DIAGNOSTIKA GMBH

Instructions for Use

SDMA human ELISA

Enzyme Immunoassay for the
Quantitative Determination of
Endogenous Symmetric Dimethylarginine (SDMA)
in human Serum or Plasma

CE IVD

REF EA214/96

Σ 12 x 8

2 – 8 °C

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Table of Contents

Symbols

IVD	In Vitro Diagnostic Medical Device	CE	EC Declaration of conformity
CONT	Content		Expiry Date
LOT	Lot Number		Store at
	Manufactured by	Σ	Sufficient for ... determinations
REF	Catalogue Number		Consult Instructions for Use

Hazard Pictograms


Warning


Danger

1 Introduction and Principle of the Test

Dosing of most drugs must be adapted in renal insufficiency, making accurate assessment of renal function an essential component of diagnostics in clinical medicine. Furthermore, even modest impairment of renal function has been recognized as a cardiovascular risk factor. As the most commonly used marker of renal excretory function, serum creatinine concentration, does not adequately respond to mild to moderate impairment of renal function, more sensitive markers for renal excretory function are urgently seeked, especially in mild stages of renal impairment. SDMA is a methylated derivative of the amino acid L-arginine (symmetric dimethylarginine). SDMA is eliminated from the body exclusively by renal excretion; therefore SDMA plasma concentration is tightly related to renal function. Thus, quantification of plasma SDMA is an adequate means to assess renal function, as could be demonstrated in a series of recent clinical trials: In 18 clinical studies involving more than 2,100 patients systemic SDMA concentrations were highly correlated with inulin clearance as well as with various clearance estimates and better corresponded to mild renal function impairment than serum creatinine.

Thus, SDMA exhibits properties of a reliable marker of renal function. Furthermore, there is evidence showing that elevated SDMA levels, as they may occur in renal function impairment, may prospectively indicate future risk of cardiovascular disease and mortality independently of the level of renal impairment.

The competitive SDMA human ELISA uses the microtiter plate format. SDMA is bound to the solid phase of the microtiter plate. SDMA in the samples is acylated and competes with solid phase bound SDMA for a fixed number of rabbit anti-SDMA antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase SDMA is detected by anti-rabbit / peroxidase. The substrate TMB / peroxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase SDMA is inversely proportional to the SDMA concentration of the sample.

2 Precautions

- For in vitro diagnostic use only.
- Wear protective clothing, disposable gloves, and safety goggles while performing the test.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.
- Individual components of different lots and test kits should not be interchanged. The expiry dates and storage conditions stated on the packaging and the labels of the individual components must be observed.
- Some of the components contain hazardous substances. These components bear the appropriate hazard symbol on their label. Further information can be found in Contents of the Kit and on the relevant safety data sheets.
- Avoid contact with individual reagents.
- Dispose of waste according to state and local environmental protection regulations.

3 Storage and Stability

On arrival, store the kit at 2-8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.

Do not use components beyond the expiration date shown on the labels.

Do not mix various lots of any kit component within an individual assay.

4 Contents of the Kit

MT-Strips **STRIPS** 12 strips

8 wells each, break apart, precoated with SDMA

Standards (1 - 6) **CAL 1 - 6** 6 vials

4 ml each, ready for use

Standard	1	2	3	4	5	6
µmol/l	0	0.2	0.4	0.7	1.2	3
ng/ml	0	40	81	141	242	606

Control 1 & 2 **CON 1 & 2** 2 vials
each 4 ml ready for use, Range: see QC certificate

Acylation Reagent **ACYL-REAG** 3 vials
lyoph., dissolve contents in 3 ml Solvent before use

Acylation Buffer **ACYL-BUFF** 1 vial
3.5 ml, ready for use, blue coloured


Warning

Solvent **SOLVENT** 1 vial
10 ml ready for use, contains DMSO,
Please note that Solvent reacts with many plastic materials including plastic trays; Solvent does not react with normal pipette tips and with glass devices


Danger


Warning

Antiserum **AS** 1 vial
7 ml, ready for use, Rabbit-anti-N-acyl-SDMA, yellow coloured

