Cp (Rat) ELISA Kit

Catalog Number KA2502

96 assays

Version: 01

Intended for research use only

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Introduction

Intended Use

Enzyme Immunoassay for the Quantitative Determination of Rat Ceruloplasmin in Serum or Plasma.

Background

Ceruloplasmin is an acute phase protein that is elevated in serum as a result of injury, infection or disease. Studies indicate that ceruloplasmin levels increase 10-20 fold during chronic disease in rats. Measurement of ceruloplasmin provides a convenient marker of inflammation and disease.

Principle of the Assay

Cp (Rat) ELISA Kit is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified anti-rat ceruloplasmin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-rat 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. This result in ceruloplasmin molecules being 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 at room temperature. 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 optical density is measured spectrophotometrically at 450 nm. The concentration of ceruloplasmin is proportional to the optical density of the test sample.

General Information

Materials Supplied

List of component

Component	Amount
Anti-rat ceruloplasmin antibody coated microtiter plate	96 wells (12 detachable strips of 8)
Ceruloplasmin stock	Lyophilized
10x Diluent	25 ml
Enzyme Conjugate Reagent	11 ml
20x Wash Solution	50 ml
TMB Reagent	11 ml
Stop Solution (1N HCl)	11 ml

Storage Instruction

The kit should be stored at 2-8°C and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable for six months from the date of purchase.

Materials Required but Not Supplied

- ✓ Precision pipettes and tips.
- ✓ Distilled or deionized 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 at 450 nm wavelength, with a bandwidth of 10 nm or less and an optical density range of 0-4
- ✓ Graph paper (PC graphing software is optional)

Precautions for Use

- Health and safety Precautions
- ✓ Avoid contact with 1N HCl (stop solution). It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.

- ✓ Do not use reagents after expiration date and do not mix or use components from different kits.
- ✓ Replace caps on reagents immediately. Do not switch caps.
- ✓ Do not pipette reagents by mouth.

- Procedural Notes
- ✓ All reagents should be allowed to reach room temperature (18- 25°C) before use.
- ✓ Serum or plasma samples should be diluted ~10,000 fold with diluent in order to obtain values within the standard range.

- Limitations of the procedure
- ✓ 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.

Assay Protocol

Reagent Preparation

- 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 deionized 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 deionized water.

- Standard Preparation

1. The rat ceruloplasmin standard 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 to obtain a 7.5 µg/ml rat ceruloplasmin stock. (the reconstituted standard should be aliquoted and frozen at -20°C after reconstitution if further use is intended).
2. Label 8 polypropylene or glass tubes as 300, 150, 75, 37.5, 18.8, 9.4, 4.7 and 0 ng/ml.
3. Dispense 960 µl of diluent into the tube labeled 300 ng/ml and 500 µl of diluent into the tubes labeled 150, 75, 37.5, 18.8, 9.4, 4.7 and 0 ng/ml.
4. Prepare a 300 ng/ml standard by diluting and mixing 40 µl of the reconstituted 7.5 µg/ml standard with 960 µl of diluent in the appropriately labeled tube.
5. Prepare a 150 ng/ml standard by diluting and mixing 500 µl of the 300 ng/ml standard with 500 µl of diluent in the tube labeled 150 ng/ml. Similarly prepare the 75, 37.5, 18.8, 9.4, 4.7 ng/ml standards by serial dilution.

Sample Preparation

General Note: Studies indicate that ceruloplasmin is present in normal rat serum at a concentration of ~ 0.5 mg/ml. In order to obtain values within the range of the standard 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 standards 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 (18-25°C) for 45 minutes.
4. Empty and wash the microtiter wells 5 times with 1x wash solution. This may 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 absorbent 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 (18-25°C) for 45 minutes.
8. Wash as detailed in 4 above.
9. Strike the wells sharply onto absorbent paper or paper towels to remove 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 (18-25°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.

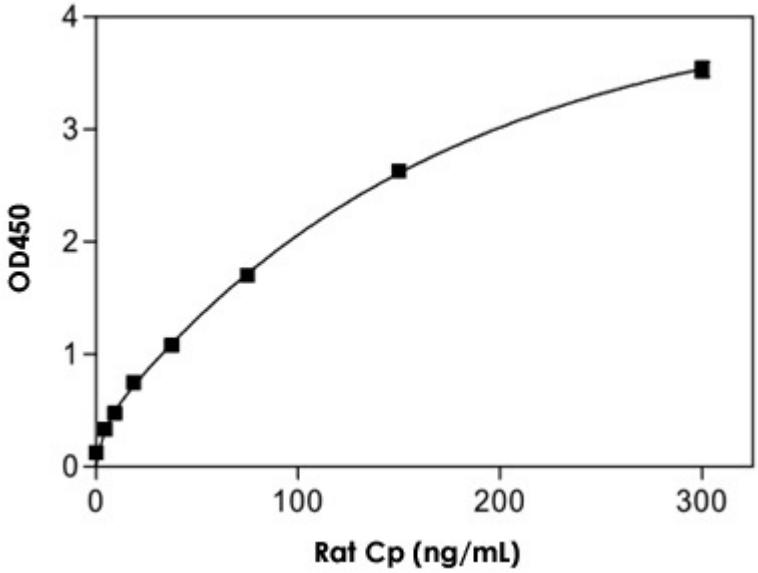
Data Analysis

Calculation of Results

1. Calculate the average absorbance values (A_{450}) for each set of reference standards, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard 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 standard curve.
4. Multiply the derived concentrations 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 standard curve when tested at a dilution of 10,000. Samples should be diluted appropriately and re-tested.

A typical standard curve with optical density readings at 450nm 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 standard curve in each experiment.

Ceruloplasmin (ng/ml)	Absorbance (450 nm)
300	3.533
150	2.627
75	1.702
37.5	1.080
18.8	0.748
9.4	0.474
4.7	0.331
0	0.124


OD450
4
3
2
1
0
0
100
200
300
Rat Cp (ng/mL)

Resources

Plate Layout

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								