Manual

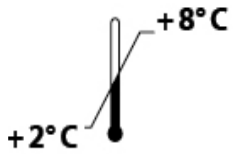
Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
info@listarfish.it
www.listarfish.it

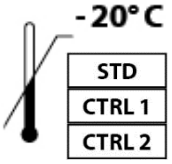
SDMA ELISA

For the in vitro determination of SDMA in human, canine and feline serum, EDTA and Li-heparin plasma

Valid from 2019-07-17

REF **K 7780**





IVD CE

Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
Tel.: + 49 6251 70190-0 Fax: + 49 6251 70190-363
e.mail: info@immundiagnostik.com www.immundiagnostik.com

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1. INTENDED USE

This Immundiagnostik AG assay is intended for the quantitative determination of SDMA in human, canine and feline serum, EDTA and Li-heparin plasma. 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 the 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 with an increased cardiovascular risk.

Indication

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

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit Components	Quantity
K 7780	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 7780	STD	Standards, ready-to-use (0, 0.1, 0.3, 0.6, 1.5, 4.0 µM)	6 x 500 µl
K 7780	CTRL 1	Control, ready-to-use (see specification for range)	1 x 500 µl

K 7780	CTRL 2	Control, ready-to-use (see specification for range)	1 x 500 µl
K 0006.C.100	WASHBUF A	Wash buffer concentrate, 10x	2 x 100 ml
K 7780	AB	SDMA antibody, ready-to-use	1 x 6 ml
K 7780	CONJ	Conjugate, ready-to-use	1 x 12 ml
K 0012.15	DERBUF	Reaction buffer, ready-to-use	1 x 15 ml
K 7780	DER	Derivatisation reagent, ready-to-use	1 x 6 ml
K 0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipets and 10-1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label.
- **Preparation of the wash buffer**: The **wash buffer concentrate (WASHBUF A)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF A + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF A** is stable at **2-8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF A) can be stored in a closed flask at **2-8 °C for 1 month**.

- Store **standards and controls (STD/CTRL)** frozen at **-20 °C**. They are stable at -20 °C until the expiry date stated on the label. Thaw before use in the test and mix well. Re-freeze standards and controls after use.
- The ready-to-use **derivatisation reagent (DER**) is alredy dissolved in DMSO and is stable at **2-8 °C** until the expiry date stated on the label. DMSO crystallises at 2-8 °C. Before opening the DER, bring to room temperature and ensure that all crystals are dissolved. Please note: DMSO attacks all plastics but not polypropylene products and laboratory glass.
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2-8 °C.**

6. STORAGE AND PREPARATION OF SAMPLES

Serum, EDTA and Li-heparin plasma

- Freshly collected serum, EDTA and Li-heparin plasma samples can be stored for up to 48 hours at room temperature or for 3 days at 2-8 °C. For longer storage keep samples frozen at -20 °C.
- The samples are analysed **undiluted**.

  If the sample volume is less than 50 µl, we recommend a 1:2 dilution in reaction buffer (25 µl sample + 25 µl DERBUF). This dilution factor must be considered in data evaluation.
- For sample preparation, a derivatisation reagent for derivatisation of SDMA is added (see sample preparation procedure).

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of SDMA. The assay is based on the method of competitive enzyme linked immunoassays.

The sample preparation includes the addition of a derivatisation reagent for SDMA derivatisation. Afterwards, the treated samples and the polyclonal SDMA antiserum are incubated in wells of a microtiter plate coated with SDMA derivative (tracer). During the incubation period, the target SDMA in the sample competes with the tracer, immobilised on the wall of the microtiter wells, for the binding of the polyclonal antibodies.

During the second incubation step, a peroxidase conjugated antibody is added to detect the anti-SDMA antibodies. After washing away the unbound components,

tetramethylbenzidine (TMB) is added as a peroxidase substrate. Finally, the enzymatic reaction is terminated by an acidic stop solution. The colour changes from blue to yellow and the absorbance is measured in a photometer at 450 nm. The intensity of the yellow colour 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 standards. SDMA, present in the patient samples, is determined directly from this curve.

Sample preparation procedure

Bring **all reagents and samples to room temperature** (15-30 °C) and mix well.

Derivatisation of standards, controls and samples is carried out in single analysis in vials (e.g. 1.5 ml polypropylene vials).

