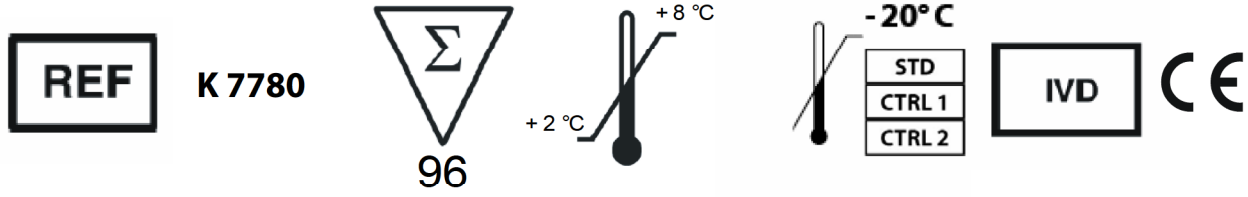
Manual

SDMA ELISA Kit

For the determination of SDMA in human EDTA-plasma and serum

Valid from 08.12.2009



1. INTENDED USE

The SDMA ELISA Kit is intended for the quantitative determination of symmetric dimethylarginine (SDMA) in human EDTA-plasma and serum. For *in vitro* diagnostic use only.

2. INTRODUCTION

The dosage of most drugs must be adapted in renal insufficiency, making accurate assessment of renal function a prerequisite in clinical medicine. Furthermore, even a modest decline in renal function has been recognized as a cardiovascular risk.

In clinical practice serum creatinine is typically used to asses renal function, but this serum creatinine does not increase at modest decline in renal function. Consequently, there is an ongoing search for suitable endogenous markers of renal function.

SDMA is a methylated derivative of L-Arginine which is strictly eliminated by renal extraction, thus SDMA plasma level is strongly correlated to renal function. In 18 studies with more than 2136 patients systemic SDMA concentrations correlated highly with inuline clearance, as well as with various clearance estimates combined and serum creatinine. With respect to this SDMA exhibits properties of a reliable marker of renal dysfunction.

Moreover, there are hints that increased SDMA correlates with total sequential organ failure indicating both renal and hepatic failure and an increased cardiovascular risk.

Indication

- Renal failure
- Cardiovascular risk in renal dysfunction
- Hypertension in renal dysfunction

3. PRINCIPLE OF THE TEST

This assay is based on the method of competitive enzyme linked immunoassays. The sample preparation includes the addition of an derivatization-reagent for SDMA coupling. Afterwards, the treated samples and the polyclonal SDMA-antiserum are incubated in wells of microplate coated with SDMA-derivative (tracer). During the incubation period, the target SDMA in the sample competes with the tracer immobilized on the wall of the microtiter wells for the binding of the polyclonal antibodies. The SDMA in the sample displaces the antibodies out of the binding to the tracer. Therefore the concentration of the tracer-bound antibody is inverse proportional to the SDMA concentration in the sample. During the second incubation step, a peroxidase-conjugated antibody is added to each microtiter well to detect the anti-SDMA antibodies. After washing away the unbound components, tetramethylbenzidine (TMB) is added as a substrate for peroxidase. Finally, the enzymatic reaction is terminated by an acidic stop solution. The color changes from blue to yellow and the absorbance is measured in the photometer at 450 nm. The intensity of the yellow color is inverse proportional to the SDMA concentration in the sample; this means high SDMA concentration in the sample reduces the concentration of tracer-bound antibodies and lowers the photometric signal.

A dose response curve of absorbance unit (optical density, OD at 450 nm) vs. concentration is generated using the values obtained from the standard. SDMA present in the patient samples is determined directly from this curve.