Enzyme Conjugate **CONJ** 1 vial
13 ml, ready for use,
Goat-anti-rabbit-IgG-peroxidase

Wash Buffer **WASH** 1 vial
20 ml, conc. (50x), Dilute with dist. water to 1000 ml total volume

Substrate **SUB** 1 vial
13 ml TMB Solution, ready for use

Stop Solution **STOP** 1 vial
13 ml, ready for use, contains 0.3M sulphuric acid, not corrosive

Reaction Plate **ACYL-PLATE** 1 piece
For acylation

Equalizing Reagent **EQUA-REAG** 1 vial
lyoph., dissolve contents with 21 ml dist. water,
dissolve carefully to minimize foam formation

Foil **FOIL** 2 pieces

Ready for use

Additional materials and equipment required but not provided:

- Pipettes (20, 50, 100 and 200 µl,)
- Multipette
- Orbital shaker
- Microplate washing device
- Microplate photometer (450 nm)
- Vortex mixer, roll mixer

5 Sample Collection

5.1 Serum and Plasma

The test can be performed with serum as well as with EDTA plasma.

Hemolytic and lipemic samples should not be used.

The samples can be stored up to 6 hours at 2 – 8 °C. For a longer storage (up to 18 months) the samples must be kept frozen at -20 °C

Repeated freezing and thawing should be avoided.

6 Preparation of Reagents and Samples

6.1 Microtiter strips

Before opening the packet of strip wells STRIPS, allow it to stand at room temperature for at least 10 minutes. After opening, keep any unused wells in the original foil packet with the desiccant provided. Reseal carefully and store at 2 – 8 °C.

6.2 Wash Buffer

Dilute the contents of WASH with dist. water to a total volume of 1000 ml, mix briefly. Stored at 2 – 8 °C, the diluted wash buffer is stable for up to 4 weeks. We recommend preparing only the required volume of wash buffer for each run, when splitting the kit into several runs.

6.3 Equalizing Reagent

Dissolve the contents of EQUA-REAG with 21 ml dist. water, mix shortly and leave on a roll mixer for 20 minutes. Avoid excess formation of foam. The reconstituted Equalizing Reagent should be stored frozen at -20 °C and is stable until expiry date.

6.4 Acylation Reagent

Dissolve the contents of one bottle ACYL-REAG with 3 ml Solvent SOLVENT and shake for 10 minutes on an orbital shaker. After use the reagent has to be discarded. The Acylation Reagent has always to be prepared immediately before use and is stable for minimum 3 hours. The two other bottles allow a second and third run of the test. If the whole kit is to be used in one run it is recommended to pool the dissolved contents of the two vials of Acylation Reagent.

Please note that Solvent reacts with many plastic materials including plastic trays which are used as reservoir for multichannel pipettes. Solvent does not react with normal pipette tips and with glass devices. It is recommended to use a multipette, fill it directly from the vial and add the Acylation Reagent to the wells.

All other reagents are ready for use.

6.5 Preparation of Samples (Acylation)

The wells of the Reaction Plate ACYL-PLATE for the acylation can be used only once. Please mark the respective wells before use to avoid repeated use.

1. Pipette each 20 µl standard 1 – 6 CAL 1 -6, each 20 µl control 1 & 2 CON 1 & 2 and each 20 µl patient sample into the respective wells of the Reaction Plate ACYL-PLATE.
2. Pipette 20 µl Acylation Buffer ACYL-BUFF into each well.
3. Pipette 200 µl reconstituted Equalizing Reagent EQUA-REAG into each well.
4. Mix the reaction plate for 10 seconds.
5. Prepare Acylation Reagent freshly and pipette 50 µl prepared Acylation Reagent ACYL-REAG each into each well, mix immediately.
   It is recommended to use a multipette. Fill it directly from the vial and add the Acylation Reagent to the wells.
   Colour changes to violet.
6. Incubate for 20 minutes at room temperature (approx. 20 °C) on an orbital shaker. Do not cover wells or plate, leave the plate open on the shaker.

Take 25 µl each for the SDMA human ELISA.

