






Instructions for Use

SDMA vet ELISA

Enzymimmunoassay for the
Quantitative Determination of
Endogenous Symmetric Dimethylarginine (SDMA)
in Serum or Plasma of Dogs and Cats

For Veterinary Diagnostic Only

	EA203/96
	12 x 8
	2 – 8 °C


 DLD Gesellschaft für Diagnostika und medizinische Geräte mbH
Adlerhorst 15 • 22459 Hamburg • Telefon: 040/ 555 87 10 • Fax: 040/ 555 87 111
Internet: <http://www.dld-diagnostika.de> • E-Mail: contact@dld-diagnostika.de

Table of Contents

1	Introduction and Principle of the Test	5
2	Precautions	6
3	Storage and Stability	6
4	Contents of the Kit	7
5	Sample Collection.....	9
6	Preparation of Reagents and Samples.....	9
7	Test Procedure ELISA.....	11
8	Calculation of the Results.....	12
9	Assay Characteristics.....	13
10	Literature	19
11	Changes to declare.....	19
	Pipetting Scheme - Sample Preparation	20
	Pipetting Scheme - ELISA.....	20

Symbols

	Content		Expiry Date
	Lot Number		Store at
	Manufactured by		Sufficient for ... determinations
	Catalogue Number		Consult Instructions for Use

Hazard Pictograms



Warning



Danger

1 Introduction and Principle of the Test

Symmetric dimethylarginine (SDMA) is a methylated arginine amino acid. SDMA is derived from intranuclear methylation of L-arginine residues and is released into the cytoplasm after proteolysis. SDMA is excreted by the kidneys.

Several studies have found that 1 in 3 cats and 1 in 10 dogs are likely to develop a kidney disease during their lifetime.

SDMA is an early biomarker of kidney function. It correlates well with glomerular filtration rate (GFR). On average, SDMA increases in chronic kidney disease (CKD) with 30 to 40% loss of kidney function. Creatinine, however, does not increase until 75% of kidney function is lost. SDMA will enable veterinarians to diagnose chronic kidney disease (CKD) much earlier than Creatinine or Cystatin C tests.

SDMA is specific for kidney function. It is not impacted by other diseases such as liver disease, cardiovascular disease, inflammatory disease and endocrine diseases. Another exciting feature of SDMA is that it is not impacted by muscle mass either, which simplifies diagnosing and monitoring CKD in thin geriatric animals, especially cats and animals with other diseases that cause muscle wasting.

The competitive SDMA-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 veterinary diagnostic only. For professional use only.
- Before carrying out 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 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 of this test kit 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.
- Dispose of waste according to state and local environmental protection regulations.
- Broken glass can cause injury. Be cautious with glass vials.

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 6.1. Reagents must equilibrate to room temperature before use and be 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
each 4 ml, 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** 2 vials
5 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 
 Warning
Danger

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 13 ml TMB Solution, ready for use	SUB	1 vial
Stop Solution 13 ml, ready for use, contains 0.3M sulphuric acid, not corrosive	STOP	1 vial
Reaction Plate For acylation	ACYL-PLATE	1 piece
Equalizing Reagent lyoph., dissolve contents with 21 ml dist. water, dissolve carefully to minimize foam formation	EQUA-REAG	1 vial
Foil Ready for use	FOIL	2 pieces

Additional materials and equipment required but not provided:

- Pipettes (20, 50, 100 and 200 μ l,)
- Multipipette
- 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, place 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 shortly. The diluted wash buffer must be stored at 2 – 8 °C and is stable for 4 weeks. **Should the kit be used in several runs, then prepare only the required amount of wash buffer for each run.**

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 multipipette, 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)

Bring all reagents to room temperature and mix them carefully, avoid development of foam. 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** (duplicates are recommended).
2. Pipette 20 µl Acylation Buffer **ACYL-BUFF** into each well.
3. Pipette 200 µl reconstituted Equalizing Reagent **EQUA-REAG** (see 6.3) into each well.
4. Mix the reaction plate for 10 seconds.
5. Pipette 50 µl of freshly prepared Acylation Reagent **ACYL-REAG** (see 6.4) into each well, mix immediately.
It is recommended to use a multipipette, 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 each 20 µl of the acylated samples for the SDMA-ELISA.

