**Manual**

# ADMA ELISA

***For the determination of ADMA in human serum, citrate and EDTA plasma***



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REF **KR7828**


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# 1. INTENDED USE

This Immundiagnostik assay is intended for the quantitative determination of asymmetric dimethyl-L-arginine (ADMA) in human serum, citrate and EDTA plasma. 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.

During the last years, the important 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 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.



**Possible research areas**

- 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 |
|---|---|---|---|
| KR7828 | PLATE | Microtiter plate, pre-coated | 12 x 8 wells |
| KR7828 | STD | Standards, ready-to-use (0, 0.1, 0.25, 0.5, 1.0, 2.0 µmol/l) | 6 x 1 ml |
| KR7828 | CTRL 1 | Control, ready-to-use (see specification for range) | 1 x 1 ml |
| KR7828 | 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 |
| KR7828 | AB | ADMA antibody, lyophilised | 1 vial |
| KR7828 | CONJ | Conjugate, ready-to-use | 1 x 12 ml |
| KR0012.15 | DERBUF | Reaction buffer, ready-to-use | 1 x 15 ml |
| KR7828 | DER | Derivatisation reagent, lyophilised | 1 x 100 mg |
| KR0008.07 | DMSO | Dimethylsulfoxide (DMSO) | 1 x 7 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 (100 mg) with **6 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

**Serum, citrate and EDTA plasma**

- Venous fasting blood is suited for this test system. 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.

- The serum, citrate and EDTA plasma samples are analysed **undiluted**.

  If the sample volume is less than 50 µl, we recommend a 1:2 dilution in DERBUF (reaction buffer) (25 µl sample + 25 µl DERBUF). This dilution factor must be considered in data evaluation.

- 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 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 **200 µl standard** (STD), **200 µl control** (CTRL) and **50 µl sample** in the corresponding vials. |
| 2. | Add **150 µl reaction buffer** (DERBUF) **only to the samples.** |
| 3. | Add **50 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 **250 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/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.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.



## 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**

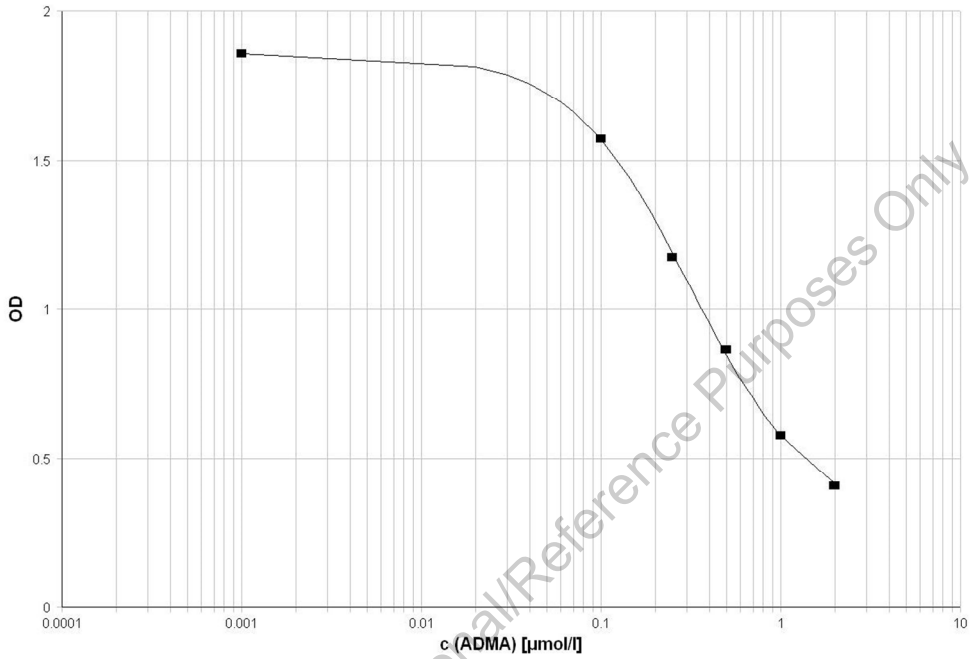
   We recommend a linear ordinate for optical density and a linear abscissa for concentration.

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, citrate and EDTA plasma**

The concentrations can be determined directly from the standard curve in µmol/l. **No factor** is required.
Exception: Consider the dilution factor for 1:2 pre-diluted samples.

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 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.

## 10. QUALITY CONTROL

Immundiagnostik 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 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.

### *Reference range*

We recommend each laboratory to establish its own reference range.

## 11. PERFORMANCE CHARACTERISTICS

### *Precision and reproducibility*

**Intra-assay (n = 12)**

| Sample | ADMA [µmol/l] | CV [%] |
|---|---|---|
| 1 | 0.29 | 7.5 |
| 2 | 0.80 | 6.4 |

**Inter-assay (n = 10)**

| Sample | ADMA [µmol/l] | CV [%] |
|---|---|---|
| 1 | 0.37 | 3.9 |
| 2 | 0.68 | 3.5 |



## 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 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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***Used symbols:***