7 Test Procedure ELISA

Bring all reagents to room temperature and mix them carefully, avoid development of foam.

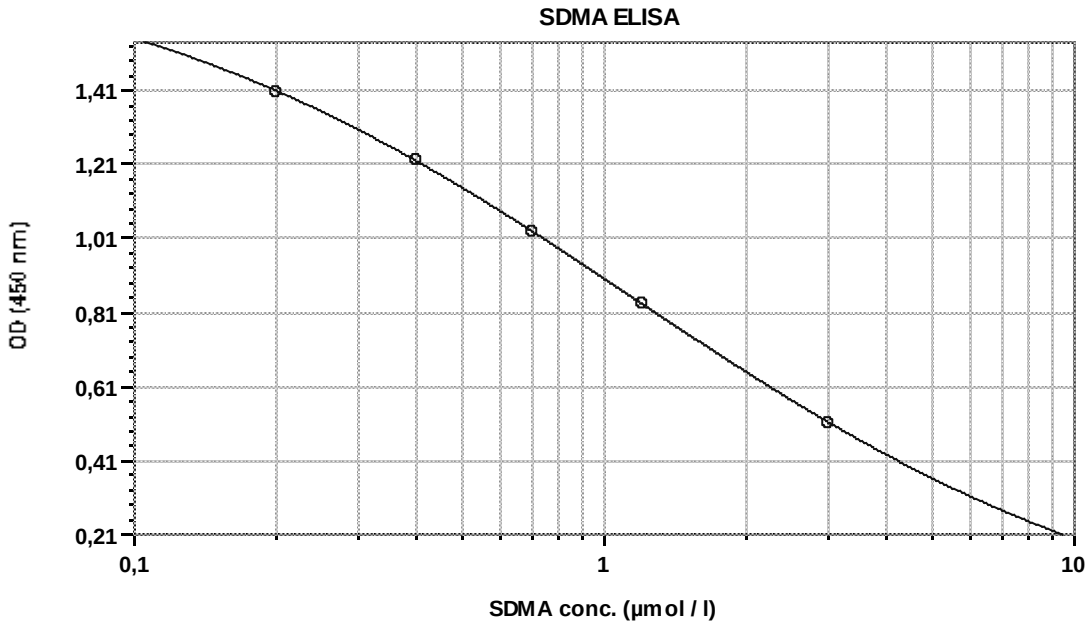
1. Pipette each 25 µl prepared Standards 1 to 6, 25 µl prepared controls and 25 µl prepared samples into the respective wells of the coated microtiter strips [STRIPS] (duplicates are recommended).
2. Pipette 50 µl Antiserum [AS] into each well.
3. Cover the plate with adhesive foil [FOIL] and incubate Microtiter Strips for 90 minutes at room temperature (20 – 25 °C) on an orbital shaker.
4. Discard or aspirate the contents of the wells and wash thoroughly with each 300 µl Wash Buffer [WASH]. Repeat the washing procedure 4 times. Remove residual liquid by tapping the inverted plate on clean absorbent paper.
5. Pipette 100 µl enzyme conjugate [CONJ] into each well.
6. Incubate for 30 minutes at room temperature on an orbital shaker.
7. Washing: Repeat step 4.
8. Pipette 100 µl Substrate [SUB] into each well and incubate for 25 ± 5 minutes at room temperature on an orbital shaker.
9. Pipette 100 µl Stop Solution [STOP] into each well.
10. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

8 Calculation of the Results

The concentration of the standards (x-axis, logarithmic) are plotted against their corresponding optical density (y-axis, linear). Cubic spline, 4 parameter or similar iteration procedures are recommended for evaluation of the standard curve.

The concentration of the controls and samples can be read directly from this standard curve by using their average optical density.

Typical standard curve:



y = ((A - D)/(1 + (x/C)^B)) + D:	A	B	C	D	R^2
Std (Standards: Concentration vs MeanValue)	1,799	0,772	1,157	-0,104	1

Conversion factor: 1 µmol/l = 202 ng/ml = 20.2 µg/dl

9 Assay Characteristics

9.1 Expected Values

The reference ranges given below should only be taken as a guideline. It is recommended that each laboratory should establish its own reference values.

