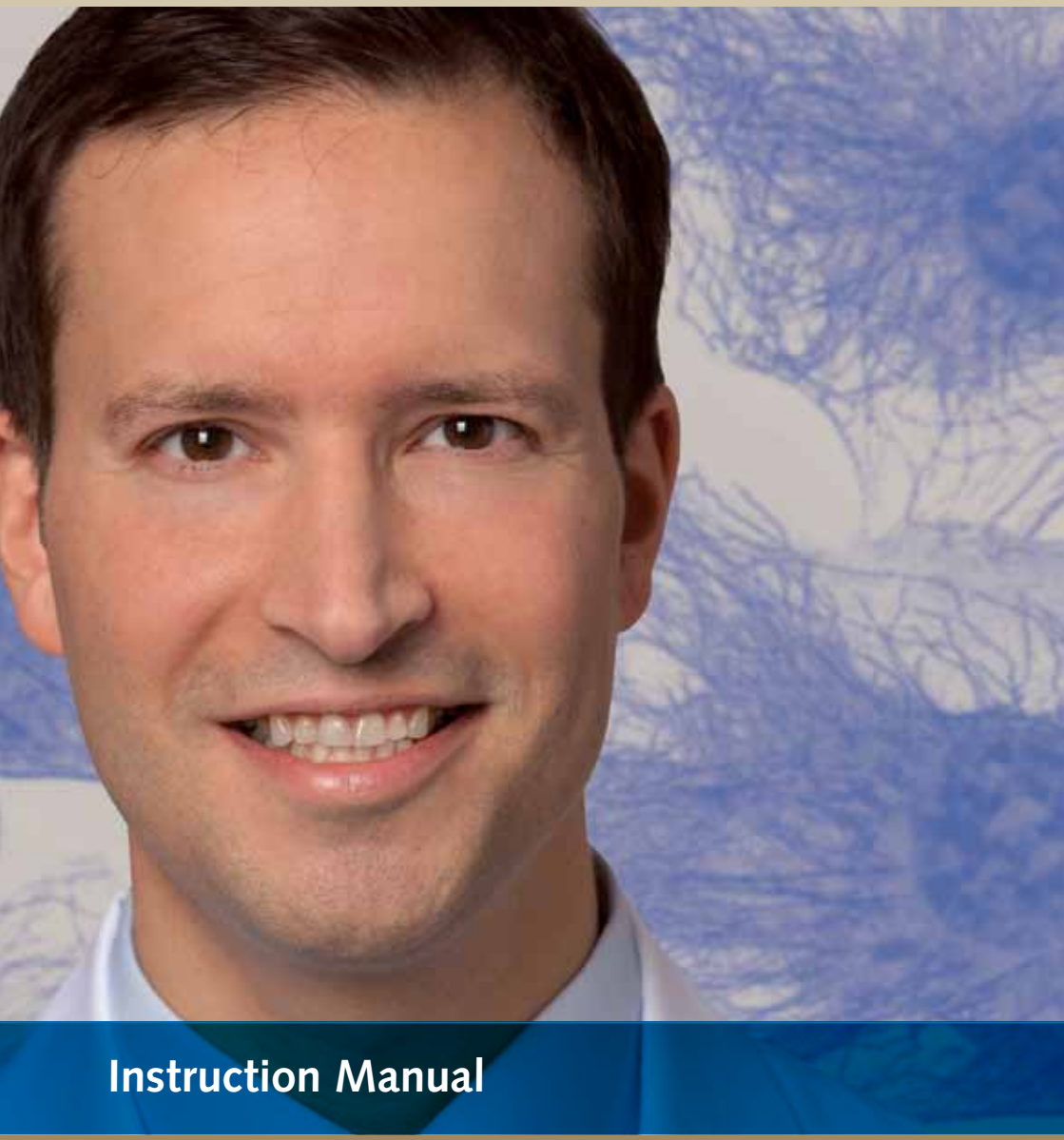
Mouse/rat ADMA direct ELISA Kit



Instruction Manual

PromoKine

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Intended Use

The ADMA ELISA Kit is intended for the quantitative determination of asymmetric dimethylarginine (ADMA) in rodent EDTA-plasma or serum and in cell culture media. It is for *research* use only.

