DIAGNOSTIKA GMBH

Instruction for use

ADMA Fast ELISA

Enzyme Immunoassay
for the Quantitative Determination of
Endogenous Asymmetric Dimethylarginine (ADMA)
in Serum or Plasma

CE

IVD

REF EA212/96

12 x 8

2 – 8 °C

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1. Introduction and Principle of the Test

The vascular endothelium plays a central role in the regulation of vascular structure and function, mainly due to the formation of endothelium-derived nitric oxide (NO). NO has been named an “endogenous anti-atherogenic molecule” due to its diverse regulatory functions in vascular homeostasis.

NO is formed by the enzyme NO synthetase (NOS) from the amino acid precursor L-arginine. NOS activity can be downregulated by asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NOS.

The effects of ADMA on NO synthesis and NO-mediated pathophysiological processes have been described in numerous experimental studies. Moreover, elevated ADMA levels in plasma have been found in clinical studies including patients with hyper-cholesterolemia, hypertension, chronic heart failure, chronic renal failure and other internal disorders.

Recent prospective and cross-sectional studies indicated that elevated ADMA levels are a risk factor for future cardiovascular events and total mortality. ADMA may have diagnostic relevance as a novel cardiovascular risk marker.

The competitive ADMA-ELISA uses the microtiter plate format. ADMA is bound to the solid phase of the microtiter plate. ADMA in the samples is acylated and competes with solid phase bound ADMA for a fixed number of rabbit anti-ADMA 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 ADMA 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 ADMA is inversely proportional to the ADMA concentration of the sample.

2. Precautions

- For in vitro use only.
- Disposable gloves should be used.
- 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.

3. Storage and Stability

On arrival, store the kit at 2-8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.
Do not use components beyond the expiration date shown on the labels.
Do not mix various lots of any kit component within an individual assay.

4. Contents of the Kit

4.1 **MT-Strips** **STRIPS** 12 strips
8 wells each, break apart
precoated with ADMA

4.2 **Standards 1 - 6** **CAL 1 – 6** 6 vials
Each 4 ml, ready for use
Concentrations:

Standard	1	2	3	4	5	6
µmol/l	0	0.2	0.45	0.7	1	3

4.3 **Control 1 & 2** **CON 1 & 2** 2 vials
Each 4 ml, ready for use
Range: see q.c. certificate

4.4 **Acylation Buffer** **ACYL-BUFF** 1 vial
3.5 ml, ready for use, colour coded blue

4.5 **Acylation Reagent** **ACYL-REAG** 3 vials
lyophilised, dissolve content
in 3 ml Solvent before use; if required
combine the contents of both vials (see also 6.)

4.6 **Antiserum** **AS** 1 vial
7 ml, ready for use, colour coded blue
Rabbit-anti-N-acyl-ADMA

4.7 **Enzyme Conjugate** **CONJ** 1 vial
13 ml, ready for use
goat anti-rabbit-IgG-peroxidase

4.8 **Wash Buffer** **WASH** 1 bottle
20 ml, concentrated
Dilute content with dist. water to 1,000 ml total volume.

4.9 **Substrate** **SUB** 1 vial
13 ml TMB solution, ready for use

4.10 **Stop Solution** **STOP** 1 vial
13 ml, ready for use
Contains 0.3 M sulphuric acid

4.11 **Reaction Plate** **ACYL-PLATE** 1 piece
for acylation

4.12 **Equalizing Reagent** **EQUA-REAG** 1 vial
lyophilized, dissolve content with 21 ml dist. water,
dissolve carefully to minimize foam formation (see also 6.)

4.13 **Solvent** **SOLVENT** 2 vials
5 ml, 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)

4.14 **Foil** **FOIL** 2 pieces
ready to use

Additional materials and equipment required but not provided:

- Pipettes 20, 25, 50, 100 and 200 µl
- Multipette
- Orbital shaker
- Microplate washing device
- Microplate photometer (450 nm)
- Vortex mixer
- Roll mixer
- Distilled water

5. Sample Collection

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 frozen at -20 °C

Repeated freezing and thawing should be avoided.

6. Preparation of Reagents and Samples

Microtiter strips **STRIPS**

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.

Wash Buffer **WASH**

Dilute the content with dist. water to a total volume of 1,000 ml.
The diluted wash buffer has to be stored at 2 - 8 °C. for a maximum of 4 weeks. For storage until expiry date as given on the label the diluted wash buffer has to be kept frozen at -20 °C.

