DIAGNOSTIKA GMBH

Instructions for Use

SDMA human ELISA

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

CE IVD

Item No. EA214/96

12 x 8

2 – 8 °C

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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

The symbols of the components of the kit are described in chapter 4 Contents of the Kit.

1 Introduction and Principle of the Test

Accurate determination of kidney function is an important part of a patient's clinical assessment due to the need to adjust the dosage of numerous drugs in cases of impaired kidney function. Even minor impairments in kidney function are associated with an increased risk of cardiovascular disease. Since the most commonly used parameter for determining kidney function, serum creatinine concentration, does not yet increase in cases of minor kidney impairment, there is a need to offer more sensitive markers of kidney function, especially in cases of minor functional impairment. SDMA is a methylated derivative of the amino acid L-arginine (symmetric dimethylarginine). SDMA is eliminated from the body exclusively through renal excretion; therefore, SDMA plasma concentration is closely correlated with renal function. Measuring SDMA therefore offers a way to sensitively determine renal function impairment, as has been demonstrated in several clinical studies: In 18 clinical studies involving over 2,100 patients, a highly significant correlation was found between SDMA plasma concentration and inulin clearance or various methods of determining glomerular filtration rate.

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 measurement methods available to date for the quantitative determination of SDMA in plasma, serum, urine, and other biological fluids were all based on the chemical detection method of high-pressure liquid chromatography (HPLC). However, this method is very time-consuming, labor-intensive, and expensive, and is therefore not suitable for routine clinical diagnostics. The SDMA human ELISA offers the advantage of specific and sensitive detection of this marker using routine methods and is superior to HPLC measurement in terms of personnel and technical effort.

The SDMA Human ELISA kit contains reagents for the quantitative determination of derivatized SDMA in plasma and serum. Derivatization takes place during sample preparation. SDMA is quantitatively converted to N-acyl-SDMA by the acylation reagent.

The SDMA human ELISA is a competitive enzyme immunoassay. Antigen bound to the solid phase and free antigen in solution compete for a defined number of antibody binding sites. When the system is in equilibrium, the unbound antigen-antibody complex is removed in a washing step and the correspondingly bound complex is detected using a peroxidase conjugate and determined via the conversion of tetramethylbenzidine (TMB). The TMB/POD reaction is stopped and measured at 450 nm. The concentration of the antigen-antibody complex bound to the solid phase is inversely proportional to the concentration of the antigen in the sample.

2 Precautions

- For in vitro diagnostic use only.
- Before performing the test, the valid instructions for use, as included in this kit, should be read completely and the content understood.
- 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.
- When handling the reagents, controls and patient samples, the current laboratory safety guidelines and good laboratory practice should be observed.
- Wear protective clothing, disposable gloves, and safety goggles while performing the test.
- Some of the components contain hazardous substances. These components bear the appropriate hazard symbol on their label. Further information can be found in 4. Contents of the Kit and on the relevant safety data sheets.
- Avoid any actions that could result in ingestion, inhalation or injection of the reagents. Never pipette by mouth.
- Avoid contact with individual reagents, as these can cause irritation and chemical burns.
- Dispose of waste according to state and local environmental protection regulations.
- Broken glass can cause injury. Be cautious with glass vials.
- The quality control guidelines in the medical laboratory regarding the inclusion of control samples and/or pooled samples should be observed.

3 Storage and Stability

The kit is shipped at ambient temperature. Upon arrival, store the kit at 2 - 8 °C to keep it stable until its expiry date. Once opened, the kit is stable until its expiry date. The shelf life of the ready-to-use reagents is indicated on the respective bottle label. For stability of prepared reagents refer to section 6.

Reagents must equilibrate to room temperature before use and refrigerated immediately after use.

