# ADMA (Asymmetric Dimethylarginine) ELISA Assay Kit

Catalog Number: ADM31-K01
96 Wells
*For Research Use Only (RUO). Not for use in clinical, diagnostic or therapeutic procedures.*
*v. 2.0 (09.18.18)*

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# 1. Introduction and Principle of the Test

The Eagle Biosciences ADMA (Asymmetric Dimethylarginine) ELISA Assay Kit is intended for the quantitative determination of ADMA (Asymmetric Dimethylarginine) in serum or plasma. The ADMA (Asymmetric Dimethylarginine) ELISA Kit is for research use only and not to be used in clinical, therapeutic or diagnostic procedures.

The vascular endothelium plays a central role in the regulation of vascular structure and function, mainly due to the formation of endothelium-derived nitric oxide (NO). NO has been named an "endogenous anti-atherogenic molecule" due to its diverse regulatory functions in vascular homeostasis. NO is formed by the enzyme NO synthetase (NOS) from the amino acid precursor L-arginine. NOS activity can be down-regulated by asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NOS.

The effects of ADMA on NO synthesis and NO-mediated pathophysiological processes have been described in numerous experimental studies. Moreover, elevated ADMA levels in plasma have been found in clinical studies including samples with hypercholesterolemia, hypertension, chronic heart failure, chronic renal failure and other internal disorders. Recent prospective and cross-sectional studies indicated that elevated ADMA levels are a risk factor for future cardiovascular events and total mortality. ADMA may have diagnostic relevance as a novel cardiovascular risk marker.

The competitive ADMA (Asymmetric Dimethylarginine) ELISA Assay Kit uses the microtiter plate format. ADMA is bound to the solid phase of the microtiter plate. ADMA in the samples is acylated and competes with solid phase bound ADMA for a fixed number of rabbit anti-ADMA antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase ADMA is detected by anti-rabbit/peroxidase. The substrate TMB / peroxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase ADMA is inversely proportional to the ADMA concentration of the sample.

# 2. Precautions

- ADMA (Asymmetric Dimethylarginine) ELISA Assay Kit is for research use only and not to be used in clinical, therapeutic or diagnostic procedures.
- Disposable gloves should be used.
- Material of animal origin used in the preparation of the ADMA (Asymmetric Dimethylarginine) ELISA Assay Kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.

## 3. Storage and Stability

- On arrival, store the ADMA (Asymmetric Dimethylarginine) ELISA Assay Kit at 2-8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.
- Do not use components beyond the expiration date shown on the labels.
- Do not mix various lots of the ADMA (Asymmetric Dimethylarginine) ELISA Assay Kit component within an individual assay.

## 4. Contents of the Kit

4.1 **MT-Strips** **STRIPS** 12 strips

8 wells each, break apart precoated with ADMA

4.2 **Standards 1 – 6** **CAL 1 - 6** 6 vials

Each 4 ml, ready for use

Concentrations:

| Standard | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| µmol/l | 0 | 0.2 | 0.45 | 0.7 | 1.0 | 3.0 |

4.3 **Control 1 & 2** **CON 1 & 2** 2 vials

Each 4 ml, ready for use Range: see q.c. certificate

4.4 **Acylation Buffer** **ACYL-BUFF** 1 bottle

3.5 ml, ready for use

4.5 **Acylation Reagent** **ACYL-REAG** 3 vials

lyophilised, dissolve content in 3.0 ml Solvent before use; if required combine the contents of both vials (see also 6.)

4.6 **Antiserum** **AS** 1 vials

7.0 ml, ready for use
Rabbit-anti-N-acyl-ADMA

4.7 **Enzyme Conjugate** **CONJ** 1 vial

13 ml, ready for use
goat anti-rabbit-IgG-peroxidase

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

4.8 **Wash Buffer** **WASH** 1 bottle
20 ml, concentrated
Dilute content with dist. water to 1,000 ml total volume.

4.10 **Stop Solution** **STOP** 1 vial
13 ml, ready for use
Contains 0.3 M sulphuric acid, not corrosive

4.11 **Reaction Plate** **ACYL-PLATE** 1 piece
for acylation

4.12 **Equalizing Reagent** **EQUA-REAG** 1 vial
lyophilzed, dissolve content with 21.0 ml dist. water, dissolve carefully to minimize foam formation(see also 6.)

