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

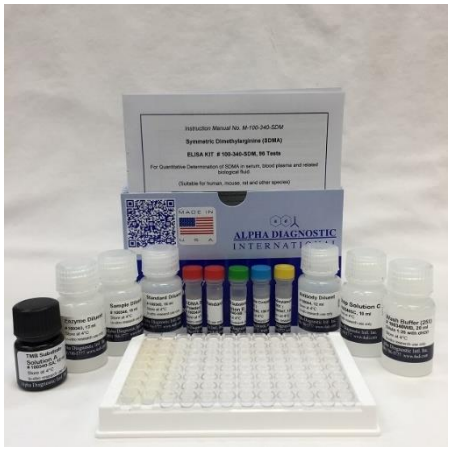
Instruction Manual No. M-100-340-SDM

Symmetric Dimethylarginine (SDMA)

ELISA KIT # 100-340-SDM, 96 Tests

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

(Suitable for human, mouse, rat and other species)



For In Vitro Research Use Only (RUO)

ALPHA DIAGNOSTIC INTERNATIONAL

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

Human SDMA ELISA KIT Cat. #100-340-SDM

Kit Contents: (reagents for 96 tests)

Components	#
Antibody coated plate (96 wells);#100341	1 plate
SDMA **Standards (Powder)**, #100342A, red cap	2 vials
Biotinylated Antibody(100X), 120 ul, #100347; Yellow cap	1 vial
Enzyme conjugate (100X), 130 ul; #100348, blue cap	1 vial
Enzyme Diluent, 12 ml; #100343	1 bottle
Antibody Diluent , 12 ml; #100344	1 bottle
Standard Diluent, 15 ml, #100345	1 bottle
Sample Diluent, 15 ml; #100346	1 bottle
Wash buffer (25X), 20 ml, dilute 1:25 with distilled water #100340WB	1 bottle
TMB Substrate **Soln A**, 10 ml # 100340SA, black cap	1 bottle
TMB Substrate **Soln B**, 1.5 ml # 100340SB; green cap	1 vial
Stop **Solution C**, 10 ml #100340SC	1 bottle
Complete Instruction Manual, M-100-340-SDM	1

Intended Use:

The SDMA ELISA Kit is a highly sensitive sandwich ELISA for the measurement of SDMA in serum, blood plasma and related biological fluid. It can be used in human, mouse, rat, cat, dog and other animals. For research use only (RUO).

Introduction

ADMA (2-Amino-5-[(amino-dimethylaminomethylene)amino]pentanoic acid, C8H18N4O2, Mol wt 202.25) is a naturally occurring chemical found in blood 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. Symmetrical Dimethylarginine (**SDMA**), an isomer of Asymmetric Dimethylarginine (ADMA). SDMA is produced by protein-arginine methyltransferase 5 (PRMT 5) and PRMT 7 (both type II methyltransferases). It is eliminated by renal excretion and does not directly inhibit NOS but is a competitor of arginine transport. SDMA reduced endothelial NO synthesis by limiting L-arginine supply to the NOS pathway. SDMA, a simpler indicator equal to serum creatinine, can serve as a marker of Glomerular filtration rate (GFR) and Coronary artery disease. Measuring SDMA overcomes the cumbersome method of using radioisotopes and inulin clearance. Chronic kidney disease (CKD) in cats is the cause of morbidity as they age, studies show that a reduced GFR corresponds to increased serum SDMA concentration. Measuring SDMA levels can be done before cats/dogs became azotemic.

PERFORMANCE CHARACTERISTICS

Assay range:100 ng/ml→1.56 ng/ml

Sensitivity: up to 0.5 ng/ml.

Specificity: No cross-reaction with other factors.

Intra assay Precision: ≤ 8%

Inter assay Precision: ≤ 12%

Recovery: 70 - 110 percent.

Storage: -20℃ [Short-term should be placed 4℃(such as two weeks)]

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^{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.

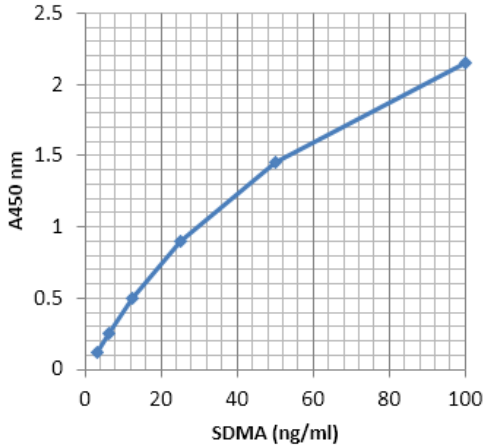
Species Reactivity

SDMA 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 SDMA and antibodies are not species specific.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds	SDMA Concn (ng/ml)	Mean A450nm
A1, A2	blanks	0.00	0.00
B1, B2	**Std. G**	1.56	0.06
C1, C2	**Std. F**	3.12	0.12
D1, D2	**Std. E**	6.25	0.25
E1, E2	**Std. D**	12.5	0.50
F1, F2	**Std. C**	25	0.90
F1, F2	**Std. B**	50	1.45
G1, G2	**Std. A**	100	2.10

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.



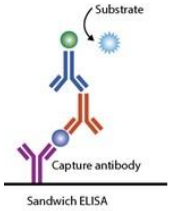
/6_ADI_ELISA_Arif

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 SDMA 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



SDMA ELISA kit is based on binding of SDMA from samples to coated antibody on the microwells, bound SDMA is detected by biotin-labeled detection antibodies followed by Streptavidin-HRP detection. After a washing step, chromogenic substrate is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of SDMA 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 SDMA 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

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

PRECAUTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute 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 and BND can be requested or obtained from the ADI website: Sample Diluent and anti-Protein G-HRP contain Proclin 300 (0.05%, v/v). http://4adi.com/objects/catalog/product/extras/ELISA-Kit-SDS-MSDS-Set-1.pdf

STORAGE AND STABILITY

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

Specimen Collection and preparation

- Freshly collected serum or plasma (EDTA, Heparin) are suitable**.** Samples may be stored at -20oC for testing.