We recommend preparing one derivatisation per standard, control and sample and transferring it in duplicate determinations into the wells of the microtiter plate.

1.	Add **200 µl standard** (STD), **200 µl control** (CTRL) and **50 µl sample** in the corresponding vials.
2.	Add **150 µl reaction buffer** (DERBUF) **only to the samples.**
3.	Add **50 µl derivatisation reagent** (DER) into each vial (STD, CTRL, sample), **mix thoroughly** by repeated inversion or several seconds on a vortex mixer. Incubate for **45 min** at room temperature (15-30 °C) on a **horizontal shaker**.

2 x 50 µl of the derivatised standards, controls and samples are used in the ELISA as duplicates.

Test procedure

Mark the positions of standards/controls/samples in duplicate on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips covered with foil at 2-8 °C. Strips are stable until expiry date stated on the label.

4.	For the analysis in duplicate, take **2 x 50 µl** of the **derivatised standards/ controls/samples** out of the vials and add into the respective wells of the microtiter plate.
5.	Add **50 µl SDMA antibody** (AB) into each well of the microtiter plate.

6.	Cover the strips and incubate for **2 hours** at room temperature (15-30 °C) on a **horizontal shaker**.
7.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8.	Add **100 µl conjugate** (CONJ) into each well.
9.	Cover the strips and incubate for **1 hour** at room temperature (15-30 °C) on a **horizontal shaker**.
10.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
11.	Add **100 µl substrate** (SUB) into each well.
12.	Incubate for **10-15 min*** at room temperature (15-30 °C) in the **dark**.
13.	Add **100 µl stop solution** (STOP) into each well and mix well.
14.	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 (690 nm) as a reference.

* The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using 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 standard must be specified with a value less than 1 (e.g. 0.001).

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 linear abscissa for concentration.

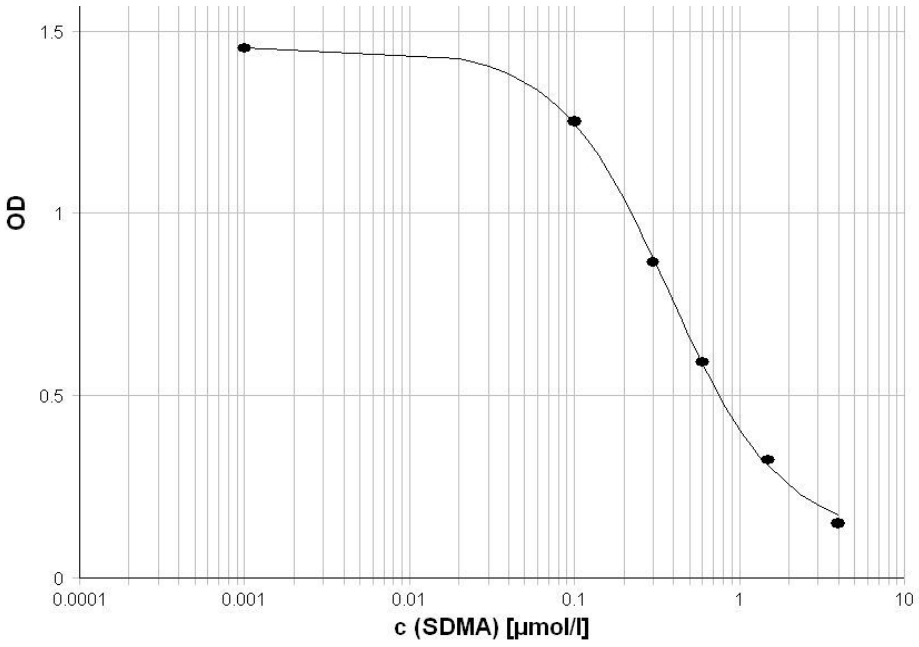
The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the program used, the duplicate values should be evaluated manually.

Serum, EDTA and Li-heparin plasma

Since the sample dilution is already considered in the standard curve, the dilution factor is 1.

In case an additional dilution factor is used, multiply the obtained result by the additionally used dilution factor.

In the following, an example of a calibration curve is given. Do not use it for the calculation of your results.



9. LIMITATIONS

Samples with concentrations above the measurement range can be diluted with reaction buffer and re-assayed. Please consider this dilution factor when calculating the results.