Introduction

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of NOsynthase. 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-arginin/NO-metabolism and to ADMA accumulation. The reasons for the deregulation of the L-arginin/NO-metabolism could only partially be elucidated. Certainly, there are multiple factors involved in the Larginin/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

Principle of the Test

This assay is based on the method of competitive enzyme linked immunoassays.
The sample preparation includes the addition of a derivatization-reagent for ADMA coupling. Afterwards, the treated samples and the polyclonal ADMA-antiserum are incubated in wells of microplate 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 each microtiter well to detect the anti-ADMA antibodies. After washing away the unbound components, tetramethylbenzidine (TMB) is added as a substrate for peroxidase. Finally, the enzymatic reaction is terminated by an acidic stop solution. The color changes from blue to yellow and the absorbance is measured in the photometer at 450 nm. The intensity of the yellow color 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 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.

Material Supplied

Content	Kit Components	Quantity
PLATE	One holder with precoated strips	12 x 8 wells
STD	Standards (diluted in reaction buffer, ready to use; 0, 0.1, 0.25, 0.5, 1.0, 2.0 µM)	6 x 1 ml
CTRL 1	Control (ready-to-use; see specification for range)	1 x 1 ml
CTRL 2	Control (ready-to-use; see specification for range)	1 x 1 ml
WASHBUF	Wash buffer concentrate (10x)	2 x 100 ml
AB	ADMA antibody (lyophilized)	1 vial
CONJ	Conjugate, ready-to-use	1 x 12 ml
DERBUF	Reaction buffer, ready-to-use	1 x 15 ml
DER	Derivatization reagent	1 x 50 mg
DMSO	Dimethylsulfoxid (DMSO)	1 x 4 ml
CODIL	Dilution buffer after derivatization	1 x 28 ml
SUB	TMB substrate, ready-to-use	1 x 15 ml
STOP	Stop solution	1 x 15 ml

Material Required but not Supplied

- Ultra-pure water*
- Precision pipettors and disposable tips to deliver 10-1000 µl
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- A multi-channel pipets or repeater pipets
- Centrifuge capable of 3,000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 450 nm or 405 nm

* We recommend using ultra-pure water (Water Type 1; ISO3696) 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.

Preparation and Storage of Reagents

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

Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.

Preparation of the wash buffer: The wash buffer concentrate (**WASHBUF**) has to be diluted with ultra pure water **1:10** before use (100 ml WASHBUF + 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 is stable at 2-8 °C until the expiry date stated on the label. Wash buffer (1:10 diluted WASHBUF) 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 derivatization 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 for use. Test reagents are stable until the expiry date (see label of test package) when stored at **2-8°C**.

Precautions

- 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.

Specimen Collection and Preparation

EDTA plasma and serum from rodents and cell culture media

- *Blood samples are stable for one week at 2-8°C. For longer storage samples should be 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 derivatization reagent (DER) for derivatization of ADMA is added (see sample preparation procedure).*

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
Derivatization of standards (STD), controls (CTRL) and samples (SAMPLE) is carried out in single analysis in vials (e.g. 1.5 ml vials) as follows:

1. Bring all reagents and samples to room temperature (15-30°C) and mix well.
2. Add **100 µl of standards (STD)**, **100 µl of ready-to-use controls (CTRL)**, and **25 µl of samples (SAMPLE)** into the corresponding vials.

3. Add **75 µl of reaction buffer (DERBUF) only to the samples** (SAMPLE).
4. Add **25 µl** of freshly prepared **derivatization reagent (DER)** into each vial (STD, CTRL, SAMPLE), mix thoroughly by repeated inversion and incubate for **45 minutes at room temperature** (15-30°C) on a horizontal shaker (180-240 rpm).
5. Afterwards add **125 µl of dilution buffer (CODIL)** into each vial, mix well and incubate for **45 minutes at room temperature** (15-30°C) on a horizontal shaker (180-240 rpm).

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

Test procedure

6. Mark the positions of standards (STD)/ controls (CTRL)/ samples (SAMPLE) in duplicate on a protocol sheet.
7. Take as many **microtiter strips (PLATE)** as needed from kit. Store unused strips covered at 2-8°C. Strips are stable until the expiry date stated on the label.
8. For the analysis in duplicate take **2 x 50 µl** of the **derivatized standards/ controls/ samples (STD/ CTRL/ SAMPLE)** out of the vials and add into the respective wells of the microtiter plate.
9. Add **50 µl ADMA antibody (AB)** into each well of the microtiter plate. Cover the strips tightly with foil.
11. Incubate **overnight (15-20 hours) at 2-8°C**.
12. 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.
13. Add 1**00 µl conjugate (CONJ)** into each well.
14. Cover the strips tightly and incubate for **1 hour at room temperature (15-30°C)** on a horizontal shaker (180-240 rpm).
15. Discard the content of each well. Wash each well **5 times with 250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
16. Add **100 µl of TMB substrate (SUB)** into each well.

