DIAGNOSTIKA GMBH

Instructions for Use

ADMA / Arginine ELISA

Enzyme Immunoassay
for the Quantitative Determination of
Endogenous Asymmetric Dimethylarginine (ADMA)
and L-Arginine (ARG) in Serum and EDTA-Plasma

RUO

For Research Use Only
Not for Use in Diagnostic Procedures

REF	EA215/96
Σ	96
+2 °C	2 – 8 °C

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Inhaltsverzeichnis

Symbols

RUO	Research use only		
CONT	Content		Expiry Date
LOT	Lot Number		Store at
	Manufactured by		Sufficient for ... determinations
REF	Catalogue Number		Consult Instructions for Use

The symbols of the components of the kit are described in chapter 4 Contents of the Kit.

1 Introduction and Principle of the Test

The ADMA/Arginine ELISA Kit contains reagents for the quantitative determination of derivatized ADMA (asymmetric dimethyl arginine) and arginine in serum or EDTA plasma. Derivatization takes place during sample preparation. ADMA is quantitatively converted into N-acyl-ADMA by the acylation reagent, and arginine is quantitatively converted into N-acyl-arginine.

The ADMA/Arginine ELISA is a competitive enzyme immunoassay. Antigen bound to the solid phase and free antigen in solution compete for a defined number of antibody binding sites. When the system is in equilibrium, the unbound antigen-antibody complex is removed in a washing step and the correspondingly bound complex is detected using an anti-rabbit IgG peroxidase conjugate and determined via the conversion of tetramethylbenzidine (TMB). The TMB/POD reaction is stopped and measured at 450 nm. The concentration of the antigen-antibody complex bound to the solid phase is inversely proportional to the concentration of the antigen in the sample.

2 Precautions

- For research use only. Not for use in diagnostic procedures. For professional use only.
- Before performing 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 have been obtained from certified healthy animals; however, the materials should be handled as potentially infectious.
- Individual components of different lots and 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, as these can cause irritation and chemical burns.
- Dispose of waste according to state and local environmental protection regulations.
- Broken glass can cause injury. Be cautious with glass vials.

3 Changes to Declare

Version _8: IFU was reformatted. Additions and changes are highlighted in gray.

Version _7: Additions and changes are highlighted in gray.

4 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 7.

Bring all reagents to room temperature before use and refrigerate immediately after use.

5 Contents of the Kit

MT-Strips 8 wells each, break apart precoated with ADMA; blue colored	**STRIPS-ADMA**	12 strips
MT-Strips 8 wells each, break apart precoated with L-Arginine; yellow colored	**STRIPS-ARG**	12 strips
Standards 1 - 6 4 ml each, ready for use	**CAL 1 – 6**	6 vials
Control 1 & 2 4 ml each, ready for use Range: see QC certificate	**CON 1 & 2**	2 vials
Acylation Buffer 3.5 ml, ready for use, color coded blue	**ACYL-BUFF**	1 vial  Warning
Acylation Reagent lyophilized, (see 7.3)	**ACYL-REAG**	3 vials
Antiserum ADMA 7 ml, ready for use, color coded blue Rabbit-anti-N-acyl-1-ADMA	**AS-ADMA**	1 vial  Warning

Antiserum Arginine **AS-ARG** 1 vial
7 ml, ready for use, color coded yellow
Rabbit-anti-N-acyl-1-Arginine
 Warning

Enzyme Conjugate **CONJ** 2 vials
13 ml, ready for use
Anti-rabbit-IgG-peroxidase
 Warning

Wash Buffer **WASH** 2 bottles
20 ml, concentrated (50x) (see 7.1)

Substrate **SUB** 2 vials
13 ml TMB solution, ready for use

Stop Solution **STOP** 2 vials
13 ml, ready for use
contains 0.3 M sulphuric acid, not corrosive

Reaction Plate **ACYL-PLATE** 1 piece
For acylation

Equalizing Reagent **EQUA-REAG** 1 vial
lyophilized, (see 7.2)

Solvent **SOLVENT** 1 vial
10 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

Self-adhesive Foil **FOIL** 4 pieces
ready for use

Additional materials and equipment required but not provided:

- Pipettes (10, 20, 25, 50, 100 and 200 µl)
- Multichannel pipette or microplate washing device
- Orbital shaker
- Multipette
- Microplate washing device
- Microplate photometer (450 nm)
- Vortex mixer
- Roll mixer
- Paper towels, pipette tips, timer

6 Sample Collection

The test can be performed with EDTA plasma and serum.