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

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., (see 6.3)

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
 Warning

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

Wash Buffer **WASH** 1 vial
20 ml, conc. (50x), (see 6.1)

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., (see 6.2)

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
- Multichannel pipette or Microplate washing device
- Microplate photometer (450 nm)
- Vortex mixer, roll mixer
- Paper towels, pipette tips, timer

5 Sample Collection and Storage

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

Hemolytic, icteric 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 Wash Buffer

Dilute the contents of WASH with dist. water to a total volume of 1000 ml, mix briefly. For further use, the diluted wash buffer must be stored at 2 – 8 °C for a maximum period of 4 weeks. Should the kit be used in several runs, then prepare only the required amount of wash buffer for each run.

6.2 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.

6.3 Acylation Reagent

The kit contains 3 vials of Acylation Reagent for multiple runs. When using the kit in one run, pool the dissolved contents of two vials. Remove the required amount of vials of Acylation Reagent ACYL-REAG from the foil pouch, leave the remaining vials inside together with the desiccant and close the pouch carefully.

Reconstitute each vial of lyophilized Acylation Reagent with 3 mL of Solvent SOLVENT and mix on a roller mixer or similar shaker for at least 5 minutes. The Acylation Reagent should be freshly prepared immediately before performing the test and is then stable for approx. 3 hours. Discard the remaining reconstituted reagent after use.

All other reagents are ready for use.

6.4 Preparation of Samples (Acylation)

Equilibrate reagents to room temperature.

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.

Duplicates are recommended.

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 (see 6.2) into each well.
4. Shake the reaction plate for 10 seconds on an orbital shaker at medium speed.
5. Pipette 50 µl of freshly prepared Acylation Reagent ACYL-REAG (see 6.3) each into each well, continue with the next step, immediately. Color changes to violet.
   Attention: Please note that Acylation Reagent reacts with many plastic materials including plastic trays. It does not react with normal pipette tips and with glass devices. Use an Eppendorf multipette or similar, fill the syringe directly from the vial and add well by well.
6. Incubate for 20 minutes at room temperature on an orbital shaker at medium speed.

Take 20 µl each for the SDMA human ELISA.

7 Test Procedure ELISA

1. Pipette each 20 µl prepared Standards 1 to 6, 20 µl prepared controls and 20 µl prepared samples into the respective wells of the coated microtiter strips [STRIPS] (duplicates are recommended).
   Leave remaining microtiter strips in the foil pouch together with the desiccant and close carefully.
2. Pipette 50 µl Antiserum [AS] into each well.
3. Seal the plate with adhesive foil [FOIL] and incubate for 90 minutes at room temperature (20 – 25 °C) on an orbital shaker at medium speed.
4. Discard or aspirate the contents of the wells and wash thoroughly with 300 µl Wash Buffer [WASH] (see 6.1) per well. Discard or aspirate the contents of the wells and remove residual liquid by tapping the inverted plate on a clean absorbent paper towel. Repeat the washing procedure 3 times.
   Alternatively, a washing device may be used.
5. Pipette 100 µl enzyme conjugate [CONJ] into each well.
6. Incubate for 30 minutes at room temperature on an orbital shaker at medium speed.
7. Washing: Repeat step 4.
8. Pipette 100 µl Substrate [SUB] into each well.
9. Incubate for 25 ± 5 minutes at room temperature (20 – 25 °C) on an orbital shaker at medium speed.
10. Pipette 100 µl Stop Solution [STOP] into each well and shake on an orbital shaker for minimum 10 seconds at medium speed.
11. 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

