Mouse Anti-ApoH Ig's (G+A+M) ELISA Procedure SUMMARY
Total Assay Time - 75 min. (30+30+15)

	Allow all reagents to reach room temperature. Arrange and label required # of coated strips. Dilute Sample Diluent 1:10 and wash buffer 1:100 with water. **Dilute** serum samples 1:100 in 1X sample diluent (5 µl of serum in 500 µl sample diluent).**Controls have been PRE-DILUTED**. Dilute required amount of HRP-Conjugate (1:100) with 1X sample diluent.
Step 1	Pipet **100 µl** of sample diluent (buffer control or blank), negative, positive controls, and *diluted* serum samples into appropriate wells *in duplicate*. Gently mix wells; Cover the plate and incubate at room temperature for **30 minutes** at room temp.
Step 2	Aspirate and wash **3 times with 1X** wash buffer. Dispense **100µl** of **1X HRP-conjugate** to each well. Mix gently, cover the plate and incubate for **30 min** at room temp.
Step 3	Aspirate and wash **5 times** with 1X wash buffer. Dispense **100µl of TMB Substrate Solution**. Mix gently, cover the plate and incubate for **15 min** at room temp. Blue color develops.
Step 4	Pipet **100 µl Stop Solution** (in H2SO4) into each well. Blue color turns yellow. Measure absorbance at 450 nm within 15 minutes.

CHECK LIST (Check box after completing each of the above steps)

Step 1	Step 2	Step 3	Step 4
Time: Start End			

KIT PROFILE

Date received: _____ **Cat #** 5000 **Lot #** _____ **Exp.** _____

Date kit opened _____ **Technician:** _____

Date used: _____ **# Strips used** _____ **# Remaining** _____

Remarks _____

Instruction Manual No. M-5000

Mouse ApoH Antibodies

ELISA KIT Cat. No. 5000

For Quantitative Determination of Anti-ApoH Ig's (G+A+M) In Mouse Serum

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Kit Contents: (Mouse Anti-ApoH Ig's ELISA KIT, Cat. No. 5000)

Kit Component, 96 tests	Cat #
Purified ApoH Coated strip plate (8 wells x 12 strips).	5001
Mouse anti-ApoH Ig's std A (0 ng/ml), 0.65 ml.	5002
Mouse anti-ApoH Ig's std B (31.2 ng/ml), 0.65ml	5003
Mouse anti-ApoH Ig's std C (62.5 ng/ml),0.65 ml.	5004
Mouse anti-ApoH Ig's std D (125 ng/ml), 0.65 ml	5005
Mouse anti-ApoH Ig's std E (250 ng/ml), 0.65 ml.	5006
Mouse anti-ApoH Ig's std F (500 ng/ml), 0.65ml	5007
Goat Anti-mouse IgG HRP conjugate (H+L), 0.120 ml , (100X)	5008
Sample Diluent, (10X), 10 ml	SD-10
Wash Buffer, (100X)10 ml	WB-100
TMB Substrate, 12 ml	80091
Stop solution, 12 ml	80101
Complete Instruction Manual.	M-5000

INTRODUCTION

Apolipoprotein H (ApoH), also known as beta(2)-glycoprotein I, is a plasma glycoprotein with its in vivo physiological and pathogenic roles being closely related to its interaction with negatively charged membranes. Although the three-dimensional crystal structure of ApoH has been recently solved, direct evidence about the spatial state of ApoH on the membrane is still lacking. Human ApoH is a single-chain molecule consisting of 326 amino acid residues and five carbohydrate chains. Apolipoprotein H has been implicated in a variety of physiologic pathways including lipoprotein metabolism, coagulation, and the production of antiphospholipid autoantibodies. Apolipoprotein H (apoH) is considered to be a necessary cofactor for the binding of certain antiphospholipid antibodies to anionic phospholipids. Some ApoH-dependent antiphospholipid antibodies also exert lupus anticoagulant (LA) activity, which seems to depend on antiphospholipid antibody epitope specificity.

Apolipoprotein H is associated with very-low-density lipoproteins, high-density lipoproteins, and chylomicrons and may play a role in triacylglycerol metabolism. ApoH preferentially binds to negatively charged phospholipids such as phosphatidyl serine (PS). Because of this property, apoH might inhibit blood coagulation and ADP dependent platelet aggregation, at least in vitro. Its precise physiological role remains to be elucidated. ApoH has also been shown to be a mediator of inflammatory changes ensuing in jeopardized human myocardium

ADI's ApoH ELISA Kit, is a quantitative assay. It has been developed to screen the presence of ApoH antibody Ig's (G+A+M) in mouse serum.