Haemolytic 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.

7 Preparation of Reagents

Equilibrate reagents to room temperature.

7.1 Wash Buffer

Dilute the content (20 ml) of 50x concentrated Wash Buffer WASH with dist. water to a total volume of 1,000 ml, mix briefly. For further use, the diluted wash buffer must be stored at 2 – 8 °C for a maximum period of 4 weeks.

Should the kit be used in several runs, then prepare only the required amount of wash buffer for each run.

7.2 Equalizing Reagent

Dissolve the content of EQUA-REAG 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 of the kit.

7.3 Acylation Reagent

Remove the required amount of vials of Acylation Reagent ACYL-REAG from the foil pouch, leave the remaining vials inside together with the desiccant and close the pouch carefully. Reconstitute each vial of lyophilized Acylation Reagent with 3 mL of Solvent SOLVENT and shake for minimum 10 minutes on an orbital shaker. The Acylation Reagent should be freshly prepared immediately before performing the test and is then stable for approx. 3 hours. The second and third vial allow a second and third run, respectively, of the test. If the whole kit is to be used in one run, it is recommended to pool the dissolved contents of two vials of Acylation Reagent. Discard the remaining reconstituted reagent after use.

All other reagents are ready for use.

8 Test Procedure ELISA

Equilibrate reagents to room temperature.

Duplicates are recommended.

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.

8.1 Preparation of Samples (Acylation)

1. Pipette each 20 µl Standard 1 to 6 CAL 1-6, Control 1 & 2 CON 1 & 2 and sample into the respective wells of the Reaction Plate ACYL-PLATE.
2. Pipette 20 µl Acylation Buffer ACYL-BUFF into each well.
3. Pipette 200 µl Equalizing Reagent EQUA-REAG (see 7.2) into each well.
   Mix for 10 seconds on an orbital shaker at medium speed.
4. Prepare Acylation Reagent ACYL-REAG (see 7.3) 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 ACYL-REAG into each well and continue with the next step, immediately. The color changes to violet.
5. Incubate for 20 minutes at room temperature (20 - 25 °C) on an orbital shaker at medium speed.

Take 25 µl each for the ADMA ELISA and 10 µl each for the Arginine ELISA.

8.2 ADMA ELISA

1. Pipette each 25 µl prepared Standards, prepared Controls and prepared samples into the respective wells of the coated microtiter strips STRIPS-ADMA (blue colored).
   Leave remaining microtiter strips in the foil pouch together with the desiccant and close thoroughly.
2. Pipette 50 µl ADMA-Antiserum AS-ADMA into each well.
3. Cover the plate with adhesive foil FOIL and incubate for 90 minutes at room temperature (20 - 25 °C) on an orbital shaker at medium speed.
4. Discard or aspirate the contents of the wells and wash thoroughly with 300 µl Wash Buffer WASH (see 7.1) per well. Discard or aspirate the contents of the wells and remove residual liquid by tapping the inverted plate on a clean absorbent paper towel. Repeat the washing procedure 3 times.
   Alternatively, a washing device may be used.
5. Pipette 100 µl Enzyme Conjugate CONJ into each well.
6. Incubate for 30 minutes at room temperature (20 – 25 °C) on an orbital shaker at medium speed.
7. Washing: Repeat step 4.
8. Pipette 100 µl Substrate SUB into each well.
9. Incubate for 25 ± 5 minutes at room temperature (20 – 25 °C) on an orbital shaker at medium speed.
10. Pipette 100 µl Stop Solution STOP into each well and shake on an orbital shaker for minimum 10 seconds at medium speed.
11. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

8.3 Arginine-ELISA

1. Pipette each 10 µl prepared Standards, prepared controls and prepared samples into the respective wells of the coated microtiter strips STRIPS-ARG (yellow colored).
   Leave remaining microtiter strips in the foil pouch together with the desiccant and close thoroughly.
2. Pipette each 50 µl Arginine-Antiserum AS-ARG into each well.
3. Cover the plate with adhesive foil FOIL and incubate for 90 minutes at room temperature (20 - 25 °C) on an orbital shaker at medium speed.
4. Discard or aspirate the contents of the wells and wash thoroughly with 300 µl Wash Buffer WASH (see 7.1) per well. Discard or aspirate the contents of the wells and remove residual liquid by tapping the inverted plate on a clean absorbent paper towel. Repeat the washing procedure 3 times.
   Alternatively, a washing device may be used.
5. Pipette 100 µl Enzyme Conjugate CONJ into each well.
6. Incubate for 30 minutes at room temperature (20 – 25 °C) on an orbital shaker at medium speed.
7. Washing: Repeat step 4.
8. Pipette 100 µl Substrate SUB into each well.
9. Incubate for 25 ± 5 minutes at room temperature (20 – 25 °C) on an orbital shaker at medium speed.
10. Pipette 100 µl Stop Solution STOP into each well and shake on an orbital shaker for minimum 10 seconds at medium speed.
11. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