17. Incubate for 10-14 minutes at room temperature (15-30°C) in the dark*.
18. Add **100 µl of stop solution (STOP)** into each well, mix thoroughly.
19. 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 color change is temperature sensitive. We recommend to observe the color change and to stop the reaction upon good differentiation.

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 duplicate values should be examined before automatically evaluating the results. If this option is not available within the used program, the duplicate values should be evaluated manually. The concentration of controls and patient samples can be determined directly from the calibration curve in µmol/l. In the following, an example of a calibration curve is given; do not use it for the calculation of your results.

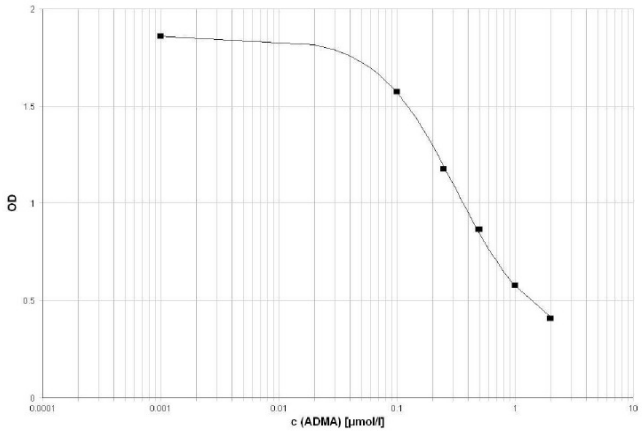
The plausibility of the duplicate values should be examined before automatically evaluating the results. If this option is not available within the used program, the duplicate values should be evaluated manually.

Serum and EDTA plasma, cell culture supernatant

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

In the following, an example of a calibration curve is given; do not use it for the calculation of your results.

Example of calibration curve



Quality Control

We recommend the use of external controls for internal quality control, if possible. Control samples should be analyzed 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 the acceptable limits.

Limitations

Samples with an OD lower than the OD of the highest standard should be diluted with reaction buffer (DERBUF) and re-assayed. Please consider this dilution factor when calculating the results.

Performance Characteristics

Precision and Reproducibility

Serum

Intra-Assay (n= 4)

Sample	ADMA [µmol/l]	Standard variation [SD]
1	0.33	7%
2	0.67	6.5%

Inter-Assay (n= 4)

Sample	ADMA [µmol/l]	Standard variation [SD]
1	0.34	7%
2	0.67	6.5%

Cell Culture Media

Intra-Assay (n= 4)

Sample	ADMA [µmol/l]	Standard variation [SD]
1	0.54	6.6%
2	1.02	4.8%

Inter-Assay (n= 4)

Probe	ADMA [µmol/l]	Standard variation [SD]
1	0.54	7.7%
2	0.99	5.5%

Analytical Sensitivity

The zero-standard was measured 6 times. The detection limit was set as B0 - 1 SD and estimated to be 0.05 µmol/l.

Sample	ADMA Mean value [OD]	Standard variation (SD)	Detection limit [µmol/l]
0	2.45	0.08	0.05

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	
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

Dilution	Measured [µmol/l]	Expected [µmol/l]	Recovery [%]
original		1.15	
1:2	0.76	0.71	94
1:4	0.38	0.36	95

Cell Culture Media

Dilution	Measured [µmol/l]	Expected [µmol/l]	Recovery [%]
original		0.98	
1:2	0.49	0.44	90
1:4	0.25	0.21	86

Specificty / Cross Reactivity

Specificity 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.

SDMA < 0.2 %
L-Arginin < 0.01 %

References

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General Notes on the Test and Test Procedure

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC. However, it is **for *in vitro* research use only**.
- The assay should always be performed according to the enclosed manual.
- Test components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- All reagents in the test package are for research use only.
- Single components with different lot numbers should not be mixed or exchanged. Do not mix plugs and caps from different reagents
- Guidelines for medical laboratories should be observed.
- 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. PromoCell can therefore not be held responsible for any damage resulting from wrong use.
- 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, as wells from already opened microtiter plates are exposed to different conditions than sealed ones.
- Reagents should not be used beyond the expiration date stated on kit label.
- Control samples should be analysed with each run
- 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.

Ordering Information

Product Name	Product Description	Size	Catalog Number
ADMA direct ELISA Kit, mouse/rat	Mouse/Rat ADMA direct (Asymetric Dimethyl Arginine) ELISAKit	96 Tests	PK-EL-K3001

For in vitro research use only.
Not for diagnostic or therapeutic procedures.

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