

# CHICKEN CERULOPLASMIN ELISA

## Life Diagnostics, Inc., Catalog Number: CER-5

### INTRODUCTION

Ceruloplasmin, the major copper transport protein in blood, is an acute phase protein that is elevated in serum because of injury, infection or disease. In chickens, levels increase up to 40-fold after infection with *Salmonella*.<sup>1</sup> Measurement of ceruloplasmin provides a convenient marker of inflammation and disease.

### PRINCIPLE OF THE ASSAY

The assay uses affinity purified chicken ceruloplasmin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated chicken ceruloplasmin antibodies for detection. Standards and diluted samples are incubated in the microtiter wells for 45 minutes. The wells are subsequently washed. HRP conjugate is added and incubated for 45 minutes. This results in ceruloplasmin molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate. TMB is added and incubated for 20 minutes. If ceruloplasmin is present a blue color develops. Color development is stopped by the addition of Stop solution, changing the color to yellow. Absorbance is measured at 450 nm. The concentration of ceruloplasmin is proportional to absorbance and is derived from a standard curve.

### MATERIALS AND COMPONENTS

#### Materials provided with the kit:

- Ceruloplasmin antibody coated plate (12 x 8-well strips)
- HRP Conjugate, 11 ml
- Ceruloplasmin stock
- 20x Wash solution: TBS50-20, 50 ml
- 10x Diluent: YD25-10, 25 ml
- TMB: TMB11-1, 11 ml
- Stop Solution: SS11-1, 11 ml

#### Materials required but not provided:

- Pipettors and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm.
- Curve fitting software

### STORAGE

The kit should be stored at 4°C and the microtiter plate should be kept in a sealed bag with desiccant. The kit will remain stable for six months from the date of purchase.

### GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.

### 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 volume of the 10x stock with nine 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 chicken ceruloplasmin stock is provided lyophilized. Add the volume of distilled or deionized water indicated on the vial label and mix gently until dissolved. **(the reconstituted stock should be aliquoted and frozen at -20°C after reconstitution if further use is intended).**
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 labelled 50 ng/ml prepare the 50 ng/ml standard as directed on the stock vial label.
4. Dispense 250 µl of diluent into the tubes labelled 25, 12.5, 6.25, 3.13 and 1.56 ng/ml.
5. Prepare the 25 ng/ml standard by diluting and mixing 250 µl of the 50 ng/ml standard with 250 µl of diluent in the tube labeled 25 ng/ml.
6. Similarly prepare the remaining standards by two-fold serial dilution.

### SAMPLE PREPARATION

Studies at Life Diagnostics, Inc. indicate that ceruloplasmin is present in normal chicken serum at a concentration of approximately 150 µg/ml. 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 2.0 µl of the serum/plasma sample into the tube containing 198 µl of diluent. This provides a 100-fold dilution.
3. Mix 3.0 µl of the 100-fold diluted sample with the 297 µl of diluent in the second tube. This provides a 10,000-fold dilution.

### ASSAY PROCEDURE

1. Secure the desired number of 8-well strips 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 standards and samples into the wells (we recommend that standards 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  $\mu$ l 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 residual droplets.
10. Dispense 100  $\mu$ l 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  $\mu$ l 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.

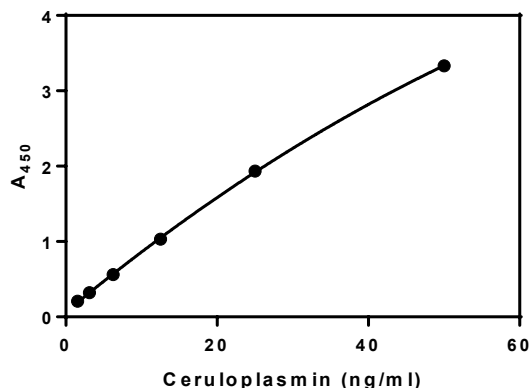
### CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus concentration.
2. Fit the standard curve to an appropriate model and derive the concentration of the samples (we recommend using a single site, total and nonspecific binding model).
3. Multiply the derived concentration by the dilution factor to determine the concentration in the sample.
4. If the  $A_{450}$  values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

### TYPICAL STANDARD CURVE

A typical standard curve is shown below. This curve is for illustration only and should not be used to calculate unknowns.

Ceruloplasmin (ng/ml)	Absorbance (450 nm)
50	3.331
25	1.934
12.5	1.031
6.25	0.563
3.13	0.321
1.56	0.207



### REFERENCES

1. Garcia KO, et al. Experimental infection of commercial layers using a Salmonella enterica serovar Gallinarium strain: Leukogram and serum acute-phase protein concentrations. Brazilian Journal of Poultry Science. 11:263-270 (2009)

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