MOUSE CLUSTERIN ELISA
Life Diagnostics, Inc., Catalog Number: CLUS-1

INTRODUCTION

Clusterin, also referred to as apolipoprotein J, sulfated glycoprotein-2, glycoprotein III and testosterone-repressed prostate message-2, is a glycoprotein of 70-80 kDa. It is comprised of one α-subunit and one β-subunit derived from proteolytic cleavage of a precursor peptide. Clusterin is expressed in many tissues and is found in serum, seminal fluid and urine. It has been identified as a potential biomarker of various forms of renal injury and prostate disease.

PRINCIPLE OF THE ASSAY

The assay uses affinity purified mouse clusterin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated mouse clusterin antibodies for detection. Standards and diluted samples are added to appropriate wells of the plate. HRP conjugated antibody is then added to the wells and the plate is incubated on a plate shaker for one hour at room temperature. This results in clusterin 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 clusterin 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 clusterin is proportional to absorbance and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:
- Clusterin antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 ml
- Clusterin 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. Reconstitute the lyophilized clusterin stock as described on the vial label. Mix gently several times over a period of 5 minutes.
2. Label 7 polypropylene tubes as 250, 125, 62.5, 31.25, 15.6, 7.8 and 3.9 ng/ml.
3. Into the tube labeled 250 ng/ml, pipette the volume of 1x diluent detailed on the stock vial label. Then add the indicated volume of stock and mix gently. This provides the 250 ng/ml standard.
4. Pipette 0.25 ml of clusterin diluent into the tubes labeled 125, 62.5, 31.25, 15.6, 7.8 and 3.9 ng/ml.
5. Prepare a 125 ng/ml standard by diluting and mixing 0.25 ml of the 250 ng/ml standard with 0.25 ml of diluent in the tube labeled 125 ng/ml.
6. Similarly prepare the 62.5, 31.25, 15.6, 7.8, 3.9 ng/ml standards by serial dilution.

Unused reconstituted clusterin stock should be frozen immediately. It remains stable in frozen form for at least 1 month at -20°C and 6 months at -70°C. Discard the working 250 – 3.9 ng/ml standards after use.

SAMPLE PREPARATION

Clusterin is present in normal mouse serum or plasma at a concentration of approximately 10 μg/ml. To obtain values within range of the standard curve we suggest that samples be diluted 100-fold by mixing 3 μl of sample with 297 μl of 1x diluent.

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. Add 100 μl of HRP-conjugate into each well.
4. Incubate on a plate shaker at 150 rpm and 25°C for one hour.
5. Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 μl/well).
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
7. Dispense 100 μl of TMB into each well.

8. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
9. After 20-minutes, stop the reaction by adding 100 μl of Stop solution to each well.
10. Gently mix. It is important to make sure that all the blue color changes to yellow.
11. 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 $\log_{10}$ of the concentration.
2. Fit the standard curve to a four-parameter logistic regression (4PL) equation (x axis = $\log_{10}$ concentration) and determine the concentration of the samples from the standard curve (remember to derive the concentration from the antilog).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum or plasma 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.

Clusterin (ng/ml)	Absorbance (450 nm)
250	1.980
125	1.401
62.5	0.900
31.25	0.594
15.6	0.391
7.8	0.289
3.9	0.221
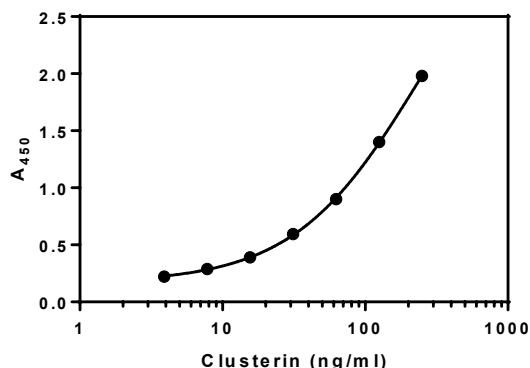
LIMITATIONS OF THE ASSAY

Urine samples can be assayed but we have not been able to fully validate the ELISA with urine due to a lack of positive control samples. Studies with clusterin-spiked urine samples indicate that best results are obtained if urine samples are either (i) diluted at least 50-fold in 1x diluent, or (ii) dialyzed against a 200-fold volume excess of de-ionized water and mixed with an equal volume of 2x diluent prior to assay. Either of these methods eliminates interfering factors. Use

of this kit for measurement of urinary clusterin levels should be performed entirely at the discretion of the researcher.

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For technical assistance please email us at
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