4. MATERIAL SUPPLIED

Catalog No	Content	Kit Components	Quantity
K7780MTP	PLATE	One holder with precoated strips	12 x 8 wells
K7780ST	STD	Standards (diluted in reaction buffer)	6 x 1 vial
K7780KO	CTRL 1 CTRL 2	Controls (diluted in reaction buffer)	2 x 1 vial
K7780WP	WASHBUF	Wash buffer concentrate (10 fold)	2 x 100 ml
K7780AK	AB	SDMA antibody (lyophilized)	1 vial
K7780K	2.AB	POD antibody (concentrate)	120 µl
K7780CSP	2.ABDIL	Conjugate stabilizing buffer	24 ml
K7780RP	DERBUF	Reaction buffer	15 ml
K7780DR	DER	Derivatization reagent	2 x 50 mg
K7780LM	DMSO	Dimethylsulfoxid (DMSO)	7 ml
K7780SL	CODIL	Dilution buffer for coupling	28 ml
K7780TMB	SUB	TMB substrate	25 ml
K7780AC	STOP	Stop solution	15 ml

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Bidistilled water (aqua bidist.)
- Precision pipettors and disposable tips to deliver 10-1000 µl
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 10000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 450 nm
  (reference wave length 620 or 690 nm)

6. PREPARATION AND STORAGE OF REAGENTS

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each assay**. The kit can be used up to 2 times within the expiry date stated on the label.

- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.

- The **Wash buffer concentrate (WASHBUF)** should be diluted with aqua bidist. **1:10** before use (100 ml concentrate + 900 ml aqua bidist.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at room temperature or at 37°C using a water bath before dilution of the buffer solutions. The **buffer concentrate** is stable at **2-8°C** until the expiry date stated on the label. Diluted **buffer solution** can be stored in a closed flask at **2-8°C for one month**.

- **Standards (STD) and Controls (CTRL1, CTRL2)** are already diluted in the **reaction buffer (DERBUF).** Store Standards and Controls frozen at -20°C, thaw before use in the test, and re-freeze immediately after use. Standards and Controls can be re-frozen up to 3 times.

- **DMSO** could crystallize at 4°C. Dissolve the crystals at 20-25°C in a water bath.

- The content of one vial of **derivatization reagent (DER) (50 mg)** must be dissolved in **3 ml DMSO.** Put the vial on a horizontal shaker for 5 min. After use, the rest of the reagent should be discarded. DER must be **prepared immediately before use**. The ELISA kit can be separated into two performances by the two DER vials. Please note: **DMSO attacks all plastics but not polypropylene products and laboratory glass.**

- The **SDMA antibody (AB)** must be dissolved in **11.2 ml of diluted wash buffer**. Therefore, as first, the content of the AB vial must be reconstituted with 1.2 ml of diluted wash buffer for 5 minutes. Then the obtained AB solution is quantitatively transferred into a separate vial and 10 ml of diluted wash buffer is added. **Diluted SDMA antibody (AB) is stable over a longer period of time. It can be stored at 2-8°C for 4 weeks.**

- The **POD antibody (2.AB)** must be diluted **1:200** in **conjugate stabilizing buffer (2.ABDIL)** (110 µl 2.AB + 22 ml 2.ABDIL). The undiluted POD antibody (2.AB) is stable at **2-8°C** until the expiry date stated on the label. **Diluted POD antibody (2.AB) is not stable over a longer period. It can be stored at 2-8°C for only 5 days.**

- All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at 2-8°C.

7. PRECAUTIONS

- For *in vitro* diagnostic use only.
- Human materials used in kit components were tested and found to be negative for HIV and Hepatitis B. However, for safety reasons, all kit components should be treated as if potentially infectious.
- Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.
- Reagents should not be used beyond the expiration date shown on kit label.

8. SPECIMEN COLLECTION AND PREPARATION

EDTA-plasma and serum

- Venous fasting blood is suited for this test system. Samples are stable for one week at 2-8°C. For longer storage samples should be frozen at –20°C up to the measurement.
- Lipemic or hemolytic samples may give erroneous results and should not be used for analysis.
- The **EDTA-plasma and serum** samples are analyzed without any dilution.*

  *If the sample volume is less than 50 µl, a 1:1 dilution in DERBUF (reaction buffer) is recommended (25 µl sample + 25 µl DERBUF). This dilution factor should be considered for data evaluation.

