

ELISA kits available from ADI:

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, Haptoglobin, 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

Goat: IgG

Rabbit: CRP, IgG

Sheep: IgG

Instruction Manual No. 600-720-A2M

Mouse α 2-Macroglobulin (A2M) ELISA

ELISA KIT Cat. #. 600-720-A2M

For Quantitative Determination of Mouse α 2-Macroglobulin in Serum and Plasma



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Mouse α 2-Macroglobulin ELISA KIT Cat. # 600-720-A2M

Kit Components, 96 tests	
Anti-Mouse α -Macroglobulin antibody coated strip plate (8 wells x 12 strips) #600720-1	1 plate
Reference Standard, lyophilized, Reconstitute with distilled water according to vial label. #600720-2	1 vial
Enzyme Conjugate, 11 ml, #600720-3	1 bottle
Sample Diluent, 30 ml, #600720-4	1 bottle
Wash Buffer (20x), 50 ml, #600720-WB	1 bottle
TMB Substrate, 11 ml, #600720-TMB	1 bottle
Stop solution, 11 ml, #600720-SS	1 bottle
Instruction Manual, #M-600720	1 manual

INTRODUCTION

Alpha-2-Macroglobulin, also known as α 2-macroglobulin and abbreviated as α 2M and **A2M**, is a large plasma protein found in the blood. It is produced by the liver, and is a major component of the alpha-2 band in protein electrophoresis. A2M is composed of four identical subunits bound together by -S-S- bonds. It is able to inactivate an enormous variety of proteinases (including serine-, cysteine-, aspartic- and metalloproteinases).

A2M has in its structure a 35 amino acid "bait" region. Proteinases binding and cleaving the bait region become bound to A2M. The proteinase-A2M complex is recognized by macrophage receptors and cleared from the system. It functions as an inhibitor of coagulation by inhibiting thrombin. It functions as an inhibitor of fibrinolysis by inhibiting plasmin and kallikrein

A2M levels are increased in nephrotic syndrome, a condition wherein the kidneys start to leak out some of the smaller blood proteins. Because of its size, A2M is retained in the bloodstream. Increased production of all proteins means A2M concentration increases. This increase has little adverse effect on the health, but is used as a diagnostic clue. Longstanding chronic renal failure can lead to amyloid by A2M.

It is a negative acute phase reactant, the levels of which decrease in mouse serum or plasma as a result of inflammation. It has also been demonstrated that mouse A2M levels increase significantly with age, after gonadectomy and during pregnancy.

ADI's Mouse A2M ELISA provides is a rapid, specific and sensitive assay for measuring Mouse A2M in serum or other biological fluids.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Draw the standard curve on a graph paper by plotting net absorbance values of standards against appropriate α -Macroglobulin concentrations. Read off the α -Macroglobulin concentrations of the control and subject samples. Multiply the values by the dilution factor of the samples. If samples were diluted 1:100,000 then the values must be multiplied by 100,000 and results are expressed as mg/ml.

PERFORMANCE CHARACTERISTICS

Detection Limit: The minimum Mouse α -Macroglobulin 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: Mouse α -Macroglobulin levels in serum may vary from 1-3 u/ml to above in normal animals and increase in inflammation, acute phase response and arthritis. Each laboratory should establish testing ranges for the animal population being investigated.

Specificity: The antibodies used in this kit are specific for Mouse α -Macroglobulin with no cross-reactivity with other Mouse α -Macroglobulin serum proteins.

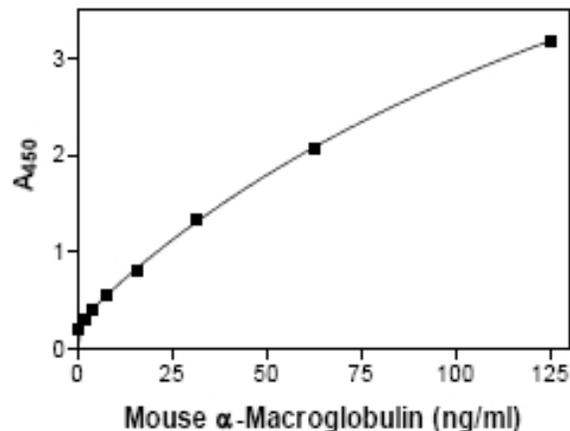
Species Crossreactivity: Utility of the kit to detect α -Macroglobulin from other species (crossreactivity) has not been studied. ADI has an ELISA kits for rat α -Macroglobulin.

