



## **ADMA Direct (mouse/rat) ELISA Kit**

*Manufactured by Immundiagnostik AG.*

### **ALX-850-327-KI01**

**96 wells (~80 tests)**

**(Version 01: May 21, 2008)**

#### **NORTH AMERICA**

AXXORA, LLC  
6181 Cornerstone Court East  
Suite 103  
San Diego, CA 92121-4727  
Phone: (858) 550-8828  
Fax: (858) 550-8825  
E-mail: [axxora-usa@axxora.com](mailto:axxora-usa@axxora.com)

#### **SWITZERLAND/REST OF THE WORLD**

ALEXIS CORPORATION  
Industriestrasse 17, Postfach  
CH-4415 Lausen / Switzerland  
Phone: +41 61 926 89 89  
Fax: +41 61 926 89 79  
E-mail: [alexis-ch@alexis-corp.com](mailto:alexis-ch@alexis-corp.com)

#### **GERMANY**

AXXORA DEUTSCHLAND GmbH  
Marie-Curie-Strasse 8  
DE-79539 Lörrach  
Phone: (07621) 5500 522  
Fax: (07621) 5500 523  
E-mail: [axxora-de@axxora.com](mailto:axxora-de@axxora.com)

#### **UK & IRELAND**

AXXORA (UK) LTD.  
P.O. Box 6757  
Bingham, Nottingham NG13 8LS  
Phone: +44 1949 836111  
Fax: +44 1949 836222  
E-mail: [axxora-uk@axxora.com](mailto:axxora-uk@axxora.com)

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



Manual

# ADMA *direct* (mouse/rat) ELISA Kit

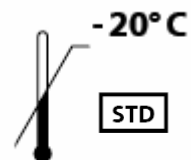
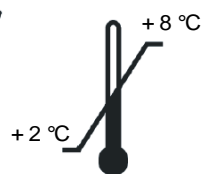
*For the determination of ADMA in EDTA-plasma and serum  
For animal experimental studies  
(not suitable for human samples)*

For research use only

Valid from 21.05.2008



K 3001



STD



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## 1. INTENDED USE

The ADMA ELISA Kit is intended for the quantitative determination of asymmetric dimethylarginine (ADMA) in EDTA-plasma and serum from mouse and rat. It is for research use only.

## 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 celltypes, 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 with cardiovascular morbidity and mortality. 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 L-arginin/NO-metabolism regulation as for example elevation of free superoxid 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. PRINCIPLE OF THE TEST**

This assay is based on the method of competitive enzyme linked immunoassays. The sample preparation includes the addition of an acylation-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. The ADMA in the sample displaces the antibodies out of the binding to the tracer. Therefore the concentration of the tracer-bound antibody is inverse proportional to the ADMA concentration in the sample. 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.

#### 4. MATERIAL SUPPLIED

Catalog No	Content	Kit Components	Quantity
K3001MTP	PLATE	One holder with precoated strips	12 x 8 wells
K3001MC	CFD	Centrifugal filter device	48 devices
K3001ST	STD	Standards (diluted in reaction buffer)	6 x 2 vials
K3001KO	CTRL 1+ CTRL 2	Controls (diluted in reaction buffer)	2 x 2 vials
K3001WP	WASHBUF	Wash buffer concentrate (10 fold)	2 x 100 ml
K3001AK	AB	ADMA antibody (lyophilized)	2 x 1 vial
K3001AVR	ABBUF	ADMA antibody dilution buffer (lyophilized)	4 x 80 mg
K3001K	2.AB	POD antibody (concentrate)	120 µl
K3001CSP	2.ABDIL	Conjugate stabilizing buffer	24 ml
K3001RP	DERBUF	Reaction buffer	10 ml
K3001DR	DER	Acylation reagent	4 x 10 mg
K3001LM	DMSO	Dimethylsulfoxide (DMSO)	1,5 ml
K3001SL	CODIL	Dilution buffer for coupling (lyophilized)	2 x 5 ml
K3001TMB	SUB	TMB substrate	2 x 15 ml
K3001AC	STOP	Stop solution	15 ml

## 5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Bidistilled water (aqua bidist.)
- Laboratory balance
- Precision pipettors and disposable tips to deliver 10-1000  $\mu$ l
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 14000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 450 nm  
(reference wave length 620 or 690 nm)

## 6. PREPARATION AND STORAGE OF REAGENTS

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each assay.** The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with a volume less than **100  $\mu$ l** should be centrifuged before use to avoid loss of volume.
- The **Wash buffer concentrate (WASHBUF)** should be diluted with aqua bidist. **1:10** before use (100 ml concentrate + 900 ml aqua bidist.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at room temperature or at 37°C using a water bath before dilution of the buffer solutions. The **buffer concentrate** is stable at **2-8°C** until the expiry date stated on the label. Diluted **buffer solution** can be stored in a closed flask at **2-8°C for one month**.
- **Standards (STD) and Controls (CTRL1, CTRL2)** are already diluted in the **reaction buffer (DERBUF)**. Store Standards and Controls frozen at -20°C, thaw before use in the test, and re-freeze immediately after use. Standards and Controls can be re-frozen up to 3 times.
- **DMSO** could crystallize at 4°C. Dissolve the crystals at 20-25°C in a water bath.