  Samples with visible amounts of **precipitates** should be **centrifuged** at least for 5 min at 10000 x g. The resulting supernatant is used in the assay.
- For sample preparation, a DER for coupling of SDMA is added (details are given in the sample preparation procedure).

9. ASSAY PROCEDURE

Procedural notes

- Quality control guidelines should be observed.
- Incubation time, incubation temperature and pipetting volumes of the different components are defined by the producer. Any variations of the test procedure, that are not coordinated with the producer, may influence the test results. Immundiagnostik AG can therefore not be held responsible for any damage resulting from this.
- The assay should always be performed according to the enclosed manual.

Sample preparation procedure

Coupling of standards (STD), controls (CTRL) and samples (SAMPLE) are carried out in single analysis.

1.	Bring all reagents and samples to room temperature (18-26°C)
2.	Add **200 µl of ready to use standards (STD), 200 µl of ready to use controls (CTRL)** and **50 µl of samples (SAMPLE)** in the corresponding vial

3.	Add **150 µl of reaction buffer (DERBUF) only to the samples (SAMPLE)**
4.	Add **50 µl** of freshly prepared **derivatization reagent (DER)** into each vial (standards, controls and samples), mix well and incubate **for 45 min on a shaker (180-240 rpm) at room temperature (18-26°C)**
5.	Afterwards add **250 µl of dilution buffer (CODIL)** into each vial, mix well and incubate for **45 min on a shaker (180-240 rpm) at room temperature (18-26°C)**

2 x 100 µl of each treated sample (STD, CTRL, SAMPLE) are used in the ELISA in duplicate.

Test procedure

6.	Mark the positions of **standards (STD)/controls (CTRL)/ samples (SAMPLE)** in duplicate on a protocol sheet
7.	Take as many microtiter strips (PLATE) as needed from kit. Store unused strips covered at 2-8°C. Strips are stable until the expiry date stated on the label
8.	Wash each well **5 times** by dispensing **250 µl** of diluted **wash buffer** into each well. After the final washing step, the inverted microtiter plate (PLATE) should be firmly tapped on absorbent paper to remove excess solution
9.	For the analysis in duplicate, take **2 x 100 µl of standard (STD)/control (CTRL)/samples (SAMPLE)** out of the vial and add into the respective well of the microtiter plate (PLATE)
10.	Add **100 µl** diluted **SDMA antibody (AB)** into each well. Cover the plate tightly

11.	Incubate overnight **(15-20 hours) at 2-8°C**
12.	Aspirate the contents of each well. Wash each well **5 times** by dispensing **250 µl** of diluted **wash buffer** into each well. After the final washing step, the inverted microtiter plate (PLATE) should be firmly tapped on absorbent paper to remove excess solution
13.	Add **200 µl** diluted **POD antibody (2. AB)** into each well
14.	Cover plate tightly and incubate for **1 hour at room temperature (18-26°C)** on a horizontal shaker (180-240 rpm)
15.	Aspirate the contents of each well. Wash each well **5 times** by dispensing **250 µl** of diluted **wash buffer** into each well. After the final washing step, the inverted microtiter plate (PLATE) should be firmly tapped on absorbent paper to remove excess solution
16.	Add **200 µl** of **TMB substrate (SUB)** into each well
17.	Incubate for **8-12 min at room temperature (18-26°C)** in the dark*
18.	Add **100 µl of stop solution (STOP)** into each well, mix thoroughly
19.	Determine absorption **immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as a reference

*The intensity of the color change is temperature sensitive. We recommend to observe the color change and to stop the reaction upon good differentiation.

10. EVALUATION OF RESULTS

If the test is performed in strict compliance with the manufacturer's instructions, e.g. with the exact volumes for standards, controls and samples/sample treatment, standards, controls and samples are equally diluted. Therefore, **no dilution factor is required for calculation of the results.** **

**At a 1:1 dilution, the dilution factor should be considered.

The following algorithms can be used alternatively to calculate the results. We recommend to use the "4-parameter-algorithm".

