Human Ceruloplasmin
ELISA Quantitation Kit

Manual

Catalog number: 40-288-20074F

For the quantitative determination of human Ceruloplasmin levels in serum or other biological samples.

This kit is for research use only, and is not for use in diagnostic procedures.

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Kit Contents:

Coating Antibody (Catalog # 15-288-20074F)

Affinity-purified Chicken anti-Human Ceruloplasmin
Concentration: 0.2 mg/ml
Volume: 1 ml
Working Dilution: 1/100

Calibrator (Catalog # 10-288-20074)

Pure Human Ceruloplasmin Antigen
Concentration: 1 mg/ml
Quantity: 100 µl
Working Range: 250 – 3.9 ng/ml

HRP Detection Antibody (Catalog # 27-288-20074F)

Affinity-purified Chicken anti-Human Ceruloplasmin – HRP Conjugate
Concentration: 0.6 mg/ml
Quantity: 100 µl

Buffers, Substrate and Plates not included.

Notes:

Range of Detection: 250 – 3.9 ng/ml

Storage: Aliquot to 10 tubes stored at -20º C. Shelf life is one year from date of receipt.

Assay Condition: The kit performance has been optimized for the stated protocol using the materials listed and standard dilutions from 250-3.9 ng/ml of human Ceruloplasmin. *For alternative assay conditions, the operator must determine appropriate dilutions of reagents.* ELISA assay reactivity is sensitive to any variation in operator, pipetting and washing techniques, incubation time or temperature, composition or reagents, and kit age. Adjustments may be required to position the standard curve and/or samples in the desired detection range.

Specificity: By immunoelectrophoresis and ELISA the antibodies in this kit react specifically with human HDL, not with other human serum proteins. The following factors prepared in the detection range of this kit, 250-3.9 ng/mL were assayed and exhibited no cross-reactivity or interference.

Human Serum Albumin
Human Transferrin
Human IgG

Country of Origin: United States of America

Assay Use: For in vitro laboratory use only. Not for any clinical, therapeutic, or diagnostic use in humans or animals. Not for animal or human consumption.

Human Ceruloplasmin Quantitative ELISA Protocol

Buffer Preparation

1. Prepare the following buffers:

A. Coating Buffer, 0.05 M Carbonate-Bicarbonate, pH 9.6
B. Wash Solution, 0.05% Tween 20 in PBS, pH 7.4
C. Blocking (Postcoat) Solution, 50 mM Tris, 0.14 M NaCl, 1% BSA, pH 8.0
D. Sample/Conjugate Diluents, 50 mM Tris, 0.14 M NaCl, 1% BSA, 0.05% Tween 20, pH 8.0
E. Enzyme Substrate, TMB (OPD or ABTS can be used)
F. Stopping Solution, 2 M H_2SO_4 or other appropriate solution

Step-by-Step Method (Perform all steps at room temperature)

1. Coat with Capture Antibody

A. Determine the number of single wells needed. Standards, samples, blanks and/or controls should be analyzed in duplicate. Insert the required number of microtiter well strips into a holder.
B. Dilute 1 µl capture antibody to 100 µl Coating Buffer for each well to be coated. (Example: for 32 wells dilute 34 µl to 3.4 ml)
C. Incubate coated plate for 60 minutes.
D. After incubation, aspirate the Capture Antibody solution from each well.
E. Wash each well with Wash Solution as follows:
   1. Fill each well with Wash Solution
   2. Remove Wash Solution by aspiration
   3. Repeat for a total of 3 washes.

2. Blocking (Postcoat)

A. Add 200 µl of Blocking (Postcoat) Solution to each well.
B. Incubate 30 minutes.
C. After incubation, remove the Blocking (Postcoat) Solution and wash each well three times as in Step 1.E.

3. Standards and Samples

A. Dilute the standards in Sample Diluent according to the chart below:

Step	ng/ml	Calibrator	Sample Diluent
1	10000	5 ul	495 ul
2	250	25 ul from step 1	975 ul
3	125	500 ul from step 2	500 ul
4	62.5	500 ul from step 3	500 ul
5	31.25	500 ul from step 4	500 ul
6	15.625	500 ul from step 5	500 ul
7	7.8	500 ul from step 6	500 ul
8	3.9	500 ul from step 7	500 ul

B. Dilute the samples, based on the expected concentration of the analyte, to fall within the concentration range of the standards.
C. Transfer 100 µl of standard or sample to assigned wells.
D. Incubate plate 60 minutes.
E. After incubation, remove samples and standards and wash each well 5 times as in Step 1.E.

