KAMIYA BIOMEDICAL COMPANY

Chicken Ceruloplasmin ELISA

For the quantitative determination of ceruloplasmin in chicken serum

Cat. No. KT-773

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
The **K-ASSAY®** Chicken Ceruloplasmin ELISA is an enzyme immunoassay for the quantitative determination of ceruloplasmin in chicken serum. For research use only.

INTRODUCTION
Ceruloplasmin is an acute phase protein that is elevated in serum following injury, infection or disease. In chickens, levels increase up to 40-fold after infection with Salmonella. Measurement of ceruloplasmin provides a convenient marker of inflammation and disease.

PRINCIPLE
The **K-ASSAY®** Chicken Ceruloplasmin ELISA uses affinity purified chicken ceruloplasmin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated chicken ceruloplasmin antibodies for detection. The test sample is diluted and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. Ceruloplasmin molecules, if present, are sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies and TMB Reagent is added and incubated for 20 minutes. This resuls in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured at 450 nm. The concentration of ceruloplasmin is proportional to the optical density of the test sample and is determined from a calibration curve.

COMPONENTS
- Anti-chicken ceruloplasmin antibody coated microtiter plate with 96 wells (12 detachable strips of 8-wells)
- Enzyme Conjugate Reagent, 11 mL
- Ceruloplasmin stock (lyophilized)
- 10X Diluent, 25 mL
- 20X Wash Solution, 50 mL
- TMB Reagent (One-Step), 11 mL
- Stop Solution (1N HCl), 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Precision pipettes and tips.
- Distilled or de-ionized water.
- Polypropylene or glass tubes.
- Vortex mixer.
- Absorbent paper or paper towels.
- Micro-Plate incubator/shaker with an approximate mixing speed of 150 rpm.
- A microtiter plate reader capable of reading at 450 nm.
- PC graphing software or graph paper.

GENERAL INSTRUCTIONS
All reagents should be allowed to reach room temperature (18-25°C) before use.

DILUENT PREPARATION

The diluent is provided as a 10X stock. Prior to use estimate the final volume of diluent required for your assay and dilute one (1) volume of the 10X stock with nine (9) volumes of distilled or de-ionized water.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20X stock. Prior to use dilute the contents of the bottle (50 mL) with 950 mL of distilled or de-ionized water.

CALIBRATOR PREPARATION

1. The chicken ceruloplasmin calibrator is provided as a lyophilized stock. Add the volume of distilled or de-ionized water indicated on the vial label and mix gently until dissolved.
2. Label 6 polypropylene or glass tubes as 50, 25, 12.5, 6.25, 3.13 and 1.56 ng/mL.
3. In the tube labeled 50 ng/mL prepare the 50 ng/mL calibrator as directed on the stock vial label.
4. Dispense 250 µL of diluent into the tubes labeled 25, 12.5, 6.25, 3.13 and 1.56 ng/mL.
5. Prepare the 25 ng/mL calibrator by diluting and mixing 250 µL of the 50 ng/mL calibrator with 250 µL of diluent in the tube labeled 25 ng/mL. Similarly prepare the remaining calibrators by serial dilution.

Note: The reconstituted stock should be aliquoted and frozen at -20°C after reconstitution if further use is intended.

SAMPLE PREPARATION

General note: Studies indicate that ceruloplasmin is present in normal chicken serum at a concentration of ~ 150 µg/mL. In order to obtain values within the range of the calibration curve we suggest that samples initially be diluted 10,000 fold using the following procedure for each sample to be tested:

1. Dispense 198 µL and 297 µL of diluent into separate tubes.
2. Pipette and mix 2 µL of the serum/plasma sample into the tube containing 198 µL of diluent. This provides a 100 fold diluted sample.
3. Mix 3 µL of the 100 fold diluted sample with the 297 µL of diluent in the second tube. This provides a 10,000 fold dilution of the sample.
4. Repeat this procedure for each sample to be tested.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of calibrators and samples into the wells (we recommend that samples be tested in duplicate.
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 45 minutes.
4. Empty and wash the microtiter wells 5 times with 1X wash solution. This may be performed using either a plate washer (400 µL/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
5. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets.
6. Add 100 µL of enzyme conjugate reagent into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 45 minutes.
8. Wash as detailed in 4 above.
9. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets.
10. Dispense 100 µL of TMB Reagent into each well.
11. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 20 minutes.
12. Stop the reaction by adding 100 µL of Stop Solution to each well.
13. Gently mix. It is important to make sure that all the blue color changes to yellow.
14. Read the optical density at 450 nm with a microtiter plate reader within 5 minutes.

CALCULATION OF RESULTS

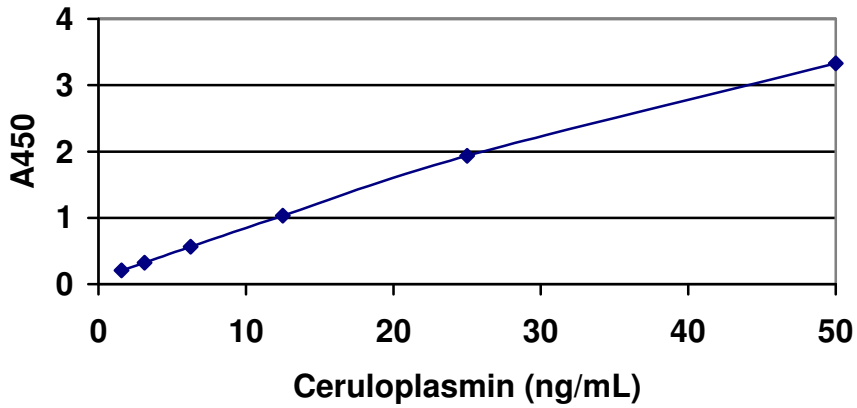
1. Calculate the average absorbance values (A_{450}) for each set of reference calibrators, and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of ceruloplasmin in ng/mL from the calibration curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of ceruloplasmin in the serum/plasma sample.
5. If available, PC graphing software may be used for the above steps.

6. If the OD_{450} values of samples fall outside the calibration curve when tested at a 10,000 fold dilution, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve with optical density readings at 450 nm on the Y-axis against ceruloplasmin concentrations on the X-axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and calibration curve in each experiment.

Ceruloplasmin (ng/mL)	A450
50	3.331
25	1.934
12.5	1.031
6.25	0.563
3.13	0.321
1.56	0.207



STORAGE

The kit should be stored at 4℃ and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. The expiration date of the kit is indicated on the box label.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

FOR RESEARCH USE ONLY

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