7 Test Procedure ELISA

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

7.1 Sample Incubation

Transfer each 20 µl acylated Standards 1 to 6, 20 µl acylated controls and 20 µl acylated samples from the Reaction Plate into the respective wells of the coated microtiter strips **STRIPS**.

Pipette 50 µl Antiserum **AS** into each well.

Cover the plate with adhesive foil **FOIL** and incubate Microtiter Strips for 90 minutes at room temperature (20 – 25 °C) on an orbital shaker.

7.2 Washing

Discard or aspirate the contents of the wells and wash thoroughly with each 300 µl diluted Wash Buffer **WASH** (see 6.2). Repeat the washing procedure 4 times. Remove residual liquid by tapping the inverted plate on clean absorbent paper.

7.3 Conjugate Incubation

Pipette 100 µl enzyme conjugate **CONJ** into each well.

Incubate for 30 minutes at room temperature on an orbital shaker.

7.4 Washing

Repeat step 7.2.

7.5 Substrate Incubation

Pipette 100 µl Substrate **SUB** into each well and incubate for 25 ± 5 minutes at room temperature on an orbital shaker.

7.6 Stopping

Pipette 100 µl Stop Solution **STOP** into each well and mix on an orbital shaker for approx. 30 seconds.

7.7 Reading

Within 15 minutes, read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer.

8 Calculation of the Results

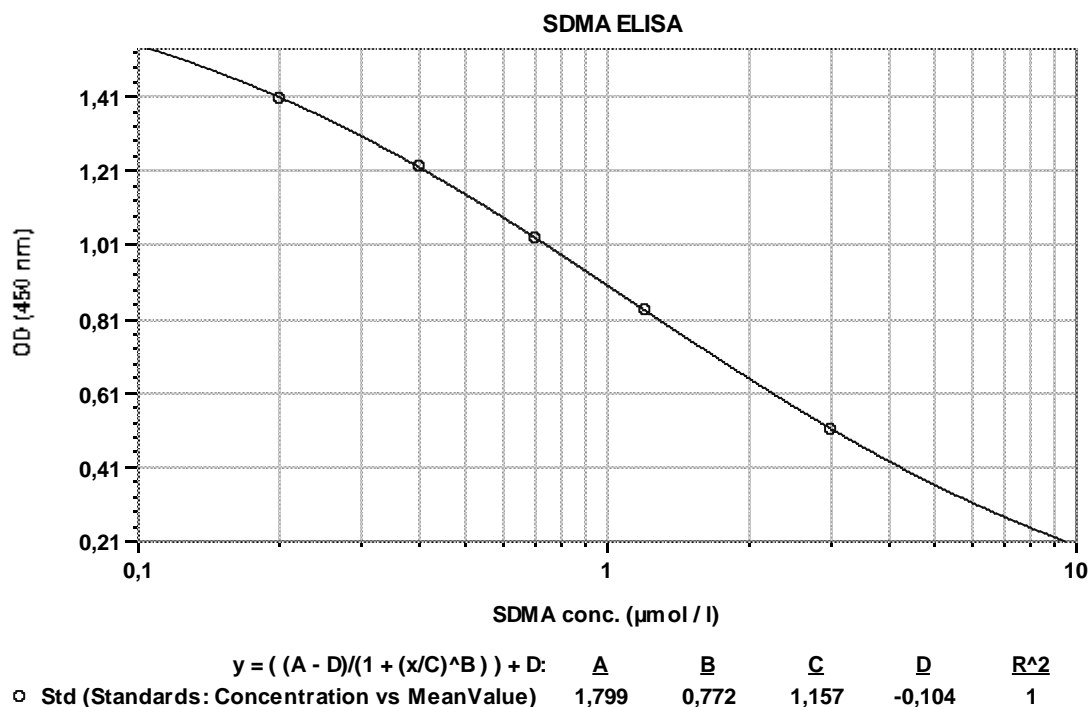
Standard	1	2	3	4	5	6
µmol/l	0	0.2	0.4	0.7	1.2	3
ng/ml	0	40	81	141	242	606

