Version 08-10/25

User's Manual

SDMA ELISA Kit

REF **DEIA4784**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This CD assay is intended for the quantitative determination of symmetric dimethylarginine (SDMA) in human EDTA plasma and serum.

General Description

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 with an increased cardiovascular risk.

Indication

• Renal failure

• Cardiovascular risk in renal dysfunction

• Hypertension in renal dysfunction

Principles of Testing

This assay is based on the method of competitive enzyme linked immunoassays. The sample preparation includes the addition of a derivatization reagent for SDMA derivatization. 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 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 unbound components tetramethylbenzidine (TMB) is added as a peroxidase substrate. Finally, the enzymatic reaction is terminated by an acidic stop solution. The color changes from blue to yellow and the absorbance is measured in a 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 standards. SDMA present in the patient samples is determined directly from this curve.

Materials Required But Not Supplied

• Ultra pure water*

• Calibrated precision pipets and 10-1000 µl tips

• Foil to cover the microtiter plate

• Horizontal microtiter plate shaker

• Multi-channel pipets or repeater pipets

• Vortex

• Centrifuge, 3000 g

• Standard laboratory glass or plastic vials, cups, etc.

• Microtiter plate reader

* CD recommends the use of Ultra Pure 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).

Storage

2-8°C

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.

• Lipemic or hemolytic samples may give erroneous results and should not be used for analysis.

• Samples with visible amounts of precipitates should be centrifuged.

• The EDTA-plasma and serum samples are analyzed undiluted.

If the sample volume is less than 50 µl, we recommend a 1:2 dilution in DERBUF (reaction buffer) (25 µl sample + 25 µl DERBUF). This dilution factor must be considered in data evaluation.

• For sample preparation a derivatization reagent (DER) for derivatization of SDMA is added (details are given in the sample preparation procedure).

Reagent Preparation

• To run the assay more than once ensure that reagents are stored at the conditions stated on the label. Prepare only the appropriate amount necessary for each run. 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.

• Dilute the ELISA wash buffer concentrate (WASHBUF) with ultra pure water

1: 0 before use (100 ml WASHBUF + 900 ml ultra pure water), mix well. Crystals may occur due to high salt concentration in the stock solution. The crystals must be redissolved at room temperature or at 37°C using a water bath before dilution. 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 reaction buffer (DERBUF). Store Standards and Controls frozen at -20°C, thaw before use in the test and mix well. Re-freeze standards and controls immediately after use. They can be re-frozen up to 3 times.

• DMSO could crystallize at 4°C. Dissolve the crystals at room temperature or in a water bath.

• Dissolve the content of one vial of derivatization reagent (DER) (50 mg) in 3 ml DMSO. Put the vial on a horizontal shaker for 5 min. DER must be prepared immediately before use. When more than one vial is to be used combine the contents and mix prior to use. Discard any rest of the reagent after use. The ELISA kit can be separated into two performances by providing two DER vials. Please note: DMSO attacks all plastics but not polypropylene products and laboratory glass.

• Dissolve the content of one vial of SDMA antibody (AB) in 5.6 ml of diluted wash buffer. For this, reconstitute the content of one AB vial with 0.6 ml of diluted wash buffer and allow to dissolve for 5 minutes. Then transfer quantitatively the obtained AB solution into a separate vial and add 5 ml of diluted wash buffer. The ELISA kit can be separated into two performances by providing two AB vials. Diluted SDMA antibody can be stored at 2-8°C for one month.

• Dilute the peroxidase conjugate (CONJ) 1:201 with conjugate stabilizing buffer (CONJBUF) (e.g. 110 µl CONJ + 22 ml CONJBUF; prepare only the required amount). The undiluted POD conjugate is stable at 2-8°C until the expiry date stated on the label. Diluted POD conjugate can be stored at 2-8°C for 1 week.

• 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.

Assay Procedure

Sample preparation procedure

Derivatization of standards (STD), controls (CTRL) and samples (SAMPLE) is carried out in single determination in vials (e.g. 1.5 ml vials) as follows:

1. Bring all reagents and samples to room temperature (15-30°C) and mix well.
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 vials.
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 (STD, CTRL, SAMPLE), mix thoroughly by repeated inversion or several seconds on a vortex mixer and incubate for 45 min at room temperature (15-30°C) on a horizontal shaker (180-240 rpm).
5. Afterwards add 250 µl of dilution buffer (CODIL) into each vial, mix well and incubate for 45 min at room temperature (15-30°C) on a horizontal shaker (180-240 rpm).

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

Test procedure

6. Mark the positions of standards (STD)/ controls (CTRL)/samples (SAMPLE) in duplicate on a protocol sheet.

7. Take as many microtiter plate 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.

Please note: Do not wash the plate.

