DIAGNOSTIKA GMBH

Instructions for Use

ADMA Fast ELISA

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

RUO

For Research Use Only
Not for Use in Diagnostic Procedures

Example Version

REF	EA212/96
Σ	12 x 8
+2 +8 °C	2 – 8 °C

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Symbols

RUO	For Research Use Only		
CONT	Content		Expiry Date
LOT	Lot Number	+2 +8 °C	Store at
	Manufactured by	Σ	Sufficient for ... determinations
REF	Catalogue Number		Consult Instructions for Use

Hazard Pictograms


Warning


Danger

1 Principle of the Test

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

The ADMA Fast ELISA is a competitive enzyme immunoassay. The acylated ADMA in the samples and the N-acyl-ADMA bound to the microtiter wells compete for a fixed number of rabbit anti-N-acyl-ADMA binding sites. When the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing. The remaining antibody bound to the solid phase ADMA is detected by adding peroxidase conjugated anti-rabbit-IgG. After washing, the TMB substrate is added and the TMB / peroxidase reaction is monitored at 450 nm. The amount of detected antibody bound to the solid phase ADMA is inversely proportional to the ADMA concentration of the sample.

2 Precautions

- For research use only. For professional use only.
- Before carrying out 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 animals certified as healthy but these materials should be handled as potentially infectious.
- Individual components of different lots and test 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.
- Avoid any actions that could result in ingestion, inhalation or injection of the reagents. Never pipette by mouth.
- Wear protective clothing, disposable gloves, and safety goggles while performing the test.
- Some components of this kit are containing hazardous reagents. These components are marked with the adequate hazard label. Further information is in section 4 and in the corresponding MSDS.
- Avoid contact with individual reagents.
- Dispose of waste according to state and local environmental protection regulations.
- Broken glass can cause injury. Be cautious with glass vials.

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3 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 6.

Reagents must equilibrate to room temperature before use and be refrigerated immediately after use.

4 Contents of the Kit

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

Standards (1 - 6) — **CAL 1 - 6** — 6 vials
each 4 ml, ready for use

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

Control 1 & 2 — **CON 1 & 2** — 2 vials
4 ml each, ready for use,
Range: see QC Certificate

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

Acylation Reagent — **ACYL-REAG** — 3 vials
lyoph., dissolve content in 3 ml Solvent before use; if required pool the vials (see section 6)

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

Enzyme Conjugate — **CONJ** — 1 vial
13 ml, ready for use,
Anti-rabbit-IgG-peroxidase

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Wash Buffer — **WASH** — 1 bottle
20 ml, 50x conc., Dilute content with dist. water to 1000 ml total volume (see section 6)

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

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

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

Equalizing reagent — **EQUA-REAG** — 1 vial
Lyoph., dissolve content with 21 ml dist. water, mix carefully to minimize foam formation (see section 6)

Solvent — **SOLVENT** — 2 vials
5 ml, contains DMSO
(Please note that Solvent reacts with many plastic materials including plastic trays. It does not react with normal pipette tips and with glass devices)


Danger


Warning

Foil — **FOIL** — 2 pieces
ready for use

Example Version

Additional materials and equipment required but not provided:

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

5 Sample Collection and Storage

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

Equilibrate reagents to room temperature

6.1 Microtiter strips

Before opening the packet of strip wells STRIPS, allow it to equilibrate to 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.

6.2 Wash Buffer

Dilute the content (20 ml) of the 50x concentrated WASH with dist. water to a total volume of 1000 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.

6.3 Equalizing Reagent

Dissolve the content of EQUA-REAG with 21 ml dist. water, vortex briefly and mix for at least 20 minutes on a roller mixer or similar shaker until completely dissolved. Thereby, avoid excessive formation of foam. The reconstituted Equalizing Reagent should be stored frozen at -20 °C and is stable until expiry date as given on the label.

6.4 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 mix on a roller mixer or similar

Example Version

shaker for at least 10 minutes. The Acylation Reagent must be freshly prepared immediately before performing the test and is then stable for approx. 3 hours. Discard the remaining reconstituted reagent after use. The second and third vial 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 or with glass devices.

All other reagents are ready for use.

6.5 Preparation of Samples (Acylation)

The wells of the reaction plate ACYL-PLATE can be used only once. Please mark the respective wells before use to avoid repeated use. Duplicates are recommended. Equilibrate reagents to room temperature and mix carefully, thereby avoid formation of foam.

1. Pipette each **20 µl standard 1 – 6** CAL 1-6, each **20 µl control 1 & 2** CON 1 & 2 and each **20 µl 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 prepared Equalizing Reagent** EQUA-REAG into each well.
4. Shake the reaction plate for 10 seconds on an orbital shaker.
5. Prepare Acylation Reagent ACYL-REAG 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 colour changes to violet.
6. Incubate for 20 minutes at room temperature on an orbital shaker at medium speed.

Take 25 µl each for the ELISA.

