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ab213973
Human SDMA ELISA kit

For the quantitative determination of SDMA in human EDTA plasma and serum samples.

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

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1. Overview

The Human SDMA ELISA kit (ab213973) is intended for the quantitative determination of symmetric dimethylarginine (SDMA) in human EDTA-plasma and serum.

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

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 the 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 samples is determined directly from this curve.

2. Protocol Summary

Prepare Standards, Controls and Samples
Add derivatization reagent
Add dilution buffer and incubate (RT)

Wash plate

Add Standards, Controls and Samples to wells

Add diluted SDMA antibody

Incubate overnight (+2-8°C)

Wash plate

Add diluted POD antibody

Incubate (RT)

Wash plate

Add TMB substrate solution and incubate (RT)

Add Stop solution and read plate at 450 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All ELISA kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store standards and controls at -20°C immediately upon receipt. Store rest of the ELISA kit at +2-8°C.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- ELISA kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition (Before prep)	Storage Condition (After prep)
SDMA coated microplate (12x 8 well strips)	96 wells	+2-8°C	+2-8°C
Standard 1 (0.0 µM)	1 vial	-20°C	-20°C
Standard 2 (0.1 µM)	1 vial	-20°C	-20°C
Standard 3 (0.3 µM)	1 vial	-20°C	-20°C
Standard 4 (0.6 µM)	1 vial	-20°C	-20°C
Standard 5 (1.5 µM)	1 vial	-20°C	-20°C
Standard 6 (4.0 µM)	1 vial	-20°C	-20°C
Control 1	1 vial	-20°C	-20°C
Control 2	1 vial	-20°C	-20°C
Wash buffer concentrate (10X)	2 x 100 mL	+2-8°C	+2-8°C
SDMA antibody	6 mL	+2-8°C	+2-8°C
Conjugate	12 mL	+2-8°C	+2-8°C
Reaction buffer	15 mL	+2-8°C	+2-8°C
Derivatization reagent	6 mL	+2-8°C	+2-8°C
TMB Substrate	15 mL	+2-8°C	+2-8°C
Stop Solution	15 mL	+2-8°C	+2-8°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 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) is recommended.
- Precision pipettes for volumes between 10 µL and 1,000 µL
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3,000 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).

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- This ELISA kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 SDMA coated microplate (12x 8 well strips):

One plate (break-apart strips) of 96 wells. Ready to use. Store at +2-8°C.

9.2 Standards:

6 vials. Ready to use. Diluted in reaction buffer. Store at -20°C. (0.0, 0.1, 0.3, 0.6, 1.5, 4.0 µM)
Thaw before use in the test, and re-freeze immediately after use. Standards can be re-frozen up to 3 times.

9.3 Control 1 and 2:

Ready to use. Diluted in reaction buffer. Store at -20°C. Thaw before use in the test, and re-freeze immediately after use. Controls can be re-frozen up to 3 times.

9.4 Wash buffer concentrate (10X):

2X 100 mL. Store at +2-8°C. Dilute the Wash buffer concentrate with ultra-pure water 1:10 before use (100 mL concentrate + 900 mL ultra-pure water), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be dissolved 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.

9.5 SDMA antibody (lyophilized):

Dissolve the SDMA antibody in 5.6 mL of diluted wash buffer as follows: At first, dissolve the content of one vial with 0.6 mL of diluted wash buffer for 5 minutes. Then, transfer quantitatively the obtained solution into a separate vial and add 5 mL of diluted wash buffer. Diluted SDMA antibody can be stored at +2-8°C for 4 weeks.

9.6 Conjugate:

12 mL. Ready to use. Store at +2-8°C.

9.7 Reaction buffer:

15 mL. Ready to use. Store at +2-8°C.

9.8 Derivatization reagent:

The derivatization reagent is already 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 derivatization reagent, bring to room temperature and ensure that all crystals are dissolved. Please note: DMSO attacks all plastics but not polypropylene products and laboratory glass.

9.9 TMB Substrate:

25 mL. Ready to use. Store at +2-8°C.

9.10 Stop Solution:

15 mL. Ready to use. Store at +2-8°C.

10. Sample Preparation

- The assay is suitable for the measurement of SDMA in human EDTA plasma and serum samples.
- 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.
- The EDTA-plasma and serum samples are analyzed without any dilution. If the sample volume is less than 50 µl, a 1:2 dilution in reaction buffer is recommended (25 µl sample + 25 µL reaction buffer). This dilution factor must be considered for data evaluation. Samples with visible amounts of precipitates should be centrifuged. The resulting supernatant is used in the assay.
- For sample preparation, a derivatization reagent for derivatization of SDMA is added (details are given in the sample preparation procedure).

11. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at +2-8°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or "edge effects" have not been observed with this assay.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.

12.1 Sample Preparation Procedure

12.1.1 Add 200 µL of ready to use Standards, 200 µL of ready to use Controls and 50 µL of samples in the corresponding vial.

12.1.2 Add 150 µL of Reaction buffer to the Samples only.

12.1.3 Add 50 µL of derivatization reagent into each vial (standards, controls and samples), mix well and incubate for 45 minutes on a shaker (180-240 rpm) at room temperature (18-26°C).