8. For the analysis in duplicate take 2 x 100 µl of the derivatized standards/ controls/ samples (STD/ CTRL/ SAMPLE) out of the vials and add into the respective wells.
9. Add 100 µl of diluted SDMA antibody (AB) into each well. Cover the plate tightly.
10. Incubate overnight (15-20 hours) at 2-8°C.
11. Aspirate or decant the contents of each well. Wash each well 5 x with 250 µl of diluted wash buffer. After the final washing step the inverted microtiter plate should be firmly tapped on absorbent paper.
12. Add 200 µl of diluted peroxidase conjugate (CONJ) into each well.
13. Cover the plate tightly and incubate for 1 hour at room temperature (15-30°C) on a horizontal shaker (180-240 rpm).
14. Aspirate or decant the contents of each well. Wash each well 5 x with 250 µl of diluted wash buffer. After the final washing step the inverted microtiter plate should be firmly tapped on absorbent paper.
15. Add 200 µl of TMB substrate (SUB) into each well.
16. Incubate for 13-18 min at room temperature (15-30°C) in the dark*.
17. Add 100 µl of stop solution (STOP) into each well, mix thoroughly.
18. 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 color change is temperature sensitive. We recommend to observe the color change and to stop the reaction upon good differentiation.

Quality Control

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

Control samples should be analyzed 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.

Calculation

If the test is performed in strict compliance with the manufacturer's instructions no dilution factor is required for the calculation of results.

Exception: Consider the dilution factor for 1:2 pre-diluted samples.

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

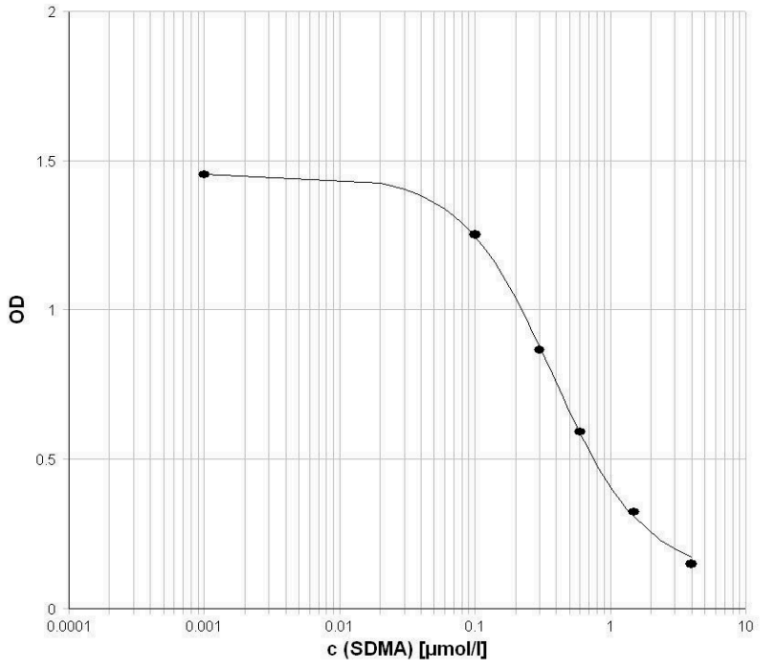
We recommend a linear ordinate for optical density and a linear abscissa for concentration.

The plausibility of the duplicate values should be examined before automatically evaluating the results. If this option is not available within the used program, the duplicate values should be evaluated manually.

Typical Standard Curve

The concentration of controls and patient samples can be determined directly from the calibration curve in µ mol/l. In the following, an example of a calibration curve is given; do not use it for the calculation of your results.

Example of calibration curve



Precision

Intra-assay (n=12)

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	6
2	0.63	7

Detection Range

Based on internal studies with serum samples of apparently healthy persons (n=40) a mean value of 0.47 µ mol/l was calculated. The standard deviation was 0.07 µmol/l.

Mean value ± 2 x standard deviation: 0.47 ± 0.14 µmol/l

Normal range: 0.33 - 0.61 µmol/l

We recommend each laboratory to establish its own reference range.

Sensitivity

The zero-standard was measured 20 times. The detection limit was set as B0 - 2 SD and estimated to be 0.05 µmol/l.

Sample	SDMA mean value [OD]	2 x standard deviation (2 SD)	Detection limit [µmol/l]
zero-standard	2.3	0.05	0.05

Specificity

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

ADMA: < 0.5 %

NMMA: < 0.5 %

L-arginine: < 0.02 %

Recovery

Spiking recovery

One serum sample was spiked with different SDMA concentrations and measured in this assay. The mean recovery rate for all concentrations was 101.5 % (n=6).

Spike [µmol/l]	SDMA expected [µmol/l]	SDMA measured [µmol/l]	recovery [%]
0		0.75	
0.5	1.25	1.26	101
1	1.75	1.79	102

Dilution recovery

One spiked sample was diluted with reaction buffer. The mean recovery was 93 % (n=6).

dilution	SDMA expected [µmol/l]	SDMA measured [µmol/l]	recovery [%]
original		1.75	
1:2	0.88	0.85	96
1:4	0.44	0.39	89

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 color 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.

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 analyzed with each run.

• Reagents should not be used beyond the expiration date stated on 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.

Limitations

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