Equalizing Reagent **EQUA-REAG**

Dissolve the content with 21 ml dist. water, mix shortly and leave on a roll mixer or orbital shaker for 20 minutes. Handle carefully in order to minimize foam formation. The reconstituted Equalizing Reagent should be stored frozen at -20 °C and is stable until expiry date as given on the label.

Acylation Reagent **ACYL-REAG**

Dissolve the content of one bottle in 3 ml Solvent and shake for 10 minutes on a roll mixer or 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 a maximum of 3 hours. The second and third bottle 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. Solvent does not react with normal pipette tips and with glass devices.

All other reagents are ready for use.

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 standard 1 - 6, each 20 µl control 1 & 2 and each 20 µl patient sample into the respective wells of the Reaction Plate.

2. Pipette 20 µl Acylation Buffer into all wells.

3. Pipette 200 µl Equalizing Reagent into all wells.

4. Mix the reaction plate for 10 seconds.

5. Prepare Acylation Reagent just before use.
   For pipetting please use a multipette or similar device, fill the syringe directly from the vial with dissolved Acylation Reagent and add well by well. Please note that dissolved Acylation Reagent reacts with many plastic materials including plastic trays. It does not react with normal pipette tips and with glass devices.
   Pipette 50 µl prepared Acylation Reagent each into all wells and continue with step 5. **immediately**. Colour changes to violet.

6. Incubate for 20 minutes at room temperature (approx. 20 °C) on an orbital shaker with medium frequency.

Take each 25 µl for the ADMA-ELISA.

7. Test Procedure ELISA

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

7.1 Sample Incubation

Pipette each 25 µl prepared Standards 1 to 6, 25 µl prepared controls and 25 µl prepared samples into the respective wells of the coated microtiter strips (duplicates are recommended).

Pipette each 50 µl Antiserum into all wells and shake shortly on an orbital shaker.

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

7.2 Washing

Discard or aspirate the contents of the wells and wash thoroughly with each 300 µl prepared Wash Buffer (Shake shortly on an orbital shaker). Repeat the washing procedure 4 times. Remove residual liquid by tapping the inverted plate on clean absorbent paper.

7.3 Conjugate Incubation

Pipette each 100 µl enzyme conjugate into all wells.
Incubate for 30 minutes at room temperature on an orbital shaker with medium frequency.

7.4 Washing

Repeat step 7.2.

7.5 Substrate Incubation

Pipette each 100 µl Substrate into all wells and incubate for minutes 25 ± 5 minutes at room temperature on an orbital shaker with medium frequency.

7.6 Stopping

Pipette each 100 µl Stop Solution into all wells.

7.7 Reading

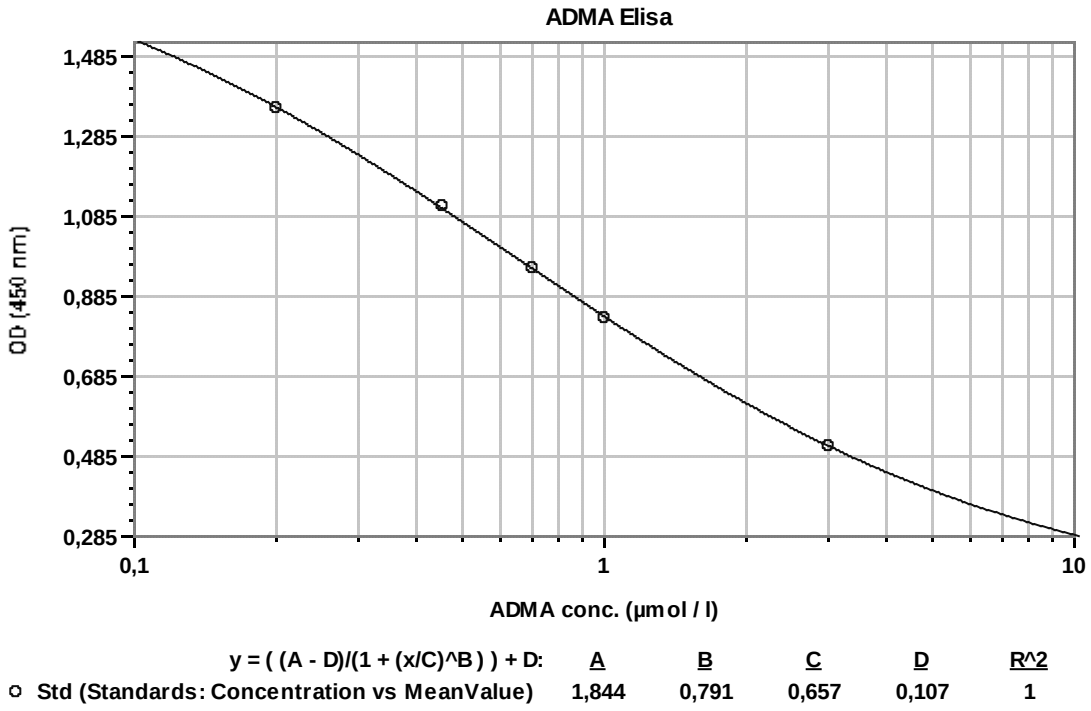
Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