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

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:



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 Expected Values (Serum, EDTA-Plasma)

Dogs: 0.30 – 0.65 $\mu\text{mol/l}$ (6.0 – 13 $\mu\text{g/dl}$)

Cats: 0.30 – 0.75 $\mu\text{mol/l}$ (6.0 – 15 $\mu\text{g/dl}$)

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

9.2 Sensitivity

0.03 $\mu\text{mol/l}$ (0.6 $\mu\text{g/dl}$)

9.3 Recovery

9.3.1 Recovery Cat

Increasing amounts of SDMA were added to a cat serum sample. Each spiked sample was assayed. The analytical recovery of SDMA was estimated at 10 different concentrations by using the theoretically expected and the actually measured values. The mean recovery from all concentrations was 101%.

added [$\mu\text{mol/l}$]	measured [$\mu\text{mol/l}$]	expected [$\mu\text{mol/l}$]	% recovery
0.00	0.58		
0.12	0.68	0.70	97
0.24	0.77	0.82	91
0.35	0.86	0.93	92
0.45	0.91	1.03	88
0.55	0.99	1.13	88
0.65	1.24	1.23	101
0.77	1.71	1.35	127
1.04	1.90	1.62	117
1.35	1.95	1.93	101
1.65	2.29	2.23	103
		mean value	101

9.3.2 Recovery Dog

Increasing amounts of SDMA were added to a dog serum sample. Each spiked sample was assayed. The analytical recovery of SDMA was estimated at 10 different concentrations by using the theoretically expected and the actually measured values. The mean recovery from all concentrations was 104 %.

added [$\mu\text{mol/l}$]	measured [$\mu\text{mol/l}$]	expected [$\mu\text{mol/l}$]	% recovery
0.00	0.54		
0.12	0.74	0.66	112
0.24	0.74	0.78	95
0.35	0.86	0.89	97
0.45	0.94	0.99	95
0.55	1.01	1.09	93
0.65	1.19	1.18	101
0.77	1.51	1.31	115
1.04	1.73	1.58	109
1.35	2.19	1.89	116
1.65	2.23	2.19	102
		mean value	104

9.4 Linearity

9.4.1 Linearity Cat

The linearity of the ELISA method was investigated using seven different dilutions of a cat serum sample. The mean linearity from all dilutions was 96%.

dilution	measured [$\mu\text{mol/l}$]	recalculated value [$\mu\text{mol/l}$]	recovery %
orig.	2.09		
4 + 1	1.73	2.16	103
2 + 1	1.30	1.95	93
1 + 1	0.95	1.90	91
1 + 2	0.59	1.77	85
1 + 3	0.52	2.08	100
1 + 5	0.33	1.98	95
1 + 7	0.28	2.24	107
mean recovery			96

9.4.2 Linearity Dog

The linearity of the ELISA method was investigated using seven different dilutions of a dog serum sample. The mean linearity from all dilutions was 92%.

dilution	measured [$\mu\text{mol/l}$]	recalculated value [$\mu\text{mol/l}$]	recovery %
orig.	1.88		
4 + 1	1.30	1.63	87
2 + 1	1.21	1.82	97
1 + 1	0.92	1.84	98
1 + 2	0.59	1.77	94
1 + 3	0.43	1.72	91
1 + 5	0.28	1.68	89
1 + 7	0.20	1.60	85
mean recovery			92

9.5 Specificity (Cross Reactivity)

Structural related components were tested for possible interference with the antisera against SDMA used in the ELISA method. The tested compounds were Arginine, Homoarginine, Monomethylarginine (NMMA) and ADMA.