Mouse Autoimmune ELISA kits available from ADI

5000 Mouse anti-ApoH Ig's (G+A+M) ELISA Kit, qualitative

5010 Mouse anti-PCNA Ig's (G+A+M) ELISA Kit, qualitative

5100 Mouse Anti-dsDNA Ig's (G+A+M) ELISA, qualitative
5110 Mouse Anti-dsDNA Ig's (G+A+M) ELISA Kit, quantitative
5120 Mouse anti-dsDNA IgG-specific ELISA Kit, quantitative
5130 Mouse anti-dsDNA IgM-specific ELISA Kit, quantitative

5200 Mouse Anti-Nuclear Antibodies (ANA) Ig's (G+A+M) ELISA Kit, qualitative
5210 Mouse Anti-Nuclear Antibodies (ANA) Ig's (G+A+M) ELISA Kit, quantitative

5300 Mouse Anti-ssDNA Ig's (G+A+M) ELISA Kit, qualitative
5310 Mouse Anti-ssDNA Ig's (G+A+M) ELISA Kit, quantitative
5320 Mouse Anti-ssDNA IgG-specific ELISA Kit, quantitative
5330 Mouse Anti-ssDNA IgM-specific ELISA Kit, quantitative

5400 Mouse Anti-Sm Ig's (G+A+M) ELISA Kit, qualitative
5405 Mouse Anti-Sm Ig's (G+A+M) ELISA Kit, quantitative
5410 Mouse Anti-nRNP Ig's (G+A+M) ELISA Kit, quantitative

5500 Mouse Anti-Cardiolipin Ig's (G+A+M) ELISA Kit, qualitative

5600 Mouse Anti-Histones Ig's (G+A+M) ELISA Kit, qualitative
5610 Mouse Anti-Histones Ig's (total) ELISA Kit, quantitative

5700 Mouse Anti-SSA/Ro Ig's (G+A+M) ELISA Kit, qualitative
5710 Mouse Anti-SSA/Ro Ig's (G+A+M) ELISA Kit, quantitative

5800 Mouse Anti-SSB/La Ig's (G+A+M) ELISA Kit, qualitative
5810 Mouse Anti-SSB Ig's (G+A+M) ELISA Kit, quantitative

5900 Mouse Circulating Immune Complexes (CIC) Ig's (G+A+M) ELISA Kit, qualitative

6000 Mouse Anti-Jo Ig's (G+A+M) ELISA Kit, qualitative
6005 Mouse Anti-Jo-1 Ig's (G+A+M) ELISA Kit, quantitative

6100 Mouse Anti-Scl70 Ig's (G+A+M) ELISA Kit, qualitative
6110 Mouse anti-Scl70 Ig's (G+A+M) ELISA Kit, quantitative

6200 Mouse RF Ig's (G+A+M) ELISA Kit, qualitative

Other ELISA kits

Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, IgE, IgM, Leptin, Resistin, Acrp30, CRP, Haptoglobin, TNF-alpha, VEGF
Rat: Albumin, CRP, IgG, IgM, Alpha 1 Acid glycoprotein
Chicken: IgG, IgM, IgY, Ovalbumin, **Turkey**: IgG, **Rabbit**: CRP, IgG
Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin, **Pig**: Albumin, IgG, IgM,
Dog: CRP, IgG, IgM, **Cat**: IgG, IgM, **Goat:** IgG, **Sheep:** IgG
Monkey: IgM, IgG, IgA, CRP
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, E2, testosterone, progesterone etc).

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards and samples. Draw the standard curve on semi-log graph paper by plotting net absorbance values of standards against appropriate Ig's (G+A+M) concentrations. Read off the Ig's concentrations of the control and treated samples. Multiply the values by the dilution factor of the samples. If samples were diluted 1:50 the values must be multiplied by 50. If the samples were diluted more than the required 1:50 dilution (i.e., 1:100) then the values multiplied by the appropriate dilution factor and the results expressed as ng/ml.

PERFORMANCE CHARACTERISTICS

Detection limit:-

Based on 8 replicate determinations of the zero standards the minimum Ig's (total) concentration detectable using this assay is 10 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

High dose hook effect:-

Ig's concentrations of up to 2500 ng/ml did not show any hook effect.

Intra-assay precision:-

Three mouse serum samples (mean Concen. 400ng, 200ng, 100ng) were run in 10 replicates. The samples showed good intra-assay precision with %CV of 3.32-5.79%.

Inter-assay precision:-

Three serum samples (400ng, 200ng, 100ng) were run in 5 independent assays. The samples showed good inter-assay precision (3.19-9.4% CV).

LINEARITY:-

Three different Mrl/lpr mouse samples were diluted (1:2, 1:4, 1:8. 1:16) and their Anti-ApoH levels determined. The samples showed excellent mean recoveries of about 90-98%.

Specificity:

The antigen used for coating is ApoH. The detection antibodies used in the kit are directed against mouse IgG (H+L), it will detect total Ig's (G+A+M).