1. *4-parameter-algorithm*

   It is recommended to use a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e.g. 0.01).

2. *Point-to-point-calculation*

   We recommend a linear ordinate for optical density and a linear abscissa for concentration.

3. *Spline-algorithm*

   We recommend a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e.g. 0.01).

   The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done manually.

Expected values

Based on internal studies of evidently healthy persons **(n=40)** a mean value of 0,47 µmol/l was estimated. The standard variation was 0,07 µmol/l.

Normal range: Serum/Plasma mean value ± 2 standard variation: 0,47 ± 0,14 µmol/l

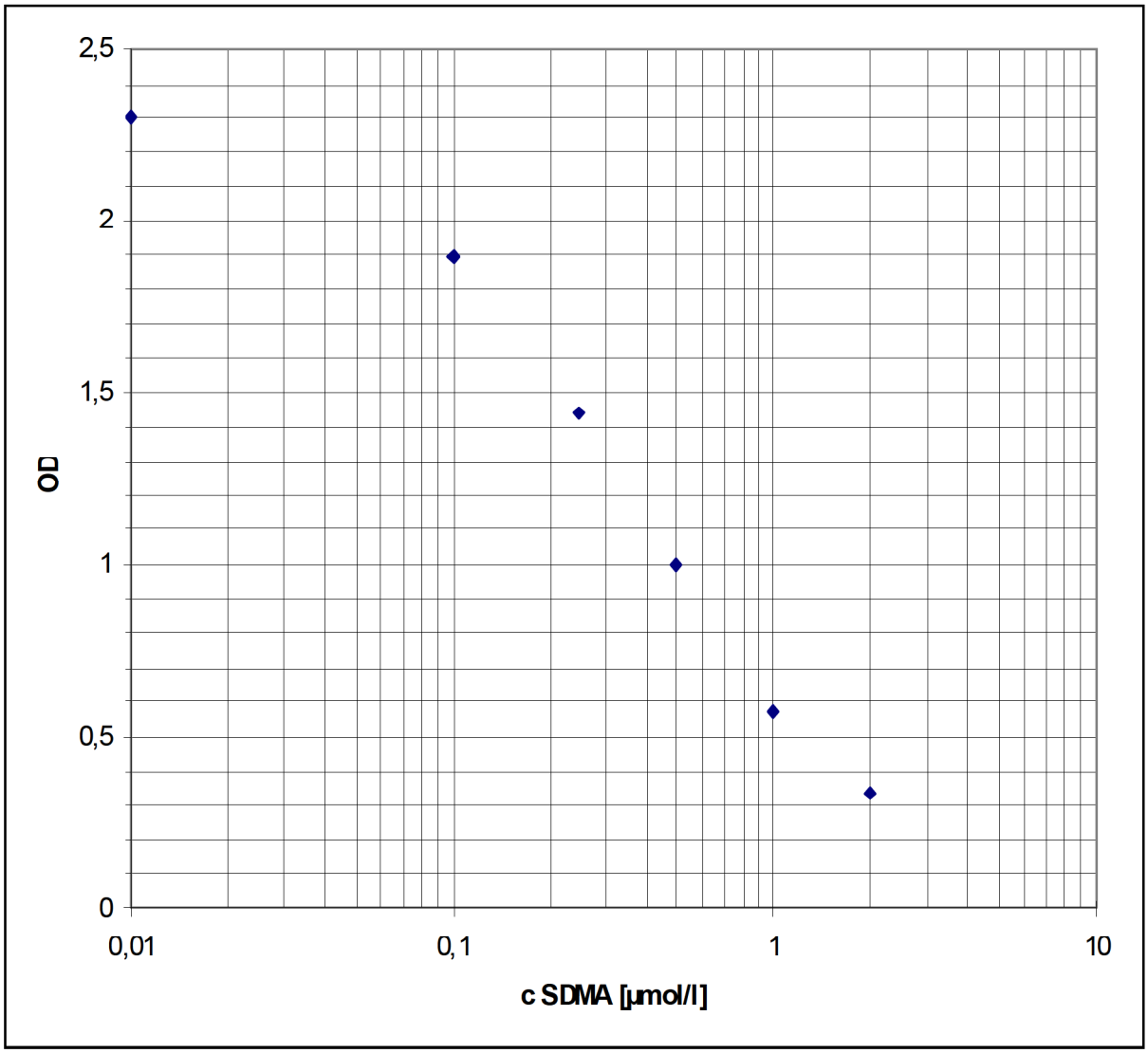
We recommend each laboratory to develop its own normal range. The values mentioned above are only for orientation and can deviate from other published data.