Matrix	Reference range
Human Serum, EDTA-Plasma,	0.30 – 0.75 µmol / l (6.0 – 15 µg / dl)

9.2 Sensitivity

Lower Detection Limit	Calculation
0.03 µmol / l	OD_{Cal1} – 3 x SD

9.3 Recovery

	Range (µmol/l)	Mean (%)	Range (%)
EDTA-Plasma	0.43 – 1.44	97	86 - 104
Serum	0.45 – 1.72	93	88 - 102

9.4 Linearity

	Range (µmol/l)	Highest Dil.	Mean (%)	Range (%)
EDTA-Plasma	0.23 – 1.72	1 : 6 with water	97	89 – 105

9.5 Specificity (Cross Reactivity)

Substance	Cross Reactivity (%)
SDMA	100
ADMA	0.74
NMMA	0.76
Homoarginine	0.04
Arginine	0.01

9.6 Reproducibility

	Range (µmol/l)	Intra Assay CV
EDTA-Plasma	0.52 – 0.82	6.2 – 4.9 %

	Range (µmol/l)	Inter Assay CV
EDTA-Plasma	0.52 – 1.21	2.0 – 8.8 %

9.7 Method Comparison

	Method	Correlation
Serum / Plasma	LC/MS	Y = 0.96 x LC/MS + 0.05; R = 0.987; N = 32

10 Changes to declare

Version _6: Changes/additions are highlighted in gray.

Version _5: IFU has been re-formatted. The manufacturer and distributor information have been changed. Component names as printed on labels were included in sections 6 and 7 and pipetting schemes to provide greater clarity. No changes have been made to components or execution of protocols. Page 11 step 10: “within 15 minutes” was included (highlighted grey).

11 Literature

- Bode-Böger S.M., Scalera F., Kielstein J.T., Martens-Lobenhoffer J., Breithardt G., Fobker M., Reinecke H.
  Symmetrical Dimethylarginine: A new combined parameter for renal function and extent of coronary artery disease
  J. Am. Soc. Nephrol. (2006) **17**: 1128-1134

- Kielstein J.T., Salpeter S.R., Bode-Böger S.M., Cooke J.P., Fliser D.
  Symmetric dimethylarginine (SDMA) as endogenous marker of renal function – a meta-analysis
  Nephrol. Dial. Transplant (2006) **21**: 2446 - 2451

- Wanby P., Teerlink T., Brudin L., Brattström L., Nilsson I., Palmqvist P., Carlsson M.
  Asymmetric dimethylarginine (ADMA) as a risk marker for stroke and TIA in a Swedish population
  Atherosclerosis (2006) 185: 271 – 277

Pipetting Scheme - Sample Preparation

		Standards	Controls	Plasma	Serum
ACYL-PLATE:					
CAL 1 - 6	µl	20			
CON 1 & 2	µl		20		
EDTA-Plasma	µl			20	
Serum	µl				20
ACYL-BUFF	µl	20	20	20	20
EQUA-REAG	µl	200	200	200	200

Shake for 10 seconds

ACYL-REAG (fresh)	µl	50	50	50	50

Incubate for 20 minutes at room temperature on an orbital shaker
Take 25 µl each for ELISA

Pipetting Scheme - ELISA

		Acyl. Standards	Acyl. Controls	Acyl. Samples
STRIPS:				
Transfer from ACYL-PLATE into STRIPS	µl	25	25	25
AS	µl	50	50	50

Cover frame with FOIL and incubate on an orbital shaker
for 90 minutes at room temperature
Wash 4 x with 300 µl WASH per well

CONJ	µl	100	100	100

Incubate for 30 minutes at room temperature on an orbital shaker
Wash 4 x with 300 µl WASH per well

SUB	µl	100	100	100

Incubate for 25 ± 5 minutes at room temperature on an orbital shaker

STOP	µl	100	100	100

Read absorbance at 450 nm (Ref. 570 – 650 nm)