9 Calculation of the Results

Standard	1	2	3	4	5	6
ADMA (µmol/l)	0	0.2	0.45	0.7	1	3
Arginine (µmol/l)	5	15	35	70	120	300

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 in µmol/l can be read directly from this standard curve by using their average optical density.

Serum arginine levels may be approximately 50% higher than the corresponding EDTA plasma levels in the same donor.

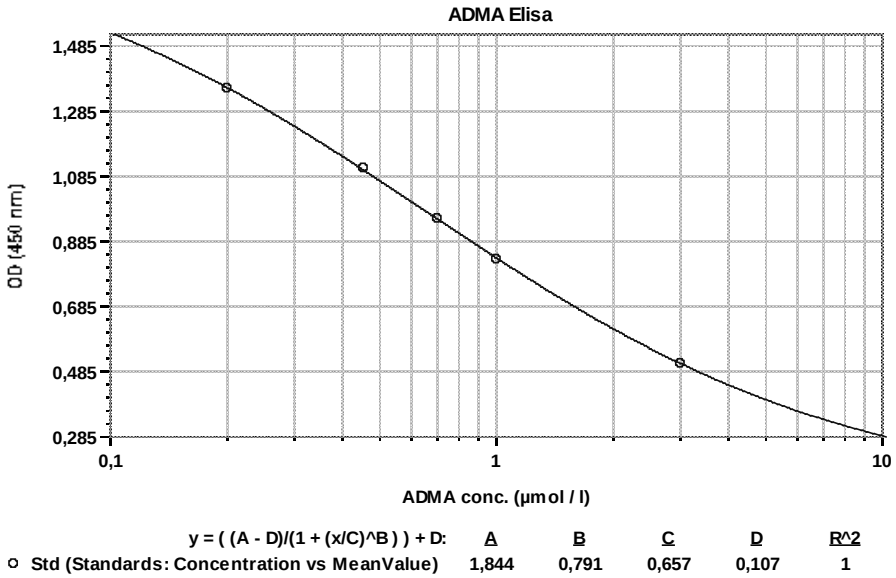
Conversion:

ADMA: 1 µmol/l = 202 ng/ml

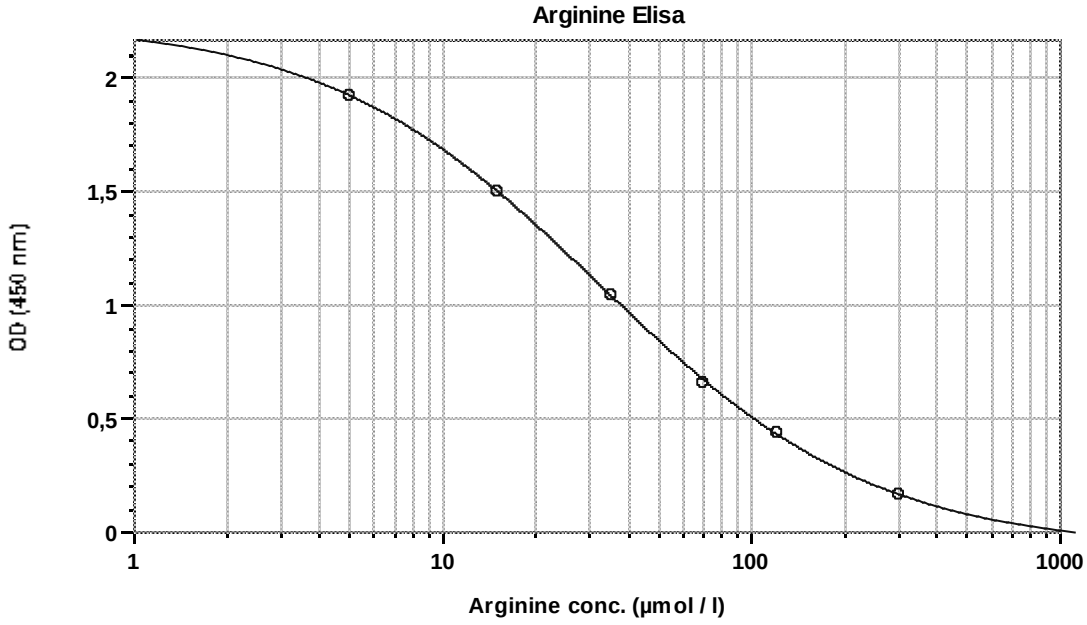
Arginine: 1 µmol/l = 174 ng/ml

Typical standard curve (do not use for calculation of results):

ADMA ELISA



Arginine ELISA:



	y = ((A - D)/(1 + (x/C)^B)) + D:	A	B	C	D	R^2
○	Std (Standards: Concentration vs MeanValue)	2,248	0,975	32,2	-0,071	1

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.

