ELISA kits available from ADI:

Catalog#	ProdDescription
610-110-RCL	Rat Clusterin ELISA Kit
600-400-CTN	Dog Cardiac Troponin 1 (Tn-I) ELISA Kit
600-410-CTN	Human Cardiac Troponin 1 (Tn-I) ELISA Kit
600-420-CTN	Monkey Cardiac Troponin 1 (Tn-I) ELISA Kit
600-430-MTN	Monkey Skeletal Muscle Troponin1 (Tn-I) ELISA Kit
600-440-CTN	Mouse Cardiac Tn-I ELISA kit for plasma samples
600-450-CTN	Mouse Cardiac Troponin 1 (Tn-I) ELISA Kit
600-470-CTN	Pig Cardiac Troponin 1 (Tn-I) ELISA Kit
600-480-CTN	Rabbit Cardiac Tn-I ELISA kit for serum samples
600-510-MTN	Rat Skeletal Muscle Troponin 1 (Tn-I) ELISA Kit
600-600-DMY	Dog Myoglobin ELISA Kit
600-610-HMY	Human Myoglobin ELISA Kit
600-620-MMY	Monkey Myoglobin ELISA Kit
600-640-PMY	Pig Myoglobin ELISA Kit
600-650-RMY	Rabbit Myoglobin ELISA Kit
600-660-RMY	Rat Myoglobin ELISA Kit

Human: Adiponectin (Acrp30 and gAcrp30), Albumin, Aldosterone, AFP, beta-amyloid 1-40/42, Angiogenin, Angiopoietin-2, beta-2M, BMP-7, C-peptide, CRP, Cox-2, Ferritin, PSA, fPSA, GH, IgG, IgM, IgE, IgG1, IgG4, Insulin, NSE, CA125, CA199, CA242, PAP, Resistin, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, estradiol, testosterone, progesterone).

Monkey: IgM, IgG, IgA, IgE
Rat: Albumin, CRP, IgG, IgM, Alpha-1- Acid glycoprotein
Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, IgE, IgM, Leptin, Resistin, Acrp30, CRP, Troponin-I, TNF-alpha

Autoimmune Antibody detection kits for ANA, ssDNA, dsDNA, Histone, Sm, RNP, SSA, SSB, Scl70, Ovalbumin, Cardiolipin, CIC

Chicken: IgG, IgM, IgY, Ovalbumin **Turkey**: IgG
Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin
Pig: Albumin, IgG, IgM **Dog:** CRP, IgG, IgM
Cat: IgG, IgM **Sheep:** IgG **Goat:** IgG **Rabbit**: CRP, IgG
See Details at the web site or Contact ADI

Instruction Manual No. M-610100-MCL

Mouse Clusterin (MCL)

ELISA KIT Cat. # 610-100-MCL

For Quantitative Determination of Clusterin in Mouse Serum/Plasma/Urine

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Mouse Clusterin (MCL) ELISA KIT
Cat. No. 610-100-MCL

Kit Components, 96 tests	
Anti- Clusterin coated strip plate (8 wells x 12 strips) #610100-1	1 plate
MCL Reference Standard, **Lyophilized**. Reconstitute in 0.20 ml, #610100-2	1 vial
Anti-Clusterin-HRP Conjugate, 11 ml # 610100-3	1 bottle
Diluent, (10X), 25 ml #610100-4	1 bottle
Wash Solution (20X), 50 ml #610100-WB	1 bottle
TMB Substrate, 11 ml #610100-TMB	1 bottle
Stop solution, 11 ml #610100-SS	1 bottle
Instruction Manual # M-610100	1 manual

INTRODUCTION

Clusterin (apolipoprotein J) is a 75 - 80 kDa disulfide-linked heterodimeric protein associated with the clearance of cellular debris and apoptosis. It is expressed in most mammalian tissues and can be found in blood plasma, milk, urine, cerebrospinal fluid and semen.

The protein itself is a disulfide-linked heterodimeric protein containing about 30% of N-linked carbohydrate rich in sialic acid. Truncated forms targeted to the nucleus have also been identified. The precursor polypeptide chain is cleaved proteolytically to remove the 22 amino acid secretory signal peptide and subsequently between residues 227/228 to generate the alpha and beta chains.