Samples with concentrations lower than the measurement range cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × *sample dilution factor to be used*

The lower limit of the measurement range can be calculated as:

analytical sensitivity × *sample dilution factor to be used*

Analytical sensitivity see chapter "Performance Characteristics".

Biotin interference

Samples containing a biotin concentration of < 40 ng/ml show a change of the results of ≤ 25 %. Higher concentrations of biotin can lead to falsely low results. Patients taking > 5 mg biotin per day should wait at least 24 hours after taking biotin to have their samples collected. Results of patients taking biotin supplements or receiving a high-dose biotin therapy should generally be interpreted along with the total clinical picture.

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of 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 samples are outside of the acceptable limits.

Reference range

Based on internal studies with human serum samples from apparently healthy persons (n = 40), a mean value of 0.47 µmol/l was estimated. The standard deviation (SD) was 0.09 µmol/l. From mean value ± 2 SD a normal range of 0.29 – 0.65 µmol/l was estimated.

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-assay (n = 10)

sample	SDMA [µmol/l]	CV [%]
1	0.27	7.5
2	0.67	4.8

Inter-assay (n = 6)

sample	SDMA [µmol/l]	CV [%]
1	0.22	9.9
2	0.63	7.4

Spiking recovery

Human

One serum sample was spiked with different SDMA concentrations and measured in this assay. The mean recovery rate was 100.4 % (n = 5).

spike [µmol/l]	expected [µmol/l]	measured [µmol/l]	recovery [%]
0		0.736	
0.5	1.236	1.220	98.7
1	1.736	1.774	102.2

Dog

Two serum samples were spiked with different SDMA concentrations. The mean recovery rate was 94.1 % (n = 2).

spike [µmol/l]	expected [µmol/l]	measured [µmol/l]	recovery [%]
0		0.526	
0.5	1.026	0.937	91.3
1	1.526	1.561	102.3
0		0.454	
0.5	0.954	0.897	94.0
1	1.454	1.288	88.6

Cat

Two serum samples were spiked with different SDMA concentrations. The mean recovery rate was 95.4 % (n = 2).

spike [µmol/l]	expected [µmol/l]	measured [µmol/l]	recovery [%]
0		0.395	
0.5	0.895	0.833	93.1
1	1.395	1.252	89.8
0		0.326	
0.5	0.826	0.818	99.0
1	1.326	1.321	99.6

Dilution recovery

Human

One spiked sample was diluted with reaction buffer. The mean recovery rate was 90.4 % (n = 5).

dilution	expected [µmol/l]	measured [µmol/l]	recovery [%]
		1.774	
1:2	0.887	0.876	98.8
1:4	0.444	0.364	82.1

Dog

Two samples were diluted with serum containing 0.126 µmol/l SDMA. The mean recovery rate was 109.0 % (n = 2).

dilution	expected [µmol/l]	measured [µmol/l]	recovery [%]
		0.526	
1:2	0.326	0.334	102.5
1:4	0.226	0.241	106.6
		0.454	
1:2	0.290	0.340	117.2
1:4	0.208	0.228	109.6

Cat

Two samples were diluted with serum containing 0.126 µmol/l SDMA. The mean recovery rate was 97.1 % (n = 2).

dilution	expected [µmol/l]	measured [µmol/l]	recovery [%]
		0.395	
1:2	0.261	0.247	94.8
1:4	0.193	0.194	100.4
		0.326	
1:2	0.226	0.231	102.2
1:4	0.176	0.160	90,9

Analytical sensitivity

The zero-standard (STD 1) was measured 40 times. The detection limit was set as B_0 - 2 SD and estimated to be 0.05 µmol/l.