PRINCIPLE OF THE TEST

Anti-ApoH ELISA kit is based on binding of Anti-ApoH from serum samples to extracted antigen immobilized on microtiter wells. After a washing step, anti-mouse IgG-HRP conjugate is added. After another washing step, to remove all the unbound enzyme conjugate, chromogenic substrate (TMB) is added and color developed. The enzymatic reaction (blue color) is directly proportional to the amount of Anti-ApoH present in the sample. The reaction is terminated by adding stopping solution (converts blue to yellow). Absorbance is then measured on a microtiter well ELISA reader at 450 nm. The concentration of Anti-ApoH Ig's (G+A+M) in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-100 µl) and multichannel pipet; Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

This ELISA Kit is intended for *in vitro research* use only. The reagents contain Proclin-300 (0.1% v/v); necessary care should be taken when disposing solutions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), H2SO4 (stop solution) and Proclin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

SAMPLE COLLECTION AND HANDLING

Blood should be collected by venipuncture, allowed to clot, and serum separated by centrifugation at room temperature. Do not heat inactivate the serum.. If sera can not be immediately assayed, these could be stored at -20^{o}C for up to six months. Avoid repeated freezing and thawing of samples.

REAGENT PREPARATION FOR THE ASSAY

Sample/Conjugate Diluent (10X). Before use, dilute 1:10 with water (1ml/10 ml water). Prepare 1 ml for every strip). Prepare diluent according to the requirement. Diluted conjugate can be stored for 1-2 weeks at 4oC.

Wash Buffer Concentrate *(100X solution)*. Before use, dilute 1:100 with distilled water. Occasionally, some salts may form crystals during storage in cold but they redissolve upon slight warming of the solution.

Goat Anti-mouse IgG-HRP Conjugate (100X). Before use, dilute 1:100 with sample diluent (10 µl/ml diluent; prepare 1 ml for every strip). Do not store diluted (1X) antibody-HRP conjugate.

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 five minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a set of standards on each plate. Addition of the HRP substrate 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.

STORAGE AND STABILITY

All kit components are stable at 2-8^{O}C until the expiration date printed on the label. The whole kit stability is at least 6 months from the date of manufacture under appropriate storage conditions. The unused strips should be stored tightly covered with adhesive film and with the desiccant in the bag.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMP. BEFORE USE). A brief summary is also given on page 7.*

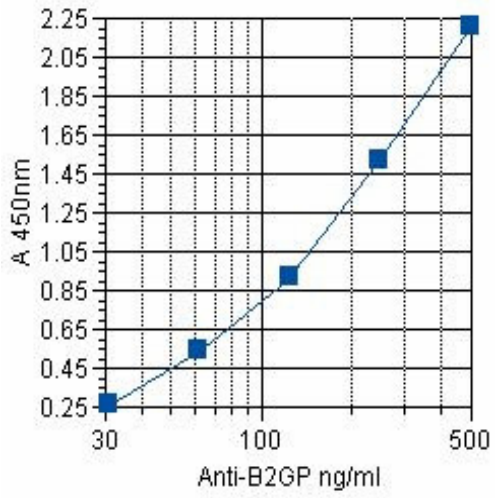
1. Label, and secure the microtiter well strips to be used on the plate. **Dilute sample/conjugate** diluent 1:10 in water. **Dilute** (1:100) serum samples (5 µl serum in 500 µl of 1X sample diluent). *A total of 200 µl of diluted sample will be required to run tests in duplicate.* **Dilute (1:100) wash buffer** concentrate with distilled water. **Dilute HRP-Conjugate** (1:100) with sample diluent.
2. Pipet **100 µl** of std A (Wells A1/A2 for use as blanks), standards B-E (B1/B2-E1/E2) and then samples into appropriate wells in *duplicate*. Mix gently, cover the plate and incubate for **30 minutes** at room temp.
3. Aspirate and **wash** the wells **3 times** with 300 µl of diluted wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
4. Add **100 µl** of diluted enzyme conjugate into each well**.** Mix gently. Cover the plate and incubate for **30 minutes** at room temp. Aspirate and **wash** the wells **5 times** as above.
5. Add **100 µl** of TMB Substrate into each well. Mix gently. Cover the plate and incubate for **15 minutes** at room temperature.
6. **Stop** the reaction by adding **100 µl** of stop solution to all wells. Mix gently. Measure the absorbance at 450 nm using an ELISA reader (The color is stable for at least 30 min). Wells with lowest color may become clearer because of color fading with time.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	*Mean A450 nm	Calculated Concn.
A1, A2	**Std A** (0 ng/ml)	0	
B1, B2	**Std B** (31.2 ng/ml)	0.26	
C1, C2	**Std C** (62.5 ng/ml)	0.54	
D1, D2	**Std D** (125 ng/ml)	0.94	
E1, E2	**Std E** (250 ng/ml)	1.42	
F1, F2	**Std F** (500 ng/ml)	2.20	
F1, F2	**Sample 1**		

***=** Average duplicate values after deducting the std zero values (0.16).

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 std. Assay curve (do not use this for calculating sample values)