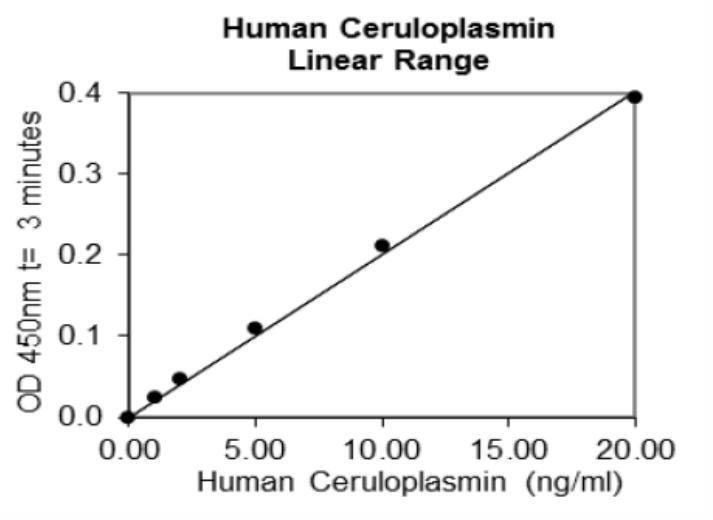
Set the absorbance at 450 nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450 nm. For best results, read plate immediately. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

7. Calculation of Results

Plot the A_{450} values against the amount of ceruloplasmin in the standards. Fit a straight line through the points 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.

A typical standard curve (EXAMPLE ONLY):





Expected Values:
The concentration of ceruloplasmin in normal human plasma is 300 µg/ml.

Performance Characteristics:

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 OD_{450}: 0.052-0.061) and calculating the corresponding concentration. The MDD was 0.333 ng/ml.

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Sample 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

Example of ELISA Plate Layout:
96 Well Plate: 22 Standard wells, 74 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	200 ng/ml	500 ng/ml	1,000 ng/ml	
B	0	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	200 ng/ml	500 ng/ml	1,000 ng/ml	
C												
D												
E												
F												
G												
H												

Important Note: This is a generic data sheet and may be subject to change. Please see the package insert shipped with your product for current data.

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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