- **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.
- **Tissue homogenate**: Take tissue slices and wash them out in 0.01MPBS; Add tissue protein extraction reagent according to proportion of 1G: 5-10ml and mix them in ice water. After being blended, mixture shall be centrifuged for 10min at 5000-10000rpm.Take supernatant tested immediately or put them at -20℃(for 1-3 months) or -80℃ (for 1-3 months) for storage.
- **Cell culture**: Take centrifugation for 10min at 1000-3000rpm.Take supernatant tested immediately or put samples at -20℃(for 1-3 months) or -80℃ (for 1-3 months) for storage.

- **For urine, ascites, cerebrospinal fluid**, etc: centrifuge for 10min at 1000-3000rpm. Take supernatant tested immediately or put samples at -20℃(for 1-3 months) or -80℃ (for 1-3 months) for storage.

- **Sample dilution**: Note: The user should refer to the references to know the probable content of the samples before decide to dilute the samples, and the diluted content of the sample must be in the best detection range of the given ELISA Kits. The dilution of the sample should be recorded in detail and samples mixed 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.

REAGENT PREPRATION

Standard Preparation

SDMA standard is supplied as powder. Add 1 ml of standard diluent and gently mix for 10-20 min on an orbital shaker or manually mix to dissolve (SDMA=100 ng/ml; Stock A). Prepare additional standards (B-F) by 2-fold serial dilution as follow:

Stds	Volume	Std Diluent	Total volume	Final. Concn
Stock A	300 ul	0	300 ul	100 ng/ml
Std B	300 ul of A	300 ul	600 ul	50 ng/ml
Std C	300 ul of B	300 ul	600 ul	25 ng/ml
Std D	300 ul of C	300 ul	600 ul	12.5 ng/ml
Std E	300 ul of D	300 ul	600 ul	6.25 ng/ml
Std F	300 ul of E	300 ul	600 ul	3.12 ng/ml
Std G	300 ul of F	300 ul	600 ul	1.56 ng/ml
(blanks)	0	300 ul	300 ul	0

Mix each tube thoroughly before the next transfer. In the above example, only 300 ul of the stds (A-G) 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. Test 100 ul/well in duplicate of 200 ul standards per test.

Prepare 1X Biotin-antibody-Dilute 1:100 stock with antibody diluent (10 ul stock in 990 ul of diluent). Prepare 1 ml for each 8-well strip and keep at room temp until used. Prepare in required amounts only and do not store 1X biotin-antibody. This reagents should be prepared before starting the test.

Prepare 1X Enzyme conjugate-Dilute 1:100 stock with enzyme diluent (10 ul stock in 990 ul of diluent). Prepare 1 ml for each 8-well strip and keep at room temp until used. Prepare in required amounts only and do not store 1X biotin-antibody. This reagents should be prepared before starting the test.

Dilute wash buffer (1:25) with distilled water (25 ml stock in 475 ml water) and store at room temp for the test. Store the remaining buffer at 4oC.

Preparation of TMB Substrate-The substrate solution is provided as 2 component solution that should be prepared 15-20 min prior to its use (at step 6, page 4). Take 900 ul of Soln A and 100 ul of Soln B and mix. Prepare 1 ml for each 8-well strip or 10 ml for full plate. Do not prepare this solution too much in advance and prepare as needed.

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 run 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 (1:25) and prepare working standards, dilute biotin-antibody and enzyme conjugate. Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

1. **A set of blank wells,** add 100 ul of standard dilution buffer or PBS (no standards).
2. Add **100 µl** of **standards or samples in duplicate** into respective wells. **Mix gently for 5-10 seconds and incubate at 37oC for 90 min**.
3. **Aspirate or remove well contents** and wash the wells **3 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.
4. Add **100 µl** of 1X **Biotin-antibody conjugate to blanks, standards and samples.** 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.
5. **Aspirate or remove well contents** and wash the wells **3 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.
6. Add **100 µl** of 1X **Enzyme-antibody conjugate to blanks, standards and samples.** Mix well contents by gentle and manual tapping of the plate against the palm for 5-10 seconds, cover the plates, and **incubate** for **30 min** at **37oC** temperature.
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.
8. Add **100 ul** of the substrate solution **mix of soln A and B** (see page 3) to all wells. Mix gently for 5-10 seconds to mix the well contents. Incubate for **30 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.
9. Add **100 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 SDMA concentration in each sample using the standards.

Quick Summary

	Blanks	Standards	Samples
Volume	100 ul or sample diluent	100 ul	100 ul
Mix gently, cover the plates, and incubate at 37℃ for 90 min. Wash 3X after incubation			
Biotin-Antibody	100 ul	100 ul	100 ul
Mix gently, cover the plates, and incubate at 37℃ for 60 min. Wash 3X after incubation			
Streptavidin-HRP	100 ul	100 ul	100 ul
Mix gently, cover the plates, and incubate at 37℃ for 30 min. Wash 5X after incubation			
Add 100 ul of substrate solution A+B Mix; mix gently and incubate at 37oC for 30 mins (blue color develops)			
Stop reaction by the addition of 100 ul stop solution to all wells (blue color turns yellow)			
Read the plate at 450nm. Plot the std curve and calculate unknown values.			