Substance	ED-50-Value ($\mu\text{mol/l}$)	Cross Reactivity (%)
SDMA	1.39	100
ADMA	84	1.2
NMMA	182	0.76
Homoarginine	2807	0.05
Arginine	8574	0.016

9.6 Reproducibility

The reproducibility of the ELISA method was investigated by determining the intra- and inter-assay-coefficient of variation (cv) by repeated measurements of different serum samples with different SDMA concentrations (concentrations in $\mu\text{mol/l}$):

9.6.1 Intra-Assay Cat

sample	n =	mean value	sd	cv (%)
K1	40	0.837	0.064	7.6
K2	40	0.862	0.049	5.7
K3	40	0.770	0.058	7.7

9.6.2 Intra-Assay Dog

sample	n =	mean value	sd	cv (%)
H1	40	0.531	0.061	11.5
H2	40	0.804	0.068	8.5
H3	40	0.776	0.046	5.9

9.6.3 Inter-Assay Cat

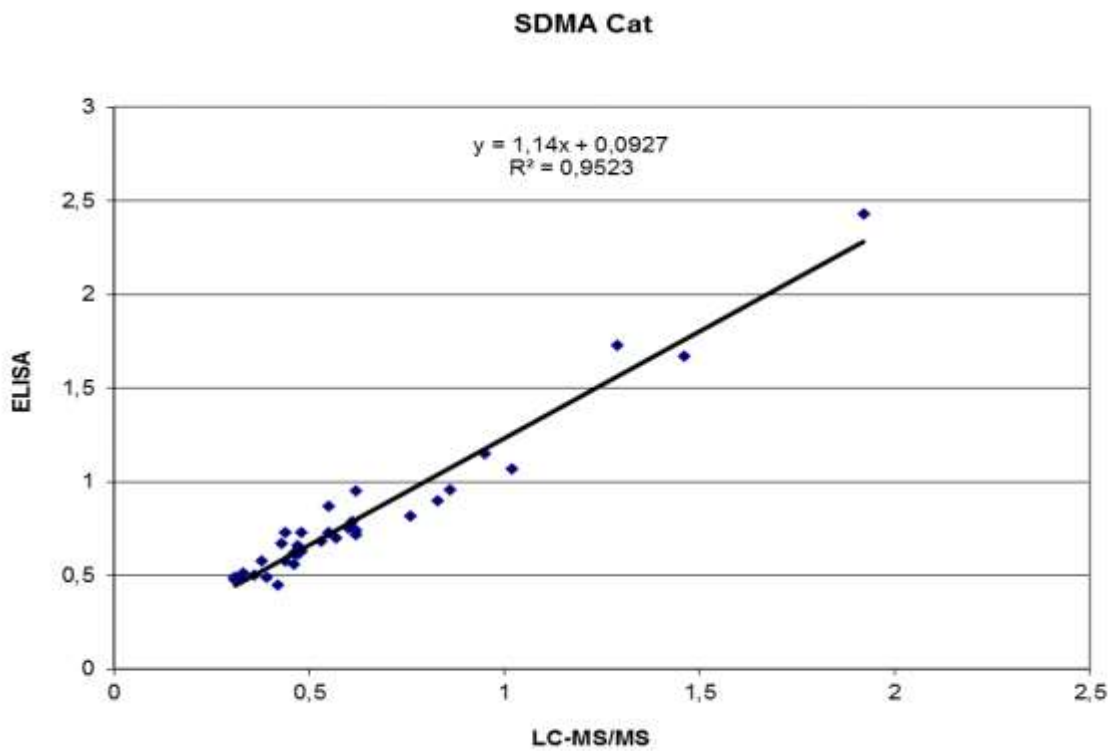
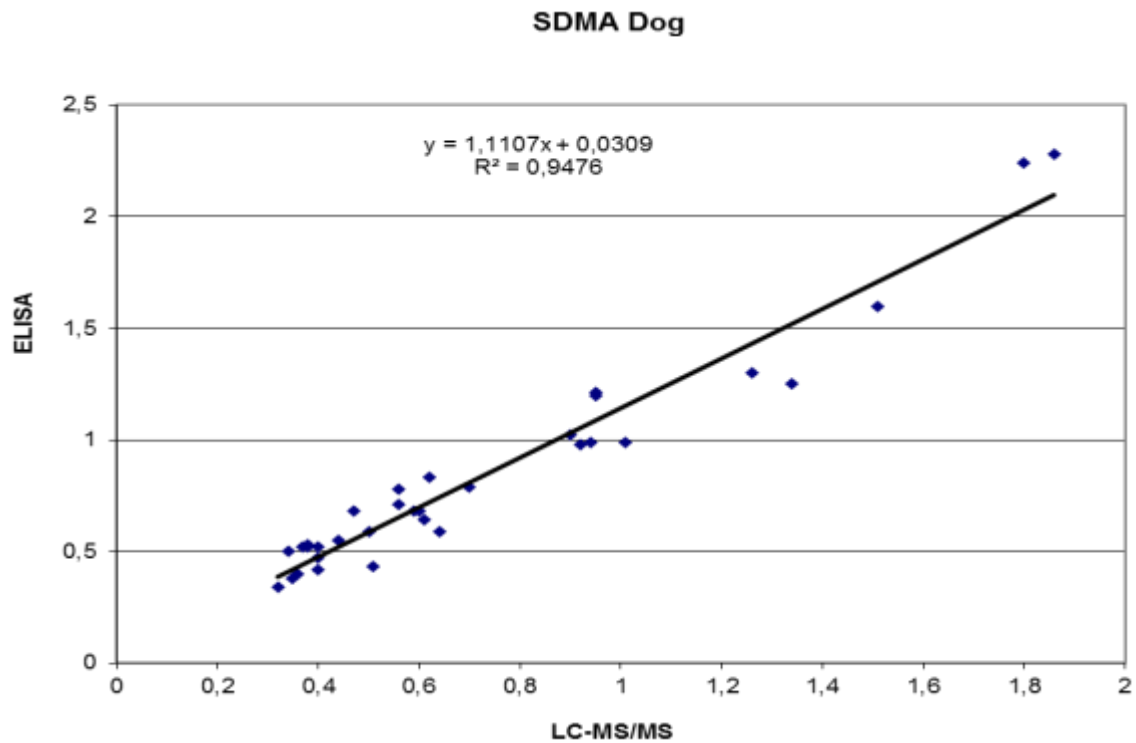
sample	n =	mean value	sd	cv (%)
K1	32	0.49	0.037	7.5
K2	32	0.59	0.043	7.3
K3	32	0.72	0.079	11.0
K4	32	0.74	0.058	7.8
K5	32	0.84	0.068	8.1
K6	32	0.84	0.982	9.7