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°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	Sample Diluent 0 ng/ml	0.167	
B1, B2	Standard A 3.9 ng/ml	0.395	
C1, C2	Standard B 7.8 ng/ml	0.552	
D1, D2	Standard C 15.6 ng/ml	0.848	
E1, E2	Standard D 31.25 ng/ml	1.419	
F1, F2	Standard E 62.5 ng/ml	2.046	
G1, G2	Standard F 125 ng/ml	3.140	
H1, H2	Sample 1	1.118	25.5 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 Std. Curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

The mouse α 2-macroglobulin ELISA kit is based on binding of mouse α -macroglobulin 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 mouse α -macroglobulin 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 mouse α -macroglobulin 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, distilled or deionized water, Micro-plate Incubator/shaker (150rpm), plate washer (recommended) and ELISA plates Reader (0-4 at 450nm) are required.

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Mouse α -Macroglobulin ELISA Kit is for research use only.

Stop Solution contains diluted 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 separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. Plasma can also be used. If sera cannot be immediately assayed, store frozen for up to six months. Avoid repeated freezing and thawing of samples. **Cell or tissues extract samples have not been optimized.**

REAGENT PREPARATION

- 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.
- Standard Preparation-**Reference standard is provided as lyophilized stock. See detailed preparation of standards on page 3.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the label. After opening the kit components, the shelf life is approximately 2 months.

SAMPLE DILUTION

Studies indicate that α 2-Macroglobulin is present in normal mouse serum at a concentration of ~ 2.5 mg/ml. The normal values may change with respect to strain, age, diet, and other factors. In order to obtain values within the range of the standard curve, we suggest that samples be diluted 50,000 fold using the following procedure for each sample to be tested:

- i) Prepare 998 μ L of water and 297 μ L of sample diluent in separate tubes.
- ii) Prepare 1:500 sample dilution by mixing 2 μ L of sample into the tube containing the 998 μ L of water.
- iii) Prepare 1:50,000 sample dilution by adding 3 μ L of the 500 fold sample dilution with the 297 μ L of diluent in second tube.

Repeat this procedure for each sample to be tested

TEST PROCEDURE

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

1. Reconstitute the lyophilized Reference Standard with the amount of distilled water indicated on the vial label. Mix gently before use. Aliquot and store unused Reference Standard at -20°C.
2. Prepare liquid standards using the following dilution scheme. Label 7 microcentrifuge tubes as 125, 62.5, 31.25, 15.6, 7.8, 3.9 and 0 ng/ml.
3. For standard F (125 ng/ml) pipette the volume of A2M diluent and add the indicated volume of A2M reference standard and mix gently. Prepare the remaining standards as shown below:

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 dilution scheme, you will have 250 μ L of negative and standards (B-F) except for 500 μ L of Standard A. You would need 200 μ L of each standard (100 μ L in duplicate).

	Mouse A2M Stds	Stock Volume	A2M diluent	Final Volume
Std F	(125 ng/ml)	500 μ L of Ref.		500 μ L
Std E	(62.5 ng/ml)	250 μ L of Std F	250 μ L	500 μ L
Std D	(31.25 ng/ml)	250 μ L of Std E	250 μ L	500 μ L
Std C	(15.6 ng/ml)	250 μ L of Std D	250 μ L	500 μ L
Std B	(7.8 ng/ml)	250 μ L of Std C	250 μ L	500 μ L
Std A	(3.9 ng/ml)	250 μ L of Std B	250 μ L	500 μ L
Sample Diluent	(0 ng/ml)	0	250 μ L	250 μ L

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

4. Pipet **100 μ L standards and diluted samples** (in duplicates) into appropriate wells.
5. Mix gently, and incubate at room temp. (20-25°C) **for 45 minutes on a plate shaker (100-150 rpm)**. If a shaker is not available then the plates can be gently mixed for 5-10 secs every 15-20 min. This may decrease the overall reaction but the incubation time may be increased by 15-30 min to achieve highest standard reading of >2.00.
6. Remove or aspirate the plate contents and **wash the wells 5 times** with 300 μ L 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. Tap the plate over fresh paper towels after washing.
7. Pipette **100 μ L of antibody-enzyme conjugate** into each well. Mix gently, and incubate for **45 minutes at room temperature on a plate shaker (100-150 rpm)**. If a shaker is not available then the plates can be gently mixed for 5-10 secs every 5 min. This may decrease the overall reaction but the incubation time may be increased by 15-30 min to achieve highest stds reading of >2.00.
8. **Repeat step 6 above for washing.**
9. Add **100 μ L of TMB Substrate** into each well. Mix gently. Cover the plate and incubate for **20 min at room temp.** Blue color develops.
10. Stop the reaction by adding **100 μ L of stop solution** to all wells. Mix gently. Blue color turns yellow.
11. **Measure the absorbance at 450 nm** using an ELISA reader. Color is stable for at least **5 minutes** after stopping.