- The content of one vial of **acylation reagent (DER) (10 mg)** must be dissolved in **300 µl DMSO**. Put the vial on a horizontal shaker for 5 min. After use, the rest of the reagent should be discarded. DER must be **prepared immediately before use**. The ELISA kit can be separated into four performances by the four DER vials. Please note: **DMSO attacks all plastics but not dispenser tips and laboratory glass**.
- The **dilution buffer for coupling (CODIL)** must be reconstituted in 5 ml diluted **wash buffer** per vial (total volume: 2 x 5 ml = 10 ml). The reconstituted dilution buffer for coupling is stable for at least 14 days after reconstitution. The ELISA kit can be separated into two performances because of the two AB vials or even into four determinations within 14 days.
- The **antibody dilution buffer (ABBUF)** must be reconstituted in **2,8 ml** of diluted **wash buffer** per vial (total volume 4 x 2,8 ml = 11,2 ml).
- The **ADMA antibody (AB)** must be dissolved in the **reconstituted antibody dilution buffer**. Therefore, each of the 2 AB vials must be reconstituted with 0,5 ml of diluted antibody dilution buffer. Incubate for 10 min, vortex, transfer the obtained solution (2 x 0,5 ml = 1,0 ml) quantitatively into a separate vial and add the residual antibody dilution buffer (10,2 ml). The ADMA antibody solution is stable for at least 14 days after reconstitution. The ELISA kit can be separated into 2 performances because of the 2 AB vials or even into 4 determinations within 14 days.
- The **POD antibody (2.AB)** must be diluted **1:200** in **conjugate stabilizing buffer (2.ABDIL)** (110 µl 2.AB + 22 ml 2.ABDIL). The undiluted POD antibody (2.AB) is stable at **2-8°C** until the expiry date stated on the label. **Diluted POD antibody (2.AB) is not stable over a longer period. It can be stored at 2-8°C for only 5 days.**
- All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at 2-8°C.

## 7. PRECAUTIONS

- For research use only.
- Human materials used in kit components were tested and found to be negative for HIV and Hepatitis B. However, for safety reasons, all kit components should be treated as if potentially infectious.

- Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.
- Reagents should not be used beyond the expiration date shown on kit label.

## **8. SPECIMEN COLLECTION AND PREPARATION**

### **EDTA-plasma and serum**

- Venous fasting blood is suited for this test system. Samples are stable for one week at 2-8°C. For longer storage samples should be frozen at -20°C up to the measurement.
- Lipemic or hemolytic samples may give erroneous results and should not be used for analysis.
- The **EDTA-plasma and serum** samples are analyzed without any dilution.
- For sample preparation, a DER for coupling of ADMA is added (details are given in the sample preparation procedure).

## **9. ASSAY PROCEDURE**

### **Procedural notes**

- Quality control guidelines should be observed.
- Incubation time, incubation temperature and pipetting volumes of the different components are defined by the producer. Any variations of the test procedure, that are not coordinated with the producer, may influence the test results. Alexis Corporation can therefore not be held reliable for any damage resulting from this.
- The assay should always be performed according to the enclosed manual.

*Sample preparation procedure*

<p>1. Pipette <b>300 µl</b> of each, <b>ready-to-used standards (STD)</b> or <b>ready-to-used standards controls (CTRL)</b>, into a centrifugal filter device (CFD)</p> <p>Pipette <b>100 µl</b> of each sample (<b>SAMPLE</b>) into a centrifugal filter device (CFD) and add <b>200 µl</b> of <b>reaction buffer (DERBUF)</b></p>
<p>2. <b>Centrifuge for 20-30 min</b> at 14000 x g and 4°C until appr. <b>250 µl filtrate</b> are collected; the residual solution remains in the centrifugal filter device</p>

2 x 100 µl of each filtrate (permeate) is used in the ELISA in duplicate.