The mature protein appears as a ≈40 kDa smear on immunoblots from reducing SDS-PAGE. The precursor form appears as a 60 kDa protein. The protein has been implicated in a variety of activities including programmed cell death, regulation of complement mediated cell lysis, membrane recycling, cell-cell adhesion and src induced transformation. As a part of the attack complex of complement, it acts as a complement inhibitor.

It is able to bind and form complexes with numerous partners such as immunoglobulins, lipids, heparin, bacteria, complement components, paraoxonase, beta amyloid, leptin and others. Clusterin has been ascribed a plethora of functions such as phagocyte recruitment, aggregation induction, complement attack prevention, apoptosis inhibition, membrane remodelling, lipid transport, hormone transport and/or scavenging and matrix metalloproteinase inhibition.

ADI's Mouse Clusterin (MCL) ELISA provides a rapid, specific and sensitive assay for measuring MCL in serum or other biological fluids.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Draw the standard curve on semi-log graph paper by plotting net absorbance values of standards against appropriate MCL concentrations. Read off the MCL concentrations of the control and patient samples. Multiply the values by the dilution factor of the samples. If samples were diluted 1:20K then the values must be multiplied by 20,000 and results are expressed as ug/ml.

If available, graphing software may be used to analyze the data. Depending on the range of the standard curve used, we find that good fits of the data may be obtained with linear regression analysis or using a two-site binding model. Alternatively, standard curves may be generated using a point-to- point fit.

PERFORMANCE CHARACTERISTICS

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

Detection Limit: The minimum MCL concentration detectable using this assay is below 3.9 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

Expected Values: Each laboratory should establish testing ranges for the animal population being investigated.

Specificity: The antibodies used in this kit are specific for Mouse MCL and have shown no cross-reactivity with other proteins.

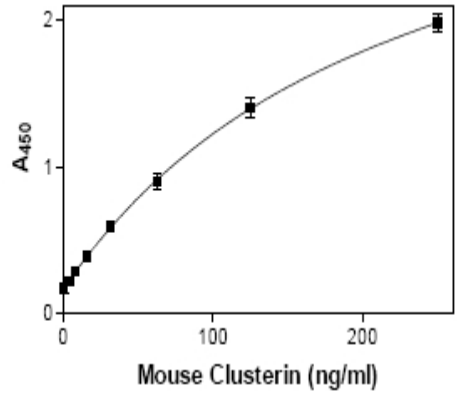
Species Crossreactivity: Cross-reactivity of Mouse MCL ELISA kit with other animals has not been tested. ADI also has a rat Clusterin ELISA kit.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 2-8^{O}C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples		Mean $A_{450\ nm}$	Calculated Concn
A1, A2	**Diluent**	0 ng/ml	0.169	
B1, B2	**Standard A**	3.9 ng/ml	0.221	
C1, C2	**Standard B**	7.8 ng/ml	0.289	
D1, D2	**Standard C**	15.6 ng/ml	0.391	
E1, E2	**Standard D**	31.2 ng/ml	0.594	
F1, F2	**Standard E**	62.5 ng/ml	0.900	
G1, G2	**Standard F**	125 ng/ml	1.401	
H1, H2	**Standard G**	250 ng/ml	1.980	
A3, A4	**Sample 1**		0.894	62 ng/ml

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical assay Curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

Mouse MCL ELISA kit is based on binding of Mouse MCL from samples to two antibodies, one immobilized on the microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of MCL present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm and the concentration of MCL in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 ul) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader.

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Mouse MCL ELISA Kit is for research use only.

Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

MSDS for TMB, sulfuric acid, if not already on file, can be requested or obtained from the ADI website.