10 Assay Characteristics

10.1 Sensitivity

	Lower Limit of Detection	Calculation
ADMA	0.03 µmol / l	OD_{Cal1} – 3 x SD
Arginin	6 µmol / l	OD_{Cal1} – 3 x SD

10.2 Specificity (Cross Reactions)

ADMA

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

Arginine

Substance	Cross Reactivity (%)
Arginine	100
ADMA	< 0.37
Homoarginine	2.92
SDMA	0.88

10.3 Recovery

ADMA

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

Arginine

	Range (µmol / l)	Mean (%)	Range (%)
EDTA-Plasma	48 – 163	97	93 - 100
Serum	82 – 211	100	96 - 103

10.4 Linearity

ADMA

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

Arginine

	Range (µmol / l)	Highest Dil.	Mean (%)	Range (%)
EDTA-Plasma	28 – 193	1 : 6 with water	102	94 - 106

10.5 Reproducibility

ADMA

	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 %

Arginine

	Range (µmol / l)	Intra-Assay-Vk
EDTA-Plasma	56 – 125	3.6 – 2.3 %

	Range (µmol / l)	Inter-Assay-Vk
EDTA-Plasma	53 – 170	3.2 – 6.3 %

10.6 Method Comparison

ADMA

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

Arginine

	Method	Correlation
Serum + EDTA-Plasma	LC/MS	Y = 0.95 x LC/MS - 0.68; R = 0.991; N = 32

10.7 Calibration

The calibration is carried out by weighing the pure substance.

10.8 Limitations of Method

The results are to be used for research use only.

Samples measured above the highest standard must be diluted with distilled water and reassayed (see 10.4) The values of diluted samples must be multiplied by the appropriate dilution factor.

10.9 Interferences

Hemolytic, lipemic and icteric specimens should not be used.

11 Literature

11.1 Literature using the ADMA-ELISA from DLD Diagnostika

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The emerging role of asymmetric dimethylarginine as a novel cardiovascular risk factor
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Plasma levels of asymmetrical dimethylarginine and adverse cardiovascular events after percutaneous coronary intervention.
Eur Heart J. 2003; 24: 1912-1919

Pipetting Scheme – Sample Preparation

For ADMA and Arginine together in ACYL-PLATE

		Standards	Controls	Plasma	Serum
ACYL-PLATE:					
CAL 1 - 6	µl	20			
CON 1 & 2	µl		20		
EDTA-Plasma	µl			20	
Serum					20
ACYL-BUFF	µl	20	20	20	20
EQUA-REAG	µl	200	200	200	200

Shake plate for 10 seconds

ACYL-REAG	µl	50	50	50	50

Immediately, shake for 20 minutes at room temperature

Take **25 µl** each for ADMA ELISA
Take **10 µl** each Arginin ELISA

Pipetting Scheme - ELISA

For ADMA and Arginine in separate microtiter plates:

		ADMA (blue) STRIPS-ADMA			**Arginin (yellow)** STRIPS-ARG		
		Acyl. Stand.	Acyl. Ctrl.	Acyl. Samples	Acyl. Stand.	Acyl. Ctrl.	Acyl. Samples
Transfer from ACYL-PLATE into STRIPS		25	25	25	10	10	10
AS-ADMA (blue)	µl	50	50	50	-	-	-
AS-ARG (yellow)	µl	-	-	-	50	50	50

Seal STRIPS with FOIL
Shake for 90 minutes at room temperature
Wash 4 x with 300 µl WASH per well

CONJ	µl	100	100	100	100	100	100

Shake for 30 minutes at room temperature
Wash 4 x with 300 µl WASH per well

SUB	µl	100	100	100	100	100	100

Shake for 25 ± 5 minutes at room temperature

STOP	µl	100	100	100	100	100	100

Shake plate for minimum 10 seconds
Read absorbance at 450 nm (ref. 570 – 650 nm)