9.6.4 Inter-Assay Dog

sample	n =	mean value	sd	cv (%)
H1	32	0.49	0.042	8.5
H2	32	0.66	0.046	7.0
H3	32	0.76	0.063	8.2
H4	32	0.93	0.069	7.4
H5	32	1.21	0.128	10.6
H6	32	1.35	0.152	11.3

9.7 Correlation ELISA to LC-MS/MS

Correlation ELISA to the LC-MS/MS method



10 Literature

- Josipa Kuleš, Petra Bilić, Blanka Beer Ljubić, Jelena Gotić, Martina Crnogaj, Mirna Brkljačić, Vladimir Mrljak
Glomerular and tubular kidney damage markers in canine babesiosis caused by *Babesia canis*
Ticks and Tick-borne Diseases (2018) **9** 1508 - 1517
- 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

11 Changes to declare

Version _13: Additions/Changes are highlighted in gray.

Version _12: Hazard symbol “Warning” was removed from POD Conjugate in section 4, as no longer required. Grey highlighting as in version _11 has been removed.

Version _11: IFU has been re-formatted. Precautions and Calculation of the Results have been complemented (highlighted in grey). Component names as printed on labels were included in pipetting schemes to provide greater clarity. No changes have been made to components or execution of protocols.

Pipetting Scheme - Sample Preparation

		Standards	Controls	Samples
ACYL-PLATE:				
CAL 1 - 6	µl	20		
CON 1 & 2	µl		20	
Sample	µl			20
ACYL-BUFF	µl	20	20	20
recon. EQUA-REAG	µl	200	200	200

Shake for 10 seconds

recon. ACYL-REAG (fresh)	µl	50	50	50
-----------------------------	----	----	----	----

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

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

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
------	----	-----	-----	-----

Within 15 minutes, read absorbance at 450 nm (ref. 570 – 650 nm)