SPECIMEN COLLECTION and HANDLING

Collect blood by venipuncture; allow clotting, and separating the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera cannot be immediately assayed, store frozen for up to six months. Plasma and urine can also be used. Avoid repeated freezing and thawing of samples. **Cell or tissues extract samples have not been optimized.**

REAGENT PREPARATION

1. **Dilute Wash Buffer (20x stock)**. Dilute the entire 50 ml with 950 ml of distilled or deionized water (total volume 1000 ml). Store at room temperature for the entire use of the kit.
2. **Diluent (10x stock)**. Dilute as needed. Mix **1 ml of diluent (10X) with 9 ml** of distilled or deionized water (**total volume 10 ml**).
3. **Reference Standard** is provided as lyophilized stock. Reconstitute as on page 3.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8oC until the expiration date printed on the label.

DILUTION OF SAMPLES

Serum or plasma samples should be diluted a 100-fold before testing. Dilute by adding 3 uL of sample to 297 uL of diluent (1X).Urine samples may show a matrix effect. So, all urine samples within an assay run should be similarly diluted. We recommend making a 50-fold dilution for urine samples by mixing 6 uL of urine with 294 uL of diluent (1X).

TEST PROCEDURE

(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

1. **Reconstitute the lyophilized MCL reference standard with 200 uL of distilled or deionized water and mix for 5 minutes. The reference concentration is given on the vial. Immediately aliquot and store** any unused reference standard at –20oC or below.

2. Prepare liquid standards using the following dilution scheme. Label 8 microcentrifuge tubes as 250, 125, 62.5, 31.2, 15.6, 7.8, 3.9 and 0 ng/ml.

3. For standard G (250 ng/ml) pipet volume of diluent (1X) and of MCL reference standard as described on the MCL reference standard vial label. Mix gently. Prepare the remaining standards as shown below.

Mouse MCL Stds	Stock Volume	MCL diluent	Final Volume
Std G (250 ng/ml)	500 uL	0	500 uL
Std F (125 ng/ml)	250 uL of Std G	250 uL	500 uL
Std E (62.5 ng/ml)	250 uL of Std F	250 uL	500 uL
Std D (31.2 ng/ml)	250 uL of Std E	250 uL	500 uL
Std C (15.6 ng/ml)	250 uL of Std D	250 uL	500 uL
Std B (7.8 ng/ml)	250 uL of Std C	250 uL	500 uL
Std A (3.9 ng/ml)	250 uL of Std B	250 uL	200 uL
Diluent (0 ng/ml)	0	250 uL	250 uL

Notes:
When preparing the serial dilutions of the standards gently mix the standards for 5-10 seconds and then take aliquots to make further dilutions. Following the above dilution scheme, you will have 250 uL of negative and all standards (B-G), and 500 ul of Std. A. You would need 200 uL of each standard (100 uL in duplicate).

Label or mark the microtiter well strips to be used on the plate.

4. Pipette **100 ul of standards and samples** into appropriate wells.

5. Pipet **100 ul of anti-MCL-HRP conjugate** into each well. Mix gently, and incubate at room temperature (20-25oC) for **60 minutes on an orbital shaker (100-150 rpm).** If an automated shaker is not available, the plate can be mixed manually every few minutes.

6. Remove or aspirate the plate contents and **wash the wells 5-6 times** with 300 ul of 1x wash buffer using an automated washer. If washing manually then dump the plate contents and tap over paper towels, add wash buffer, shake the contents of 5-10 seconds and repeat the steps. Tap the plate over fresh paper towels between each washing.

7. **Add 100 ul of TMB Substrate** into each well. Mix gently. Cover the plate and incubate for **20 minutes** at room temperature **on an orbital shaker (100-150 rpm)**. Blue color develops. This step can be reduced or increased by ± 5 minutes to keep the color within reading range. If your ELISA reader cannot read above A450 of 2.00-3.00 then reduce the incubation time.

8. Stop the reaction by adding **100 ul of stop solution** to all wells. Mix gently. Blue color turns yellow.

9. Measure the **absorbance at 450 nm** using an ELISA reader. Color is stable for at least 30 minutes after stopping.

10. Please Note: Due to plate reader differences, the high standard absorbance values may be out of range occasionally. If this occurs, absorbance values may be determined at 405 nm instead. If absorbance values exceed the high standard, the samples should be appropriately diluted and redetermined. Samples with absorbance values below those of the lowest standard should be assigned a zero MCL value