ADMA ELISA

Manufactured by Immundiagnostik AG.

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For laboratory use only. Not for human or diagnostic use.

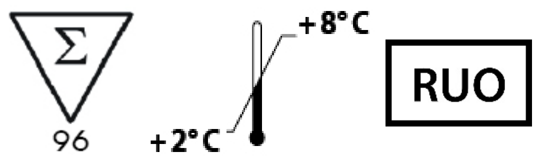
Manual

ADMA ELISA

For the in vitro determination of ADMA in EDTA plasma and serum of rodents and in cell culture media

For research use only

Valid from 2019-01-03



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1. INTENDED USE

This Immundiagnostik AG assay is intended for the quantitative determination of asymmetric dimethyl-L-arginine (ADMA) in rodent EDTA plasma or serum and in cell culture media. It is for research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of NO-synthase. It is formed during proteolysis of methylated proteins and removed by renal excretion or metabolic degradation by the enzyme dimethylarginine dimethylaminohydrolase (DDAH). Several cell types, including human endothelial and tubular cells are capable of synthesizing and metabolizing ADMA. Elevated ADMA concentrations in the blood are found in numerous diseases associated with endothelial dysfunction. For example, elevated ADMA levels in blood of dialysis patients correlate significantly with the degree of arteriosclerosis and cardiovascular risk. Furthermore, elevated ADMA levels are found in patients with hypercholesterolemia, hypertension, arteriosclerosis, chronic renal failure and chronic heart failure, and are associated with restrictions in endothelial vasodilatation.

During the last years, the important clinical relevance of the regulation of vascular tone and structure by nitric oxide (NO) has been shown. Moreover, there were reports that human endothelial cells produce ADMA as well as nitric oxide, which points to an endogenous endothelial NO-regulation by ADMA. Therefore it was assumed that hypertension, arteriosclerosis and immunological dysfunction in patients with chronic renal failure are connected to a dysfunction of the L-arginine/NO-metabolism and to ADMA accumulation. The reasons for the deregulation of the L-arginine/NO-metabolism could only partially be elucidated. Certainly, there are multiple factors involved in the L-arginine/NO-metabolism regulation as for example elevation of free superoxide radicals (O_2^-), ADMA accumulation and reduced NO-synthase activity.

Prospective clinical studies of the last years demonstrate the increased importance of ADMA as a novel cardiovascular risk factor.

Indication

- Arteriosclerosis
- Hypertension
- Chronic heart failure
- Coronary artery disease

- Hypercholesterolemia
- Chronic renal failure
- Diabetes mellitus
- Peripheral arterial occlusive disease

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit Components	Quantity
KR3001	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR3001	STD	Standards, ready-to-use (0, 0.1, 0.25, 0.5, 1.0, 2.0 µM)	6 x 1 ml
KR3001	CTRL 1	Control, ready-to-use (see specification for range)	1 x 1 ml
KR3001	CTRL 2	Control, ready-to-use (see specification for range)	1 x 1 ml
KR0006.C.100	WASHBUF A	Wash buffer concentrate, 10x	2 x 100 ml
KR3001	AB	ADMA antibody, lyophilised	1 vial
KR3001	CONJ	Conjugate, ready-to-use	1 x 12 ml
KR0012.15	DERBUF	Reaction buffer, ready-to-use	1 x 15 ml
KR3001	DER	Derivatisation reagent, lyophilised	1 x 50 mg
KR0008.04	DMSO	Dimethylsulfoxide (DMSO)	1 x 4 ml
KR0013.28	CODIL	Dilution buffer after derivatisation, ready-to-use	1 x 28 ml
KR0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
KR0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- Calibrated precision pipets and 10-1000 µl tips
- Foil to cover the microtiter plate

- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Vortex
- Centrifuge, 3000 *g*
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of 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).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label.

- **Preparation of the wash buffer**: The **wash buffer concentrate (WASHBUF A)** has to be diluted with ultra pure water **1:10** before use (100 ml WASHBUF A + 900 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF A** is stable at **2-8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF A) can be stored in a closed flask at **2-8 °C for 1 month**.

- **DMSO** crystallises at 2-8 °C. Before use, bring to room temperature to dissolve the crystals.

- The **lyophilised derivatisation reagent (DER)** is stable at **2-8 °C** until the expiry date stated on the label. Bring to room temperature before opening. Reconstitute the DER (50 mg) with **3 ml DMSO**. Allow to dissolve for 10 minutes and mix thoroughly with a vortex-mixer. **The derivatisation reagent** (reconstituted DER) **can be stored at 2-8 °C for 2 months**. Bring to room temperature before reuse. Please note: DMSO attacks all plastics but not polypropylene products and laboratory glass.

