ELISA kits available from ADI (see details at the web site)

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	**#1200**	Human Albumin (Urinary)
#1750	Human IgG (total)	**#1760**	Human IgM
#1800	Human IgE	**#1810**	Human Ferritin
#1210	Human Transferrin (Tf)	**#0020**	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		

#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	**#1830**	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	**#1510**	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		

#0030	Human Insulin	**#0040**	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#**0410**	HCG-free beta

#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	**#1110**	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	**#1700**	Human T3 (total)

100-300-SCR Serum Creatinine ELISA kit (colorimetric, all species), 96 tests, quantitative

100-305-SCR Serum Creatinine ELISA kit (colorimetric, all species), 2x96 tests, quantitative

100-310-ADM Human Asymmetrical Dimethylarginine (ADMA) ELISA Kit, 96 tests

100-320-CIT Human Citrulline (CIT) ELISA Kit, 96 tests

100-330-ARG Human Arginine (Arg) ELISA Kit, 96 tests

Instruction Manual No. M-100-310-ADM

Asymmetrical Dimethylarginine (ADMA)

ELISA KIT # 100-310-ADM

For Quantitative Determination of ADMA in serum, blood plasma and related biological fluid.

For In Vitro Research Use Only

India Contact:

Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034
Ph: +91-11-42208000, 42208111, 42208222

Mobile: +91-9810521400
Fax: +91-11-42208444
Email: customerservice@atzlabs.com
Web: www.atzlabs.com

DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Human ADMA ELISA KIT Cat. No. 100-310-ADM

For Quantitative Determination of ADMA in Human serum, plasma

Kit Contents: (reagents for 96 tests)

Components	#
Coated microwell strip plate (96 wells);#100311	1 plate
ADMA **Standard**, 0.5 ml, 80000 ng/L, (80 ng/ml) #100312A	1 vial
Standard Diluent, 3 ml, #100313	1 bottle
Streptavidin-HRP Conjugate, 6 ml, #100314	1 bottle
HRP substrate Solution A, 6 ml # 100310SA	1 bottle
HRP substrate Solution B, 6 ml # 100310SB	1 bottle
Anti-ADMA antibodies-biotin Conj, #100315; 1 ml	1 bottle
Wash buffer (30X), 20 ml, dilute 1:30 with distilled water #100310WB	1 bottle
Stop solution, 6 ml, #100310-ST	1 bottle
ELISA plate covers	2
Complete Instruction Manual, M-100-310-ADM	1

The ADMA ELISA Kit is a highly sensitive Biotin double antibody sandwich ELISA for the measurement of ADMA in human serum, blood plasma and related biological fluid. For in vitro research use only.

Introduction

Synonyms (2S-2-amino 5-[(aminodimethylaminomethylen) amino]) pentanoic acid; N, N-Dimethylarginine; at C8H18N4O2 and natural chemical matter are normally present in plasma. It is a metabolic by-product of continual protein modification processes in the cytoplasm of all human cells. This reaction is catalyzed by an enzyme set called S-adenosylmethionine protein N-methyltransferases (protein methylases I and II). After synthesis; ADMA migrates into blood plasma via extracellular space. It is closely related to L-arginine, a conditionally-essential amino acid. ADMA interferes with L-arginine in the production of nitric oxide, a key chemical involved in normal endothelial function and, by extension, cardiovascular health. High concentrations of ADMA found in some pathophysiological conditions are associated with other factors giving increased risk of atherosclerosis such as increasing age, hypercholesterolemia, hypertension, hypertriglyceridemia, diabetes mellitus, insulin insensitivity, hyperhomocysteinemia and renal failure.
ADMA has equal or very similar sensitivity and specificity to RF in untreated Rheumatoid arthritis or RA in the detection of asymptomatic endothelial dysfunction in untreated RA.

PERFORMANCE CHARACTERISTICS

Assay range :200ng/L→60000ng/L.