Controls

Control samples or serum pools should be analyzed with each run. Results, generated from the analysis of the control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits.

The concentration of controls and patient samples can be determined directly from calibration curve. In the following an example of a calibration curve is given.

Example of calibration curve



11. PERFORMANCE CHARACTERISTICS

Cross reactivity

ADMA < 0,5 % NMMA < 0,5 % L-Arginine < 0,02 %

Precision and reproducibility

Intra-Assay (n=12)		
Sample	SDMA [µmol/l]	Standard variation (SD) [%]
1	0,27	7,5
2	0,67	4,8

Inter-Assay (n=6)		
Sample	SDMA [µmol/l]	Standard variation (SD) [%]
1	0,22	6
2	0,63	7

Sensitivity

The sensitivity was set as B_0 + 2SD. The zero-standard was measured 6 times.

Sample	SDMA mean value [OD]	Standard variation (2 x SD) [%]	Detection limit [µmol/l]
0	2,3	0,05	0,05

Recovery

One sample was spiked with different SDMA concentrations and measured using this assay. The analytical recovery rate was determined by the expected and measured SDMA levels. The expected levels were calculated as the sum of the measured SDMA concentration in the original sample and the spiked SDMA amount. The mean recovery rate for all concentrations was 101 % (n=6).

Spike [µmol/l]	SDMA expected [µmol/l]	SDMA measured [µmol/l]	Recovery [%]
0	x	x=0,75	100
0,5	0,5+x=1,25	1,26	101
1	1,0+x=1,75	1,79	102

Linearity

The linearity of the ELISA was determined by the dilution of a spiked patient sample. The mean linearity was 95%.

Dilution	Expected [µmol/l]	Measured [µmol/l]	Recovery [%]
original	1,75	1,75	100
1+1	0,88	0,85	96
1+3	0,44	0,37	90

12. LIMITATIONS

Strong hemolytic and lipemic samples often show wrong concentrations. Do not to measure hemolytic and lipemic samples.

13. REFERENCES

1. Fleck C., Schweitzer F., Karge E., Busch M., Stein G. Serum concentrations of asymmetric (ADMA) and symmetric (SDMA) dimethylarginine in patients with chronic kidney diseases. Clinical Chimica Acta (2003) 336: 1 - 12
2. D'Apolito O., Paglia G., Tricarico F., Garofalo D., Pilotti A., Lamacchia O., Cignarelli M., Corso G. Development and validation of a fast quantitative method for plasma dimethylarginines analysis using liquid chromatography-tandem mass spectrometry. Clinical Biochemistry (2008) 41: 1391 - 1395
3. 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
4. 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

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.
- Test components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- All reagents in the test package are for *in vitro* diagnostic use only.
- Reagents should not be used after the date of expiry stated on the label.
- Single components with different lot numbers should not be mixed or exchanged.
- Guidelines for medical laboratories should be observed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from wrong use.

Used symbols:

	Temperature limitation	REF	Catalogue Number
IVD	In Vitro Diagnostic Medical Device		Contains sufficient for <n> tests
	Manufacturer		Use by
LOT	Lot number		