KAMIYA BIOMEDICAL COMPANY

Cat Ceruloplasmin ELISA

For the quantitative determination of ceruloplasmin in cat serum and plasma

Cat. No. KT-1879

For Research Use Only.

PRODUCT INFORMATION

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

INTRODUCTION
Ceruloplasmin is an acute phase protein that may be elevated in serum during injury, infection and disease. Studies indicate that ceruloplasmin levels increase up to 8- fold during the acute phase response in cats. Ceruloplasmin is usually measured via its oxidase activity; a somewhat cumbersome method. This ELISA allows rapid measurement of ceruloplasmin in cat serum and plasma.

PRINCIPLE
The cat ceruloplasmin ELISA uses affinity purified anti-cat ceruloplasmin for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-cat ceruloplasmin for detection. Samples are diluted and incubated alongside calibrators in wells of a 96-well plate for 45 minutes. The wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. Ceruloplasmin molecules, if present, are sandwiched between the immobilization and HRP-conjugated antibodies. The wells are then washed to remove unbound HRP-conjugate and TMB reagent is added and incubated for 20 minutes. This results in the development of a blue color. Color development is stopped by the addition of Stop solution, changing the color to yellow, and absorbance is measured at 450 nm. The concentration of ceruloplasmin is proportional to absorbance and is derived from a calibration curve.

COMPONENTS
- Anti-cat ceruloplasmin coated 96 well plate (12 x 8-well strips)
- HRP-conjugate, 11 mL
- Ceruloplasmin stock (lyophilized)
- 10X Diluent, 25 mL
- 20X Wash Solution, 50 mL
- TMB, 11 mL
- Stop solution, 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Precision pipettors and tips.
- Distilled or de-ionized water.
- Microcentrifuge tubes.
- Vortex mixer.
- Absorbent paper or paper towels.
- Micro-Plate incubator/shaker.
- Plate reader capable of measuring at 450 nm.
- Graphing software.

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

Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

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 cat 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 8 microcentrifuge tubes as 100, 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 ng/mL.
3. In the tube labeled 100 ng/mL, prepare the 100 ng/mL calibrator as described on the stock vial label.
4. Dispense 250 µL of diluent into the tubes labeled 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 ng/mL.
5. Prepare the 50 ng/mL calibrator by diluting and mixing 250 µL of the 100 ng/mL calibrator with 250 µL of diluent in the appropriate tube.
6. Similarly prepare the remaining calibrators by 2-fold serial dilution.

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

SAMPLE PREPARATION

Studies indicate that ceruloplasmin is present in cat serum at concentrations ranging from 100 to 800 µg/mL. To obtain values within the range of the calibration curve we suggest that samples initially be diluted 10,000-fold using the following procedure.

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. Unused strips should be stored in the re-sealed bag with desiccant at 4°C for future use.
2. Dispense 100 µL of calibrators and samples into the wells (we recommend that calibrators and samples be run in duplicate).
3. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 45 minutes.
4. Empty and wash the microtiter wells 5x with 1X wash solution using a plate washer (400 µL/well).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 µL of HRP-conjugate into each well.
7. Incubate on a plate shaker at 150 rpm and 25°C for 45 minutes.
8. Wash as detailed above.
9. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
10. Dispense 100 µL of TMB into each well.
11. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
12. After 20 minutes, 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 absorbance at 450 nm with a plate reader within 5 minutes.

CALCULATION OF RESULTS

1. Using graphing software, construct a calibration curve by plotting absorbance values of the calibrators versus the $\log_{10}$ of the ceruloplasmin concentration.

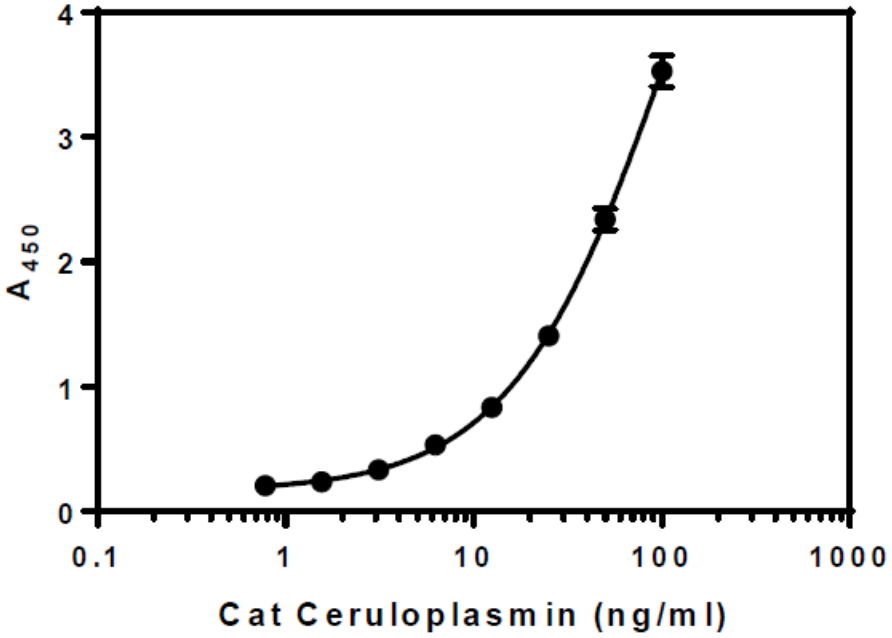
2. Fit the calibration curve to a four-parameter logistic regression (4PL) equation (x axis = $\log_{10}$ ceruloplasmin concentration).

3. Derive the corresponding concentration of ceruloplasmin in the samples from the calibration curve (remember to derive the concentration from the antilog).
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of ceruloplasmin in the serum or plasma sample.
5. If the A_{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 is shown below. This curve is for 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)	A_{450}
100	3.527
50	2.342
25	1.408
12.5	0.835
6.25	0.534
3.13	0.334
1.56	0.238
0.78	0.209



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.

FOR RESEARCH USE ONLY

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com