Sensitivity :100.21ng/L.

QUALITY CONTROL

Each laboratory should utilize controls at several levels to monitor assay performance. The controls should be treated as unknown. Values obtained should be in a agreement with the assigned values of the control. Controls can be obtained from commercially available sources but should not contain sodium azide as preservative.

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 4°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.

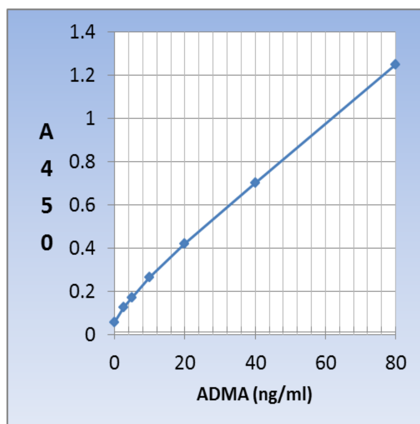
Species Reactivity

ADMA is is found in all species. The ELISA kit has been tested in human samples but the kit should work in mouse, rat, and other species as ADMA and antibodies are not species specific.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds	ADMA Concn (ng/ml)	Mean A450nm
A1, A2		0	0.058
B1, B2	**Std. A**	2.5	0.124
C1, C2	**Std. B**	5	0.172
D1, D2	**Std. C**	10	0.263
E1, E2	**Std. D**	20	0.419
F1, F2	**Std. E**	40	0.703
F1, F2	**Std. F**	80	1.25

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)

CALCULATION OF RESULTS

Calculate the Net A450 values of the duplicate (deduct zero values). Plot the Citrulline standard concentration versus the net A450 using a 4-point log-log curve. Calculate the samples values from the standard curve.

PRINCIPLE OF THE TEST

Asymmetrical Dimethylarginine (ADMA) ELISA kit is based on binding of ADMA from samples to coated antibody on the microwells, bound ADMA is detected by HRP-labeled detection antibodies. Higher concentrations of ADMA in the samples result in higher binding of enzyme (HRP) labeled antibody to the microwell plate. After a washing step, chromogenic substrate is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of ADMA 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 ADMA in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (25-100 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

LIMITATIONS

1. The Alpha Diagnostic's ADMA ELISA test is intended for *in vitro research* use only.
2. Bloody specimens are unsuitable for use, even if clarified by centrifugation, since blood flow is a likely a sign of contamination.

PRECAUTIONS

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.
TMB (substrate), H_2SO_4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).
All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

Specimen Collection and preparation

- **Samples containing NaN_3 are incompatible with the ELISA** as they inhibit the activity of Horse Radish Peroxidase (HRP).

- Upon sample collection, extraction should be carried out as soon as possible in accordance with related documents. After extraction, the experiment should be conducted immediately as well. Otherwise, the sample should be preserved **at -20** °C. Repeated freeze-thaw cycles should be avoided.
- **Serum**: Allow the serum to clot for 10-20 minutes at room temperature, then place in centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. If sediments have occurred during storage, centrifugation should be repeated.

- **Blood plasma**: In accordance with sample collection requirements, EDTA or sodium citrate should be used for anticoagulation. Add EDTA or sodium citrate

and mix them for 10-20 minutes, then place in centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. If sediments have occurred during storage, centrifugation should be repeated.

- **Urine**: Collect with sterile tube. Place in centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. If sediments have occurred during storage, centrifugation should be repeated. When collecting pleuroperitoneal fluid and cerebrospinal fluid, please follow the procedures mentioned above.

- **Cell culture** supernatant: Collect with sterile tubes when examining secrete components. Place in centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When examining the components within the cell, use PBS (PH 7.2-7.4) to dilute cell suspension to cell concentration of approximately 1 million/ml. Degrade cells through repeated freeze-thaw cycles to release interior components. Place in centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. If sediments have occ urred during storage, centrifugation should be repeated.