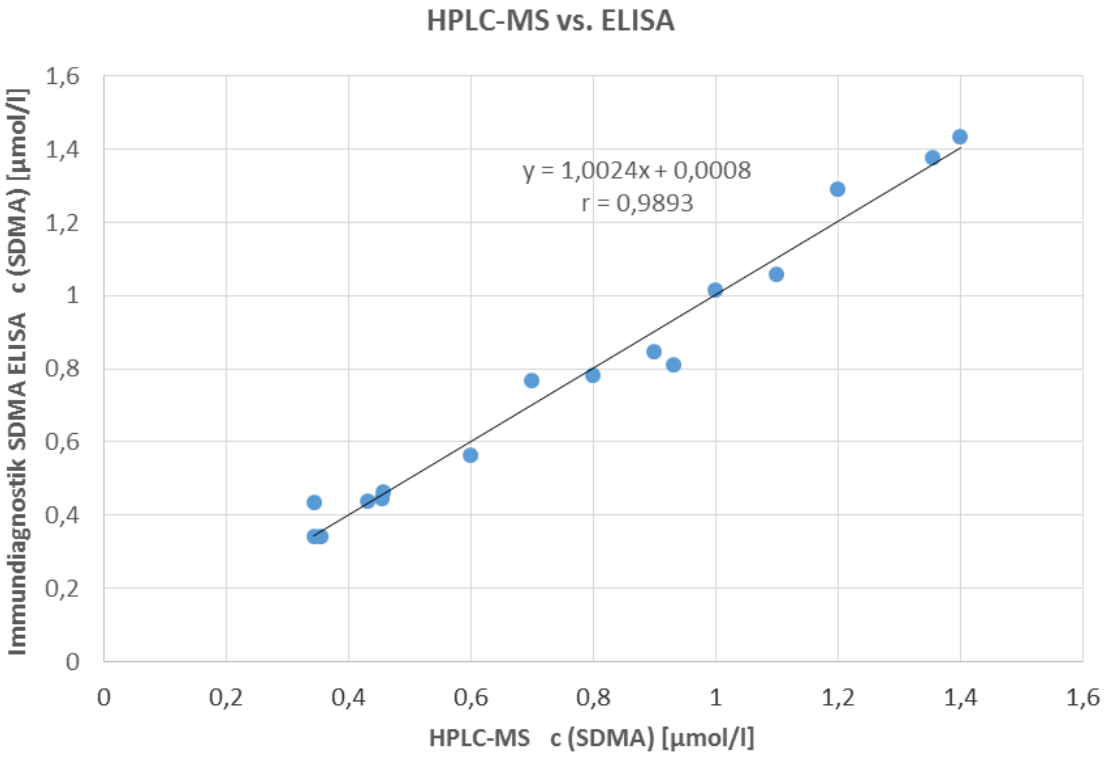
Specificity

The specificity of the antibody was tested by measuring the cross-reactivity against a range of compounds with structural similarity to SDMA. The specificity is calculated in percent in relation to the SDMA-binding activity.

ADMA	< 0.1 %
L-arginine	< 0.001 %

Correlation with HPLC-MS

16 samples were measured with this ELISA and HPLC-MS. The correlation was r = 0.99.



12. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- The stop solution consists of diluted sulfuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breathe vapour and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore, we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The guidelines for medical laboratories should be followed.
- 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 incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be sent to Immundiagnostik AG along with a written complaint.

15. REFERENCES

General literature

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* **336**: 1 – 12 (2003).

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* **41**: 1391 – 1395 (2008).
3. Kielstein JT, Salpeter SR, Bode-Böger SM, Cooke JP, Fliser D. Symmetric dimethylarginine (SDMA) as endogenous marker of renal function – a meta-analysis. *Nephrol. Dial. Transplant* **21**: 2446 - 2451(2006).
4. Bode-Böger SM, Scalera F, Kielstein JT, 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.* **17**: 1128 – 1134 (2006).

Literature using Immundiagnostik SDMA ELISA

5. Koch A, Weiskirchen R, Bruensing J, Dückers H, Buendgens L, Kunze J, Matthes M, Luedde T, Trautwein C, Tacke F. Regulation and prognostic relevance of symmetric dimethylarginine serum concentrations in critical illness and sepsis. *Mediators of Inflammation* **2013**: 413826 (2013).
6. Tenderenda-Banasiuk E, Wasilewska A, Taranta-Janusz K, Korzeniecka-Kozerska A. Asymmetric and symmetric dimethylarginine in adolescents with hyperuricemia. *Disease Markers* **35**: 407-412 (2013).

Used symbols:

 Temperature limitation

Catalogue Number

In Vitro Diagnostic Medical Device

To be used with

 Manufacturer

Contains sufficient for <n> tests

Lot number

 Use by

 Attention

 Consult instructions for use

Consult specification data sheet