- The **lyophilised ADMA antibody (AB)** is stable at **2-8 °C** until the expiry date stated on the label. Reconstitute the AB with **6 ml of wash buffer**. **ADMA antibody** (reconstituted AB) **can be stored at 2-8 °C for 2 months.**

- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2-8 °C.**

6. STORAGE AND PREPARATION OF SAMPLES

EDTA plasma and serum from rodents and cell culture media

- Blood samples are stable for one week at 2-8°C. For longer storage keep samples frozen at -20 °C.
- Lipemic or hemolytic samples may give erroneous results and should not be used for analysis.
- Plasma, serum and cell culture medium samples are analysed **undiluted**.
- For sample preparation a derivatisation reagent for derivatisation of ADMA is added (see sample preparation procedure).

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of ADMA. The assay is based on the method of competitive enzyme linked immunoassays.

The sample preparation includes the addition of a derivatisation-reagent for ADMA derivatisation. Afterwards, the treated samples and the polyclonal ADMA-antiserum are incubated in the wells of a microtiter plate coated with ADMA-derivative (tracer). During the incubation period, the target ADMA in the sample competes with the tracer immobilized on the wall of the microtiter wells for the binding of the polyclonal antibodies.

During the second incubation step a peroxidase-conjugated antibody is added to detect the anti-ADMA 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 colour changes from blue to yellow, and the absorbance is measured in the photometer at 450 nm. The intensity of the yellow colour is inverse proportional to the ADMA concentration in the sample; this means, high ADMA concentration in the sample reduces the concentration of tracer-bound antibodies and lowers the photometric signal. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. ADMA, present in the patient samples, is determined directly from this curve.

Sample preparation procedure

Bring **all reagents and samples to room temperature** (15-30 °C) and mix well.

Derivatisation of standards, controls and samples is carried out in single analysis in vials (e.g. 1.5 ml vials).

We recommend preparing one derivatisation per standard, control and sample and transferring it in duplicate determinations into the wells of the microtiter plate.

1.	Add **100 µl standard** (STD), **100 µl control** (CTRL) and **25 µl sample** in the corresponding vials.
2.	Add **75 µl reaction buffer** (DERBUF) **only to the samples.**
3.	Add **25 µl derivatisation reagent** into each vial (STD, CTRL, sample), **mix thoroughly** by repeated inversion or several seconds on a vortex mixer. Incubate for **45 min at room temperature** (15-30 °C) on a **horizontal shaker**.
4.	Add **125 µl dilution buffer** (CODIL) into each vial, mix well and incubate for **45 min** at **room temperature** (15-30 °C) on a horizontal **shaker**.

2 x 50 µl of the derivatised standards, controls and samples are used in the ELISA as duplicates.

Test procedure

Mark the positions of standards, controls and samples in duplicate on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips covered at 2-8 °C. Strips are stable until expiry date stated on the label.

5.	For the analysis in duplicate take **2 x 50 µl** of the **derivatised standards/ controls/ samples** out of the vials and add into the respective wells of the microtiter plate.
6.	Add **50 µl ADMA antibody** into each well of the microtiter plate.
7.	Cover the strips tightly with foil and incubate **overnight at 2-8 °C**.
8.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
9.	Add **100 µl conjugate** (CONJ) into each well.

10.	Cover the strips and incubate for **1 hour** at **room temperature** (15-30 °C) on a **horizontal shaker**.
11.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
12.	Add **100 µl substrate** (SUB) into each well.
13.	Incubate for **10-14 min*** at room temperature (15-30 °C) in the **dark**.
14.	Add **100 µl stop solution** (STOP) into each well and mix well.
15.	Determine **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 (690 nm) as a reference.

* The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the 4 parameter algorithm.

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 standard must be specified with a value less than 1 (e.g. 0.001).

2. **Point-to-point calculation**

   We recommend a linear ordinate for optical density and a linear abscissa for concentration.

