# Human Ceruloplasmin ELISA Kit

ARG83640 Human Ceruloplasmin ELISA Kit is an Enzyme Immunoassay kit for the quantification of Human Ceruloplasmin in serum and plasma

Catalog number: ARG83640

Package: 96 wells

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## INTRODUCTION

The protein encoded by this gene is a metalloprotein that binds most of the copper in plasma and is involved in the peroxidation of Fe(II)transferrin to Fe(III) transferrin. Mutations in this gene cause aceruloplasminemia, which results in iron accumulation and tissue damage, and is associated with diabetes and neurologic abnormalities. Two transcript variants, one protein-coding and the other not protein-coding, have been found for this gene. [provided by RefSeq, Feb 2012]

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A capture antibody specific for Human Ceruloplasmin has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Human Ceruloplasmin present is bound by the immobilized antibody. After washing away any unbound substances, added Antibody Conjugate specific for Human Ceruloplasmin to each well and incubate. After washing away any unbound substances, the TMB substrate is added to the wells and color develops in proportion to the amount of Human Ceruloplasmin bound in the initial step. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of Human Ceruloplasmin in the samples is then determined by comparing the O.D. of samples to the standard curve.

## MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Use the kit before expiration date.

| Component | Quantity | Storage information |
|---|---|---|
| Antibody Coated microplate | 1 plate | 4°C |
| Standards (lyophilized) | 1 vial | 4°C |
| 5X Diluent Buffer | 50 mL | 4°C |
| 100X Antibody Conjugate | 150 µL | 4°C |
| 20X Wash Buffer | 50 mL | 4°C |
| TMB substrate | 12 mL (ready to use) | 4°C<br>(protect from light) |
| STOP solution | 12 mL (ready to use) | 4°C |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Mixer or Ultra-Turrax
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

## TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (20-25°C).
- The reagent preparation method might be different from lot to lot, so please check the lot and follow the instructions given in this manual.
- Briefly spin down the all vials before use.
- If crystals are observed in the 20X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

## SAMPLE COLLECTION & STORAGE INFORMATION

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

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum/plasma sample.
3. Samples containing sodium azide should not be used in the assay.
4. Store all samples on ice after preparation and use immediately or aliquot and store at-80°C. Avoid repeated freeze-thaw cycles.

## REAGENT PREPARATION

- **1X Wash Buffer**: Dilute 20X Wash Buffer into **distilled water** to yield 1X Wash Buffer.
- **1X Diluent Buffer:** Dilute 5X Diluent Buffer into **distilled water** to yield 1X Wash Buffer.
- **1X Antibody Conjugate:** Dilute 100X Antibody Conjugate into **1X Diluent Buffer** to yield 1X Antibody Conjugate.
- **Standards**: Reconstitute 1 vial of Standards in 1 mL distilled water to a concentration of **200 ng/mL**. Then dilute in 1X Diluent Buffer to prepare a series diluted Standards according to the table below. Do not store the diluted Standards.

| Standard tube | Final Standards conc. (ng/mL) | Volume of 1X Diluent Buffer (µL) | Volume of 200 ng/mL Standard (µL) |
|---|---|---|---|
| S1 | 200 | 0 | 500 |
| S2 | 100 | 500 | 500 of S1 |
| S3 | 50 | 500 | 500 of S2 |
| S4 | 25 | 500 | 500 of S3 |
| S5 | 12.5 | 500 | 500 of S4 |
| S6 | 6.25 | 500 | 500 of S5 |
| S7 | 3.125 | 500 | 500 of S6 |
| S0 | 0 | 500 | 0 |

## ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

1. Add **100 µL** of **Samples** and **Standards** to the **Antibody Coated microplate**. Cover the plate with the Plate Sealer.
2. Incubate for **1 hour** at **room temperature.**
3. Aspirate each well and wash, repeating the process 3 times for a total 4 washes. Wash by filling each well with **1X Wash Buffer (300 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of the **1X Antibody Conjugate** per well. Then cover the plate with the Plate Sealer. Incubate for **20 min** at **room temperature**.
5. Aspirate and wash plate as in step 3.
6. Add **100 µL** of **TMB Substrate** to each well, including the blank wells. Incubate for **7- 15 minutes** at **room temperature** in the dark.
7. Immediately Add **100 µL** of **Stop Solution** to each well, including the blank wells. The color of the solution should change from blue to yellow.
8. Read the OD with a microplate reader at **450 nm** immediately. It is recommended reading the absorbance **within 10 minutes** after adding the stop solution.

## CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of Controls, standards and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.