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7 Test Procedure ELISA

1. Transfer **25 µl each of prepared (acylated) standards, controls and samples** from the reaction plate into the respective wells of the coated microtiter strips [STRIPS].
2. Add **50 µl Antiserum** [AS] into each well.
3. Seal 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 prepared Wash Buffer** [WASH] per well. Discard or aspirate the contents of the wells and remove residual liquid by tapping the inverted plate on a clean absorbent paper. 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 on an orbital shaker at medium speed.
7. Wash: Repeat step 4
8. Pipette **100 µl Substrate** [SUB] into each well and incubate for 25 ± 5 minutes at room temperature on an orbital shaker at medium speed.
9. Pipette **100 µl Stop Solution** [STOP] into each well. Shake for 10 seconds on an orbital shaker.
10. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

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8 Calculation of the Results

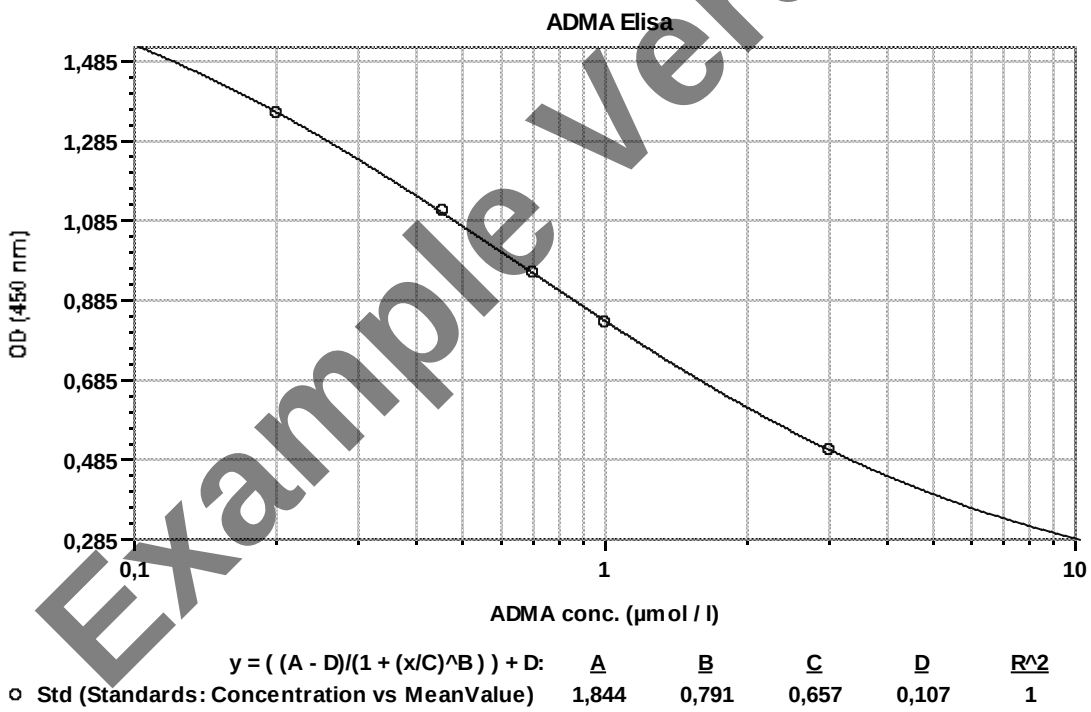
The concentration of the standards (x-axis, logarithmic) are plotted against their corresponding optical density (y-axis, linear).

When using analysing software, it is recommended to use the Four Parameter Logistic (4PL) Regression (alternatively: cubic-spline or logit-log).

The concentration of the controls and samples in µmol/l 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 (do not use for calculation of results):



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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.

9 Assay Characteristics

9.1 Analytical Sensitivity

Lower Limit of Detection	Calculation
0.03 µmol / l	ODCal1 – 3 x SD

9.2 Analytical Specificity (Cross Reactivity)

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

9.3 Recovery after Spiking

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

9.4 Linearity

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

9.5 Reproducibility

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

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

9.6 Method Comparison

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

9.7 Limitations of Method

The results are to be used for research use only.

Example Version

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 ADMA ELISA is available. Contact us via contact@dld-diagnostika.de

11 Changes to declare

Version _8: Introduction in section 1 and reference range in section 9 have been removed. Significant changes/additions are highlighted in gray.

Version _7: IFU has been re-formatted. Component names as printed on labels were included in sections 6 and 7 and pipetting schemes to provide greater clarity. No changes have been made to components or execution of protocols.

Example Version

Pipetting Scheme - Sample Preparation

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

Shake for 10 seconds

ACYL-REAG (fresh)	µl	50	50	50	50

Immediately, shake for 20 minutes at room temperature
Take 25 µl of each supernatant for the ELISA

Pipetting Scheme – ELISA

		Acyl. Standards	Acyl. Controls	Acyl. Samples
STRIPS:				
Transfer from ACYL-PLATE into STRIPS:	µl	25	25	25
AS	µl	50	50	50

Cover with FOIL and shake for 90 minutes at room temperature
Wash 4 x with 300 µl WASH per well

CONJ	µl	100	100	100

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

SUB	µl	100	100	100

Shake for 25 ± 5 minutes at room temperature

STOP	µl	100	100	100

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

Example Version