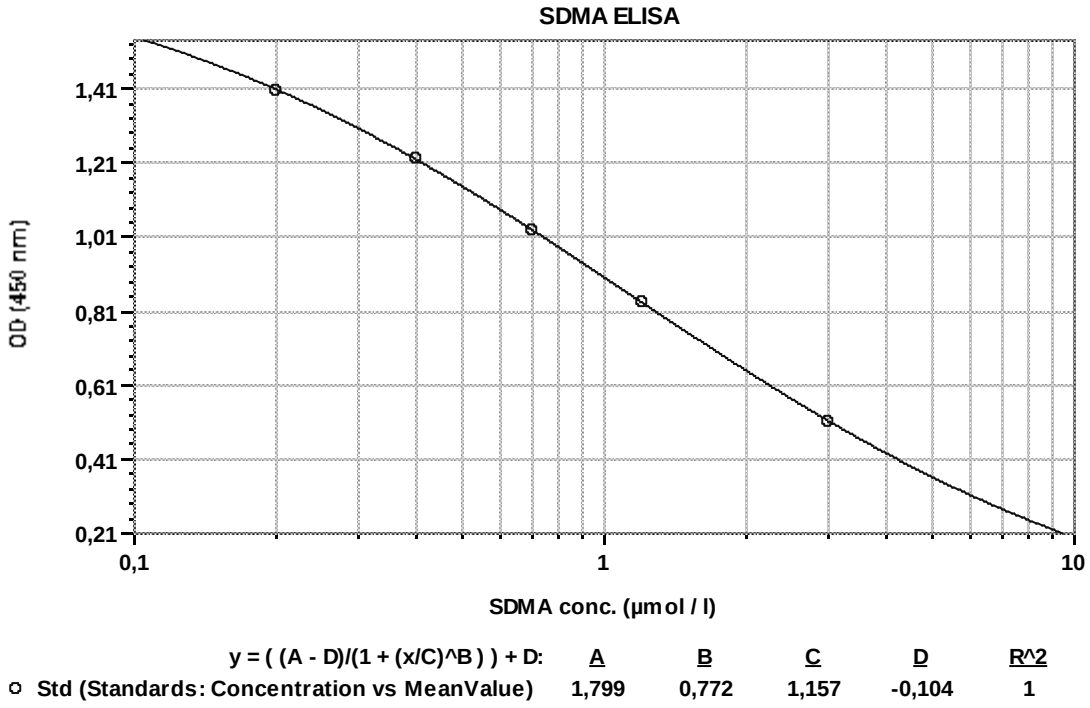
Standards	1	2	3	4	5	6
SDMA µmol/l	0	0.2	0.4	0.7	1.2	3
SDMA ng/ml	0	40	81	141	242	606

The concentration of the standards (x-axis, logarithmic) are plotted against their corresponding optical density (y-axis, linear).

A good fit is provided with 4 Parameter Logistic (alternatively Log-Logit or Cubic Spline).

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

Typical standard curve:



Conversion factor: 1 µmol/l = 202 ng/ml

Quality Control: Test results are valid only if the kit controls are within the ranges specified on the QC Certificate. Otherwise, the test should be repeated.

9 Assay Characteristics

9.1 Reference Range

The reference range 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

9.2 Sensitivity

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

9.3 Specificity (Cross Reactivity)

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

9.4 Recovery

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

9.5 Linearity

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

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 / EDTA-Plasma	LC/MS	Y = 0.96 x LC/MS + 0.05; R = 0.987; N = 32

9.8 Calibration

The calibration is carried out by weighing the pure substance. The correctness of the method was determined by comparison with reference range (9.1) and method comparison (9.7).

9.9 Limitations of Method

The results are to be seen in connection with other diagnostic procedures and the anamnesis and the resulting questions. Samples measured above the highest standard must be diluted with distilled water (9.5) and reassayed. The values of diluted samples must be multiplied by the appropriate dilution factor.

9.10 Interferences

Hemolytic, lipemic and icteric samples should not be used.

10 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

11 Changes to declare

Version _7: Changes/additions are highlighted in gray.

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).

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	µl	50	50	50	50

Immediately, shake for 20 minutes at room temperature

Pipetting Scheme - ELISA

		Acyl. Standards	Acyl. Controls	Acyl. Samples
STRIPS:				
Transfer from ACYL-PLATE into STRIPS	µl	20	20	20
AS	µl	50	50	50

Seal wells with FOIL
Shake for 90 minutes at room temperature
Wash 4 x with 300 µl WASH per well

CONJ	µl	100	100	100

Shake for 30 minutes at room temperature
Wash 4 x with 300 µl WASH per well

SUB	µl	100	100	100

Shake for 25 ± 5 minutes at room temperature

STOP	µl	100	100	100

Shake for 10 seconds
Read absorbance at 450 nm (Ref. 570 – 650 nm)