8. Calculation of the Results

On a semilogarithmic graph paper 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.

Conversion factor: 1 µmol ADMA / l = 202 ng ADMA / ml

Typical standard curve:



9. Assay Characteristics

9.1 Reference Ranges

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

	Reference Range
EDTA-Plasma, Serum	0.40 – 0.75 µmol / l

9.2 Sensitivity

Lower Limit of Detection	Calculation
0.03 µmol / l	OD_{Cal1} – 3 x SD

9.3 Specificity (Cross Reactivity)

Substance	Cross Reactivity (%)
ADMA	100
SDMA	0.05
Monomethylarginine (NMMA)	1.93
Homoarginine	< 0.01
Arginine	0.03

9.4 Recovery after Spiking

	Range (µmol / l)	Mean (%)	Range (%)
EDTA-Plasma	0.43 – 1.55	99	90 - 107
Serum	0.54 – 1.72	92	87 - 102

9.5 Linearity

	Range (µmol / l)	Highest Dil.	Mean (%)	Range (%)
EDTA-Plasma	0.23 – 1.53	1 : 6 with water	99	92 - 105

9.6 Reproducibility

	Range (µmol / l)	Intra-Assay-CV
EDTA-Plasma	0.58 – 1.04	4.9 – 5.4 %

	Range (µmol / l)	Inter-Assay-CV
EDTA-Plasma	0.57 – 1.34	4.3 – 9.6 %

9.7 Method Comparison

	Method	Correlation
Serum + Plasma	LC/MS	Y = 0.99 x LC/MS + 0.02; R = 0.983; N = 32

10. Literature

Schulze F, Wesemann R, Schwedhelm E, Sydow K, Albsmeier J, Cooke JP, Böger RH.
Determination of ADMA using a novel ELISA assay.
Clin. Chem. Lab. Med. 2004; 42: 1377-1383

Schulze F, Maas R, Freese R, Schwedhelm E, Silberhorn L, Böger RH.
Determination of a reference value for N,N-dimethyl-L-arginine in 500 subjects.
Eur. J. Clin. Invest. 2005; 35 : 622-626

A comprehensive list of publications using the DLD ADMA ELISA can be found on the web site http://www.dld-diagnostika.de in the product information about ADMA:
http://www.dld-diagnostika.de/uploads/uploads/ADMA_published_studies_DLD-Diagnostika_update_June-2014.pdf

Pipetting Scheme
Sample Preparation

		Standards	Control	Sample
Standard 1 - 6	µl	20		
Control 1 & 2	µl		20	
Patient Sample	µl			20
Acylation Buffer	µl	20	20	20
Equalizing Reagent	µl	200	200	200

shake for 10 seconds

freshly prepared Acylation Reagent	µl	50	50	50

immediately incubate for 20 minutes at room temperature
on an orbital shaker

take each 25 µl of the supernatant for the ELISA

Pipetting Scheme ELISA

		Standard	Control	Patient-Sample
Standard 1 - 6	µl	25		
Control 1 & 2	µl		25	
Patient Sample	µl			25
Antiserum	µl	50	50	50

cover with foil and incubate for 90 minutes
at room temperature on an orbital shaker

wash 4 x with each 300 µl Wash Buffer

Enzyme Conjugat	µl	100	100	100

shake for 30 minutes at room temperature

wash 4 x with each 300 µl Wash Buffer

Substrate	µl	100	100	100

shake for 25 ± 5 minutes at room temperature

Stop Solution	µl	100	100	100

read absorbance at 450 nm