2 x 50 µL of each treated sample (Standard, Control, Sample) are used in the ELISA as duplicates.

12.2 Test Procedure

12.2.1 Mark the positions of Standards, Controls and Samples in duplicate on a protocol sheet.

12.2.2 Take as many microtiter strips as needed from the kit.

12.2.3 For analysis in duplicate, take 2 x 50 µL of Standard, Control and Samples out of the vial and add into the respective well of the microtiter plate.

12.2.4 Add 50 µL of diluted SDMA antibody into each well. Cover the plate tightly.

12.2.5 Incubate for 2 hours at Room temperature (15-30°C).

12.2.6 Aspirate the contents of each well. Wash each well 5 times by dispensing 250 µL of diluted was buffer into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution.

12.2.7 Add 100 µL of conjugate antibody into each well.

12.2.8 Cover the plate tightly and incubate for 1 hour at room temperature on a horizontal plate shaker (180-240rpm).

12.2.9 Aspirate the contents of each well. Wash each well 5 times by dispensing 250 µL of diluted was buffer into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution.

12.2.10 Add 100 µL of TMB substrate into each well.

12.2.11 Incubate for 10-15 minutes at room temperature (15-30°C) in the dark.

12.2.12 Add 100 µL of Stop Solution into each well, mix thoroughly.

12.2.13 Determine the 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.

13. Calculations

If the test is performed in strict compliance with the manufacturer's instructions (i.e. with the exact volumes for standards, controls, samples, and with correct sample treatment), standards, controls, and blood samples are equally diluted. Therefore, no dilution factor is required for the calculation of results.
The following algorithms can be used alternatively to calculate the results. We recommend to use the "4-parameter-algorithm".

13.1 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. 0.001).

13.2 Point-to-point-calculation
We recommend a linear ordinate for optical density and a linear abscissa for concentration.

13.3 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. 0.001). Plausibility of the measured pairs of values should be examined before automatically evaluating the results. If this option is not available in 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 deviation was 0.07 µmol/L.

Serum/Plasma mean value ±2 standard deviation: 0.47 ± 0.14 µmol/L

Normal range: 0.33 – 0.61 µmol/L

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

Controls

Control samples 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 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 samples can be determined directly from calibration curve in µmol/L.

14. Typical data

Typical standard curve – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

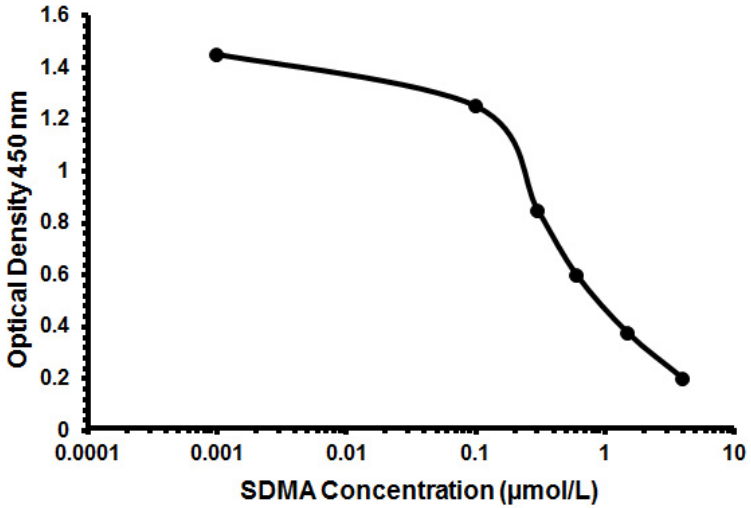

Figure 1. Human SDMA ELISA Kit (ab213973) Standard Curve

15. Typical sample values

SENSITIVITY –

The sensitivity was set as B0 + 2SD. The zero-standard was measured 20 times.

Sample	SDMA mean value [OD]	2 x standard deviation (SD)	Detection limit (µmol/L)
Zero-standard	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 mean recovery rate for all concentrations was 90.4 % (n=5).

Spike (µmol/L)	SDMA expected (µmol/L)	SDMA measured (µmol/L)	Recovery [%]
0		1.774	
0.5	0.887	0.876	98.8
0	0.444	0.364	82.1

LINEARITY –

The linearity of the ELISA was determined by the dilution of a spiked sample. The mean linearity was 93% (n=6).

Dilution	Expected (µmol/L)	Measured (µmol/L)	Recovery [%]
Original		1.75	
1 + 1	0.88	0.85	96
1 + 3	0.44	0.37	89

PRECISION –

Intra-assay precision:

Sample	Number of measures	Mean (µmol/L)	CV%
1	12	0.27	7.5
2	12	0.67	4.8

Inter-assay precision:

Sample	Number of assays	Mean (µmol/L)	CV%
1	6	0.22	6
2	6	0.63	7

Cross reactivity

ADMA < 0.1 %

L-Arginine < 0.01 %

Problem	Cause	Solution
Poor standard curve	Inaccurate Pipetting	Check Pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	All components 4°C. Keep TMB substrate solution protected from light.

16. Troubleshooting

17. Notes

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