4. HRP Detection Antibody

A. Dilute the HRP conjugate in Conjugate Diluent. Recommended starting dilution is 1:5,000 with a range of 1:3,000 to 1:6,000. (Note: Adjustments in dilution may be needed depending on substrate used, incubation time, and age of kit).
B. Transfer 100 µl to each well.
C. Incubate 60 minutes.
D. After incubation, remove HRP Conjugate and wash each well 5 times as in Step 1.E.

5. Enzyme Substrate Reaction

A. Prepare the substrate solution according to the manufacturer's recommendation.
B. Transfer 100 µl of substrate solution to each well.
C. Incubate plate 20-30 minutes.
D. To stop the TMB reaction, apply 100 µl of 2 M H_2SO_4 to each well. If using another substrate, use the stop solution recommended by manufacturer.

6. Plate Reading

Using a microtiter plate reader, read the plate at the wavelength that is appropriate for the substrate used (450 nm for TMB).

Calculation of Results

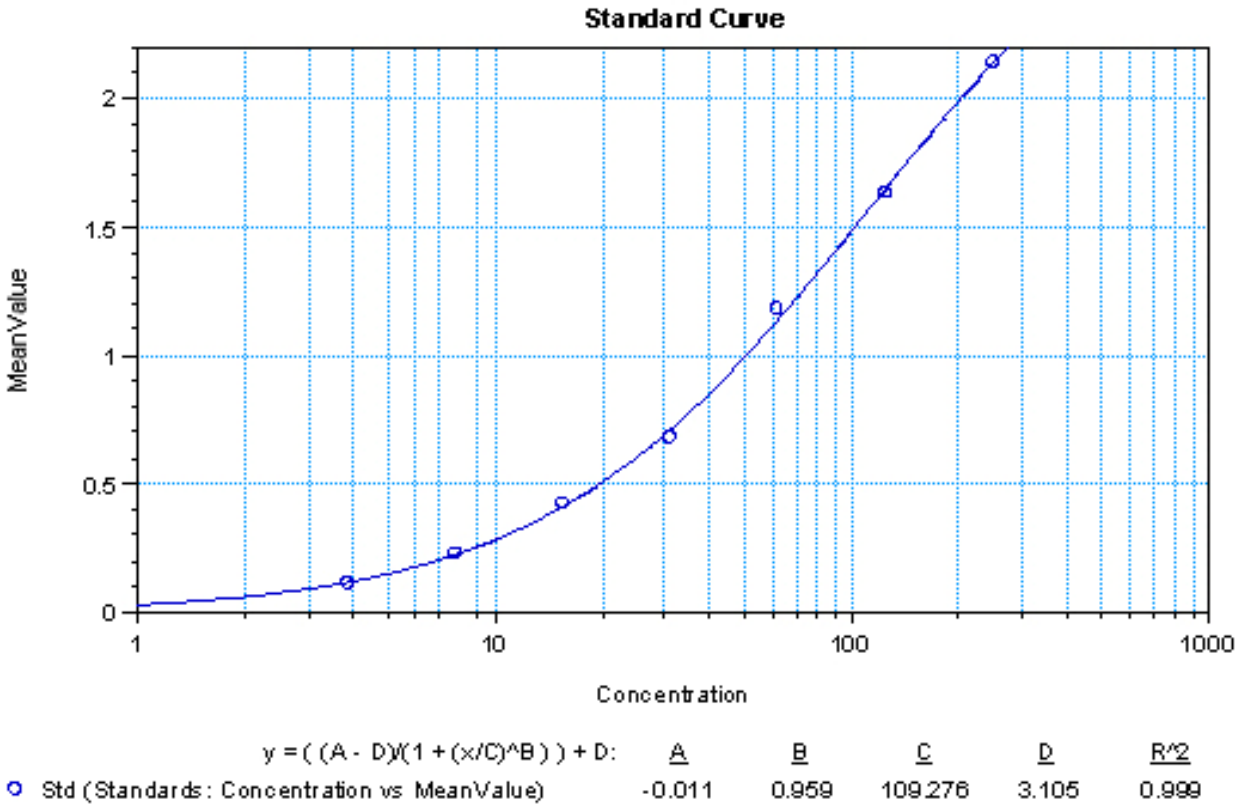
1. Average the duplicate readings from each standard, control, and sample.
2. Subtract the zero reading from each averaged value above.
3. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. Other curve fits may also be used.
4. A standard curve should be generated for each set of samples. See example below:

Standards (ng/ml)

Sample	Concentration	BackCalcConc	Wells	Values	MeanValue	Std.Dev.	CV%
St01	250	244.598	A1	2.121	2.139	0.026	1.2
		259.177	A2	2.158			
St02	125	122.409	B1	1.632	1.629	0.004	0.2
		121.59	B2	1.627			
St03	62.5	64.354	C1	1.16	1.172	0.018	1.5
		66.681	C2	1.185			
St04	31.25	31.118	D1	0.708	0.678	0.042	6.3
		27.691	D2	0.648			
St05	15.625	15.754	E1	0.41	0.421	0.016	3.9
		16.803	E2	0.433			
St06	7.813	7.611	F1	0.214	0.219	0.008	3.6
		8.032	F2	0.225			
St07	3.906	3.8	G1	0.109	0.11	0.002	1.9
		3.903	G2	0.112			

Smallest standard value: 0.110

Largest standard value: 2.139



Technical Hints

1. The Capture antibody should be diluted with coating buffer immediately prior to its addition to the wells. Coated plates are stable overnight at 4ºC when covered.
2. Change pipette tips between each addition of standard, sample and reagents to avoid cross-contamination.
3. Standards and samples should be pipetted to the bottom of the wells and all other reagents should be added to the side of the wells to avoid contamination.
4. Ensure that all buffers are not contaminated or expired. When troubleshooting ELISA results, it is recommended to prepare all new buffers in new vessels.
5. Do not add Sodium Azide to any of the buffers.
6. Sample and Conjugate dilutions should be made shortly before use.
7. Wash buffer should be aspirated from wells, as pouring wash buffer from wells may cause cross-contamination.
8. When preparing dilutions, wipe excess antibody/analyte from pipette tips to ensure accurate dilutions.
9. Incubation time of the Enzyme Substrate will depend on the substrate used and the intensity of the color change. The high standard should have an O.D. reading of between 1 and 3. The low standard should have an O.D. reading above background.
10. The Stopping solution should be added to the wells in the same order as the Enzyme Substrate.

Troubleshooting

The following are some common problems encountered with the use of ELISA kits, and some of the causes of these problems.

1. Problem: Low absorbance

- □ Incorrect dilutions or pipetting errors.
- □ Improper incubation times
- □ Improper mixing of the TMB substrate. Each component is mixed in equal parts.
- □ Wrong filter on microtiter reader. Wavelength should be 450 nm for TMB, 490 nm for OPD, or 405 nm for ABTS.
- □ Kit materials or reagents are contaminated or expired.
- □ Incorrect reagents used.

2. Problem: High Absorbance

- □ Cross contamination from other samples or positive control.
- □ Incorrect dilutions or pipetting errors.
- □ Improper washing.
- □ Wrong filter on microtiter reader.
- □ Contaminated buffers or enzyme substrate.
- □ Improper incubation times.
- □ Kit materials or reagents are contaminated or expired.

3. Problem: Poor Duplicates

- □ Poor mixing of specimens.
- □ Incorrect dilutions or pipetting errors.
- □ Technical error.
- □ Inconsistency in following ELISA protocol.
- □ Inefficient washing.

4. Problem: All wells are positive

- □ Contaminated buffers or enzyme substrate.
- □ Incorrect dilutions or pipetting errors.
- □ Kit materials or reagents are contaminated or expired.
- □ Inefficient washing.

5. Problem: All wells are negative

- □ Procedure not followed correctly.
- □ Contaminated buffers or enzyme substrate.
- □ Contaminated conjugate.
- □ Kit materials or reagents are contaminated or expired.