*Test procedure*

<p>1. Bring all reagents and samples to room temperature (18-26°C)</p>
<p>2. Mark the positions of <b>standards (STD)/controls (CTRL)/ samples (SAMPLE)</b> in duplicate on a protocol sheet</p>
<p>3. Take as many microtiter strips (PLATE) as needed from kit. Store unused strips in the closed original package bag at 2-8°C. Strips are stable until the expiry date stated on the label</p>
<p>4. Wash each well <b>5 times</b> by dispensing <b>250 µl</b> of diluted <b>Wash buffer (WASHBUF)</b> into each well. After the final washing step, the inverted microtiter plate (PLATE) should be firmly tapped on absorbent paper to remove excess solution</p>
<p>5. Add <b>100 µl</b> of <b>filtered standards (STD)/ controls (CTRL)/samples (SAMPLE)</b> in duplicate to the wells of the ELISA plate (PLATE)</p>
<p>6. Add <b>10 µl</b> of freshly prepared <b>acylation reagent (DER)</b> to all wells and incubate <b>immediately for 30 min</b> on a <b>horizontal mixer</b> at <b>room temperature (18-26°C)</b></p>
<p>7. Add <b>40 µl</b> <b>dilution buffer (CODIL)</b> to each used well of the ELISA plate (PLATE)</p>

8. Incubate for <b>5 min at room temperature (18-26°C)</b> on a horizontal mixer
10. Add <b>50 µl</b> diluted <b>ADMA antibody (AB)</b> into each well and cover the plate tightly
11. Incubate over night ( <b>15-20 hours</b> ) at <b>2-8°C</b>
9. Aspirate the contents of each well. Wash <b>5 times</b> by dispensing <b>250 µl</b> of diluted <b>Wash buffer (WASHBUF)</b> into each well. After the final washing step, the inverted microtiter plate (PLATE) should be firmly tapped on absorbent paper to remove excess solution
10. Add <b>200 µl</b> diluted <b>POD antibody (2. AB)</b> into each well
11. Cover plate tightly and incubate for <b>1 hour at room temperature (18-26°C)</b> on a horizontal mixer
12. Aspirate the contents of each well. Wash <b>5 times</b> by dispensing <b>250 µl</b> of diluted <b>Wash buffer (WASHBUF)</b> into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution
13. Add <b>200 µl</b> of <b>TMB substrate (SUB)</b> into each well
14. Incubate for <b>6-15 min at room temperature (18-26°C)</b> in the dark*
15. Add <b>100 µl</b> of <b>stop solution (STOP)</b> into each well, mix thoroughly
16. Determine absorption <b>immediately</b> with an ELISA reader at <b>450 nm</b> against 620 nm (alternatively at 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 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.

## 10. EVALUATION OF RESULTS

If the test is performed in strict compliance with the manufacturer's instructions, e.g. with the exact volumes for standards, controls and samples/sample treatment (*Sample preparation procedure*, Point 1), standards, controls and samples are equally diluted. Therefore, **no dilution factor is required for calculation of the results.**

The following algorithms can be used alternatively to calculate the results. We recommend to use 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 calibrator must be specified with a value less than 1 (e. g. 0.01).

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 logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.01).