- **Tissue sample**: Incise sample and weigh. Add a given amount of PBS (PH 7.4).Immediately freeze with liquid nitrogen for later use. Thaw sample and hold at 2-8℃. Add a given amount of PBS (PH 7.4), then homogenize the sample thoroughly by hand or with homogenizer. Place in centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. Aliquot and keep one for examination and freeze the others for later use.

REAGENT PREPRATION

Standard Preparation

ADMA standard is supplied as 80,000 ng/L or 80 ng/ml solution. Prepare additional standards by 2-fold serial dilution as follow:

Stds	Volume	Std Diluent	Total volume	Final. Concn
Stock A	240 ul	0	240 ul	80.00 ng/ml
Std B	120 ul of A	120 ul	240 ul	40.00 ng/ml
Std C	120 ul of B	120 ul	240 ul	20.00 ng/ml
Std D	120 ul of C	120 ul	240 ul	10.00 ng/ml
Std E	120 ul of D	120 ul	240 ul	5 ng/ml
Std F	120 ul of E	120 ul	240 ul	2.5 ng/ml
(blanks)	0	120 ul	120 ul	0

Mix each tube thoroughly before the next transfer. In the above example, only 120 ul of the stds (a-F) will remain that will be sufficient to run 1 test in duplicate. Do not use the working stds (B-E) beyond the assay date and prepare fresh stds if necessary.

Dilute wash buffer (1:30) with distilled water (20 ml stock in 580 ml of water). Store at 4oC.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label.

Important: If you have not used this kit before, we recommend to use 1 or 2 strips to run the standards alone to get familiar with the test and not runt the risk of making mistakes and lose sample or the whole kit.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).* Dilute wash buffer (1:30) and prepare working standards. Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

Step 1. **A set of blank wells,** add 40 ul of standard dilution buffer or PBS (no standards).

Step 2. Add **50 µl** of **standards in duplicate** into respective wells.

Step 3. Add **40 µl** of **samples in duplicate** into respective wells.

Step 4 Add **10 µl** of **Biotin-Antibody to blanks and samples (do not add to the standards).**

Step 5 Add **50 µl** of **Streptavidin-HRP conjugate to blanks, standards and samples.**

Step 6 Mix well contents by gentle and manual tapping of the plate against the palm for 5-10 seconds, cover the plates, and **incubate** for **60 min** at **37oC** temperature.

Step 7. **Aspirate or remove well contents** and wash the wells **5 times** with 300 ul of 1X wash buffer. After the last wash, invert the plate over the paper fresh towels and tap a few times to remove any traces of wash buffer.

Step 8. Add **50 ul** of **substrate solution A** followed by **50 ul of substrate solution B** to all wells. Mix gently to mix the plate. Incubate for **10 min** at **37oC** for color development. Blue color develops in standards and samples. **Notes**: It is possible to change the incubation time +3 mins so as to get the maximum yellow color A450nm to about 1.0-2.0 or within the linear range of the ELISA reader.

Step 9. Add **50 ul of stop solution** into all wells and mix gently (blue color turns yellow). **Measure absorbance at 450 nm** within 10 minutes of stopping reaction Determine ADMA concentration in each sample using the standards.

Quick Summary

	Blanks	Standards	Samples
Volume	40 ul pbs or sample diluent	50 ul	40 ul
Biotin-Antibody	10 ul	0	10 ul
Streptavidin-HRP	50 ul	50 ul	50 ul
Mix gently, cover the plates, and incubate at 370C for 60 min			
Aspirate and wash the plate 5X with 1X wash buffer			
Add 50 ul of substrate solution A followed by 50 ul of solution B, mix gently and incubate the plates for 10 mins (blue color develops)			
Stop reaction by the addition of 50 ul stop solution to all wells (blue color turns yellow)			
Read the plate at 450nm. Plot the std curve and calculate unknown values.			