Human SDMA ELISA Kit

Human SDMA ELISA Kit is an enzyme immunoassay kit for the quantification of SDMA in serum and plasma (EDTA).

Catalog number: ARG82905

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

There are several species of methylated arginine produced as a byproduct of intracellular protein methylation followed by protein degradation. Of interest are the dimethylated derivatives of arginine, symmetric dimethylarginine (SDMA) and asymmetric dimethylarginine (ADMA). While ADMA is metabolized enzymatically, SDMA is eliminated primarily by renal filtration and plasma SDMA concentration has been used as an alternate biomarker for estimating GFR in people. [Provide by IRiS: Symmetric dimethylarginine (SDMA): new biomarker of renal function in cats and dogs (2015)]

PRINCIPLE OF THE ASSAY

This assay employs the competitive quantitative enzyme immunoassay technique. First, SDMA is bound to the solid phase of the microtiter plate. SDMA in the samples or Standard 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-antibody complexes are removed by washing procedure. HRP conjugated is added to each well and incubated. After washing away any unbound antibody conjugate, a substrate solution (TMB) is added to the wells and color develops in inversely proportion to the amount of SDMA present in the samples. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of SDMA in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
SDMA Precoated Microplate	8 x 12 strip	4°C
Standards (1 – 6; 0 - 3 µmol/l)	4 mL each, 6 vials (ready to use)	4°C
Control (1 & 2)	4 mL each, 2 vials (ready to use)	4°C
Acylation Buffer (Blue coloured)	3.5 mL (ready to use)	4°C
Acylation Reagent (Lyophilized)	3 vials	4°C
Antiserum (Yellow coloured)	7 mL (ready to use)	4°C
HRP Conjugate	13 mL (ready to use)	4°C (protect from light)
Equalizing Reagent (Lyophilized)	1 vial	4°C
Solvent	5 mL	4°C
Foil	2 piece	4°C
50X Wash Buffer	20 mL	4°C
TMB Substrate	13 mL (ready to use)	4°C (protect from light)
Stop Solution	13 mL (ready to use)	4°C
Reaction Plate	1 piece	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm
- Deionized or distilled water
- Orbital shaker
- Vortex mixer, roll mixer
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (20-25°C).
- Before opening the packet of strip wells, allow it to stand at room temperature for at least 10 minutes. After opening, keep any unused wells in the original foil packet with the desiccant provided. Reseal carefully and store at 2-8 °C.
- The reagent preparation method might be different from lot to lot, so please check the lot and follow the instructions given in this manual.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.

- Please note that Solvent reacts with many plastic materials including plastic trays; Solvent does not react with normal pipette tips and with glass devices.
- If crystals are observed in the 50X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 1,000 x g for 15 minutes at 4°C.

Plasma: Collect blood with EDTA and centrifuge at 1,000 x g for 15 minutes at 4°C.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum/plasma sample.
3. 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 Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **1X Wash Buffer**: Dilute 50X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 20 mL of 50X Wash Buffer into 980 mL of distilled water to a final volume of 1 L) The 1X Wash Buffer must be stored at 2 - 8 °C and is stable for 4 weeks. For longer storage the 1X Wash Buffer has to be stored frozen at-20°C.
- **Equalizing Reagent:** Dissolve the contents with 21 mL of distilled 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.
- **Acylation Reagent:** Dissolve the contents of one bottle in 3 mL of 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. 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.

Note: 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 multipette, fill it directly from the vial and add the Acylation Reagent to the wells.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

Preparation of Samples (Acylation)

The wells of the reaction 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** of **Standard 1 - 6**, each **20 µL** of **Control 1 & 2** and each **20 µL** of **sample** into the respective wells of the Reaction Plate.
2. Pipette **20 µL** of **Acylation Buffer** into all wells.
3. Pipette **200 µL** of **Equalizing Reagent** into all wells.
4. Mix the reaction plate for **10 seconds**.
5. Prepare **Acylation Reagent** freshly and pipette **50 µL** of prepared **Acylation Reagent** each into all wells, mix immediately. It is recommended to use a multipette, 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.
7. Take each well **25µL** for the Human SDMA ELISA

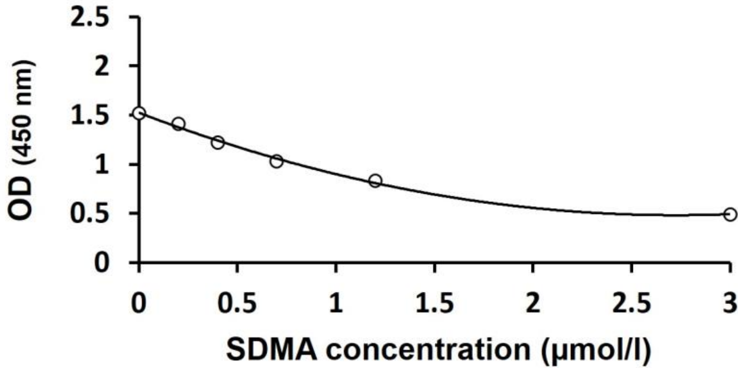
ELISA Procedure

1. Transfer **25 µL** of **acylation samples, Controls, Standards** to the respective wells of the **SDMA Precoated Microplate**. Then add **50 µL** of **Antiserum** into each well.
2. Cover the plate with the Foil. Incubate for **90 minutes** at **room temperature** on an orbital shaker.

3. Aspirate each well and wash, repeating the process 3 times for a total 4 washes. Wash by filling each well with **1X Wash Buffer (300 µL)** using a squirt bottle, manifold dispenser. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of the **HRP Conjugate** per well. Then cover the plate with the Plate Sealer. Incubate for **30 minutes** at **room temperature** on an orbital shaker.
5. Aspirate and wash plate as in step 3.
6. Add **100 µL** of **TMB Substrate** to each well. Incubate for **25 ± 5 minutes** at **room temperature** on an orbital shaker in the dark.
7. Immediately Add **100 µL** of **Stop solution** to each well. The color of the solution should change from blue to yellow.
8. Read the absorbance with a microplate reader at **O.D. 450 nm** (reference wavelength between 570 and 650 nm).

EXAMPLE OF TYPICAL STANDARD VALUES

The following figures demonstrate typical results with the Human SDMA ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



CALCULATION OF RESULTS

1. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
2. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
3. arigo provides GainData®, an in-house development ELISA data calculator,

for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

4. Conversion factor: 1 µmol/l = 202 ng/mL = 20.2 µg/dl
5. Expected value (Serum, EDTA-Plasma): Human 0.30 – 0.75 µmol/l.
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Sensitivity

0.03 µmol/l

Cross Reactivity

Substance	Cross Reactivity (%)
SDMA	100
ADMA	0.74
NMMA	0.76
Homoarginine	0.04
Arginine	0.01

Recovery (Serum & EDTA-Plasma)

86 – 104%

Precision

Intra-CV= 4.9 ~ 6.2%; Inter-CV= 2.0 ~ 8.8%