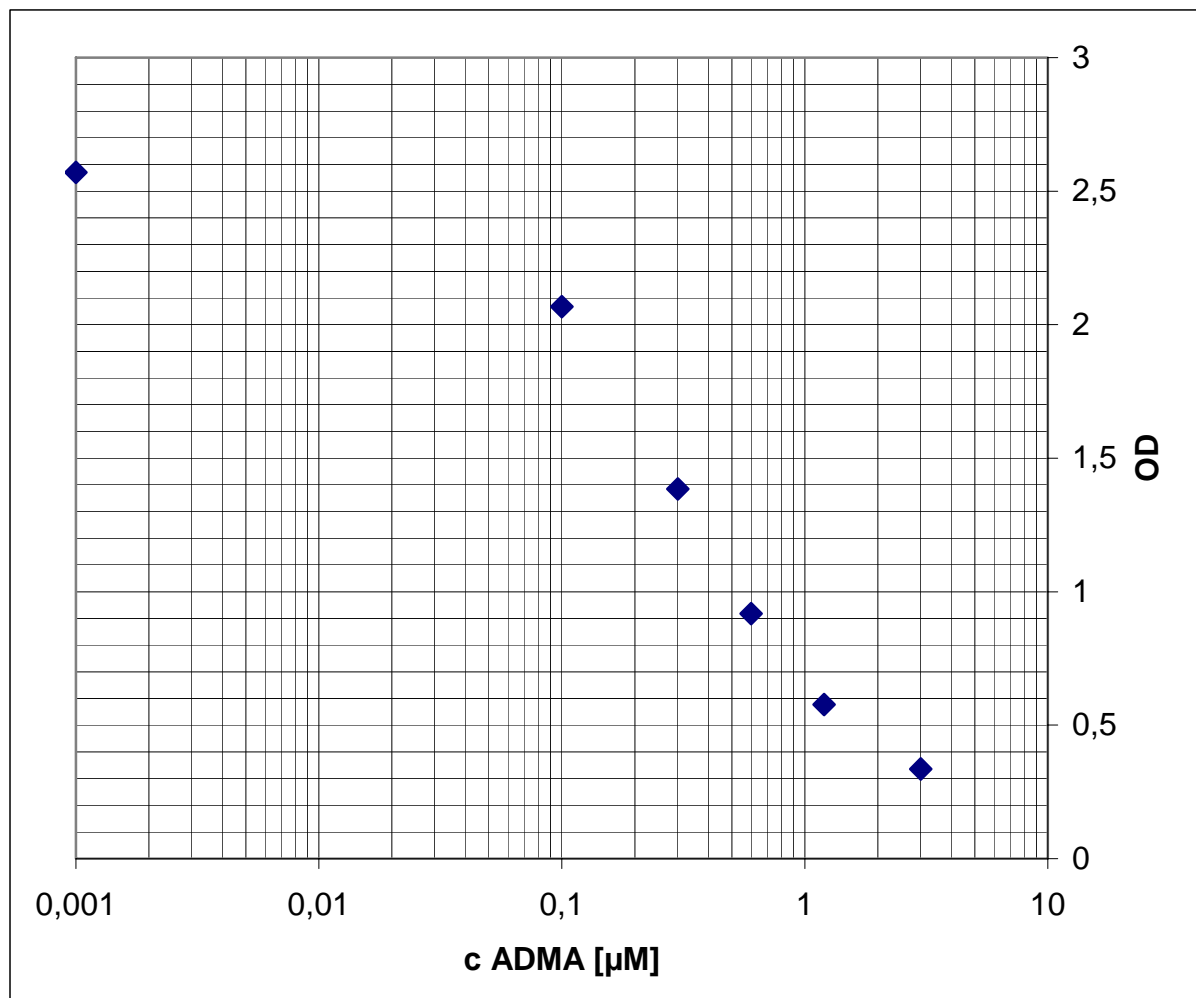
The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done manually.

## Controls

Control samples or serum pools should be analyzed with each run. Results, generated from the analysis of the 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 sample are outside the acceptable limits.

The concentration of controls and patient samples can be determined directly from calibration curve. In the following an example of a calibration curve is given.

## Example of calibration curve



## 11. PERFORMANCE CHARACTERISTICS

### *Cross reactivity*

SDMA &lt; 0,5 %

NMMA &lt; 0,5 %

L-Arginin &lt; 0,02 %

### *Precision and reproducibility*

Intra-Assay (n=6)		
Sample	ADMA [μmol/l]	Standard variation (SD)
1	0,62	0,079
2	1,10	0,115

Inter-Assay (n=6)		
Sample	ADMA [μmol/l]	Standard variation (SD)
1	0,60	0,071
2	1,15	0,105

### *Sensitivity*

The sensitivity was set as  $B_0 + 1SD$ . The zero-standard was measured 6 times.

Sample	ADMA mean value [OD]	Standard variation (SD)	Detection limit [μmol/l]
0	3,00	0,2	0,05

### Recovery

Samples were spiked with 2 different ADMA concentrations and measured using this assay. The analytical recovery rate was determined by the expected and measured ADMA levels. The expected levels were calculated as the sum of the measured ADMA concentration in the original sample and the spiked ADMA amount.

Spike [ $\mu\text{mol/l}$ ]	ADMA expected [ $\mu\text{mol/l}$ ]	ADMA measured [ $\mu\text{mol/l}$ ]	Recovery [%]
0	x	x=0,9	100
0,5	0,5+x=1,4	1,3	92,8
1,0	1,0 +x=1,9	1,6	84,2

### Linearity

The linearity of the ELISA was determined by the dilution of a spiked patient sample. The mean linearity was 115%.

Dilution	Measured [ $\mu\text{M}$ ]	Expected [ $\mu\text{M}$ ]	Recovery %
original	0,95	0,95	100
1+1	0,55	0,475	115
1+2	0,40	0,316	127

## 12. LIMITATIONS

Strong haemolytic and lipaemic samples often show wrong concentrations. Do not to measure haemolytic and lipaemic samples.

### 13. REFERENCES








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## 14. 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.
- Test components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- All reagents in the test package are for in-vitro-diagnostic use only.
- Reagents should not be used after the date of expiry stated on the label.
- Single components with different lot numbers should not be mixed können or exchanged.
- 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. Alexis Corporation can therefore not be held responsible for any damage resulting from wrong use.

21.05.2008

### Used symbols:

	Temperature limitation		Catalogue Number
	For research use only		Contains sufficient for <n> tests
	Manufacturer		Use by
	Lot number		