3. **Spline algorithm**

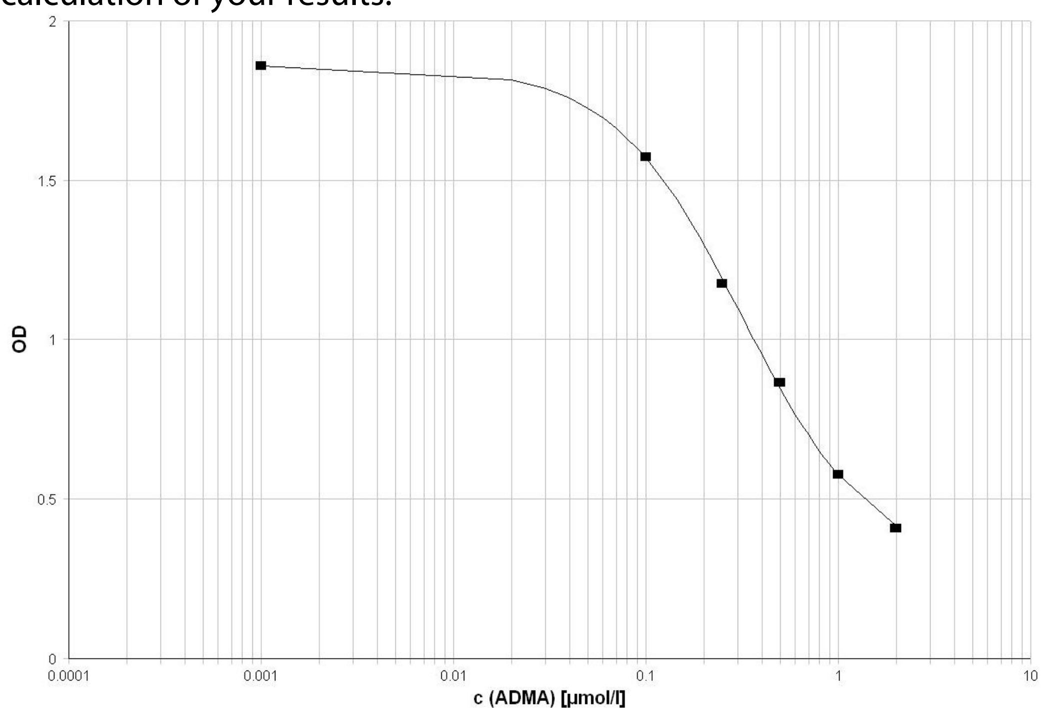
   We recommend a linear ordinate for optical density and a linear abscissa for concentration.

The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done manually.

Serum and EDTA plasma, cell culture supernatant

The concentrations can be determined directly from the standard curve in µmol/l. **No factor** is required.

In the following, an example of a standard curve is given. Do not use it for the calculation of your results.



9. LIMITATIONS

Samples with concentrations above the measurement range (see definition below) can be diluted with reaction buffer (DERBUF) and re-assayed. Please consider this dilution factor when calculating the results.

Samples with concentrations lower than the measurement range (see definition below) cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × sample dilution factor to be used

The lower limit of the measurement range can be calculated as:

LoB × sample dilution factor to be used

LoB see chapter "Performance Characteristics".

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control samples are outside of the acceptable limits.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibilityt

Serum

Intra-Assay (n = 4)

sample	ADMA [µmol/l]	CV [%]
1	0.33	7.0
2	0.67	6.5

Inter-Assay (n = 4)

sample	ADMA [µmol/l]	CV [%]
1	0.34	7.0
2	0.67	6.5

Cell culture media

Intra-Assay (n = 4)

sample	ADMA [µmol/l]	CV [%]
1	0.54	6.6
2	1.02	4.8

Inter-Assay (n = 4)

sample	ADMA [µmol/l]	CV [%]
1	0.54	7.7
2	0.99	5.5

Spiking recovery

Different ADMA concentrations were spiked to rodent serum and to cell culture medium and measured in this assay. The mean recovery rate was 92 % for serum and 104 % for cell culture medium (n = 4).

Serum

spike [µmol/l]	ADMA expected [µmol/l]	ADMA measured [µmol/l]	recovery [%]
		0.55	
0.5	1.05	0.98	93
1.0	1.55	1.41	91

Cell culture media

spike [µmol/l]	ADMA expected [µmol/l]	ADMA measured [µmol/l]	recovery [%]
		0.0	
0.5	0.5	0.55	110
1.0	1.0	0.98	98

Dilution recovery

One spiked sample, respectively, was diluted with DERBUF. The mean recovery rate was 95 % for serum and 88 % for cell culture medium.

Serum

spike [µmol/l]	ADMA expected [µmol/l]	ADMA measured [µmol/l]	recovery [%]
		1.51	
1:2	0.76	0.71	94
1:4	0.38	0.36	95

Cell culture media

spike [µmol/l]	ADMA expected [µmol/l]	ADMA measured [µmol/l]	recovery [%]
		0.98	
1:2	0.49	0.44	90
1:4	0.25	0.21	86

Analytical sensitivity

Limit of blank, LoB 0.12 µmol/l
Limit of detection, LoD 0.15 µmol/l
Limit of quantitation, LoQ 0.16 µmol/l

The evaluation was performed according to the CLSI guideline EP-17-A2. The specified accuracy goal for the LoQ was 20 % CV.

Specificity

The specificity of the antibody was tested by measuring the cross-reactivity against compounds with structural similarity to ADMA. The specificity is calculated in percent in relation to the ADMA-binding activity.

L-Arginin < 0.01 %
SDMA < 0.2 %

12. PRECAUTIONS

- All reagents in the kit package are for research use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes..
- The stop solution consists of diluted sulfuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing.

Any spill should be wiped up immediately with copious quantities of water. Do not breathe vapour and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore, we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature, and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be sent to Immundiagnostik AG along with a written complaint.

15. REFERENCES

General literature

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