4.13 **Solvent** **SOLVENT** 1 vial
5 ml, contains acetone/ DMSO
(please note that Solvent reacts with many plastic materials including plastic trays; Solvent does not react with normal pipette tips and with glass devices)

4.14 **Foil** **FOIL**
2 Pieces

**Additional materials and equipment required but not provided:**

- Pipettes (20, 25, 50, 100 and 200 µl)
- Mulitpipette
- Orbital shaker
- Microplate washing device
- Microplate photometer (450 nm)
- Vortex mixer
- Roll mixer

# 5. Sample Collection

## 5.1. Serum and Plasma

- The ADMA (Asymmetric Dimethylarginine) ELISA Assay can be performed with serum as well as with EDTA plasma.

- Hemolytic and lipemic samples should not be used in the ADMA (Asymmetric Dimethylarginine) ELISA Assay Kit.
- The samples can be stored up to 24 hours at 2 - 8 °C. For a longer storage (up to 24 months) the samples must be frozen at -20 °C
- Repeated freezing and thawing should be avoided.

# 6. Preparation of Reagents and Samples

### 6.1. Microtiter strips **STRIPS**

Before opening the packet of strip wells, allow it to stand at 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 **WASH**

Dilute the content with dist. water to a total volume of 1,000 ml. The diluted wash buffer has to be stored at 2 - 8 °C for a maximum of 4 weeks. For storage until exppiry date as give on the label the diluted wash buffer has to be kept frozen at -20°C.

### 6.3. Equalizing Reagent **EQUA-REAG**

Dissolve the content with 21.0 ml dist. water, mix shortly and leave on a roll mixer or orbital shaker for 20 minutes. Handle carefully in order to minimize foam formation. The reconstituted Equalizing Reagent should be stored frozen at -20 °C and is stable for a minimum of 1 year.

### 6.4. Acylation Reagent **ACYL-REAG**

Dissolve the content of one bottle in 3.0 ml Solvent and shake for 10 minutes on a orbital shaker. After use the reagent has to be discarded. The Acylation Reagent has always to be prepared immediately before use and is stable for a maximum of 3 hours. The second and third bottle allows 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 and with glass devices.

**Attention**

Solvent is volatile and the dissolved Acylation Reagent evaporates quickly. Therefore, please do not use a tray with big surface together with a multichannel pipette for pipetting Acylation Reagent. Rather, use an Eppendorf multipipette with a yellow tip (or similar device), fill the syringe directly from the vial with dissolved Acylation Reagent and add well by well.

All other reagents are ready for use.

### 6.5. Preparation of Samples (Acylation)

**The wells of the reaction plate for the acylation can be used only once. Please mark the respective wells before use to avoid repeated use.**

1. Pipette each 20 µl standard 1 - 6, each 20 µl control 1 & 2 and each 20 µl sample into the respective wells of the Reaction Plate.

2. Pipette 20 µl Acylation Buffer into all wells.

3. Pipette 200 µl Equalizing Reagent into all wells.

4. Mix the reaction plate for 10 seconds.

5. Prepare Acylation Reagent just before use and pipette 50 µl prepared Acylation Reagent each into all wells, mix **immediately.**

   Attention
   Solvent is volatile and the dissolved Acylation Reagent evaporates quickly. Therefore, please do not use a tray with big surface together with a multichannel pipette for pipetting Acylation Reagent. Rather, use an Eppendorf multipipette with a yellow tip (or similar device), fill the syringe directly from the vial with dissolved Acylation Reagent and add well by well.

6. Incubate for 20 minutes at room temperature (approx. 20 °C) on an orbital shaker. Do not cover the wells or the plate; leave the plate open on the shaker.

**Take each 25 µl for the ADMA ELISA.**

## 7. Test Procedure ADMA ELISA

Bring all reagents to room temperature and mix them carefully, avoid development of foam.

### 7.1 Sample Incubation

- Pipette each 25 µl prepared Standards 1 to 6, 20 µl prepared controls and 25 µl prepared samples into the respective wells of the coated microtiter strips (duplicates are recommended).
- Pipette each 50 µl Antiserum into all wells and shake shortly on an orbital shaker.
- Cover the plate with adhesive foil and incubate Microtiter Strips for 90 minutes at 20 to 25°C on an orbital shaker with medium frequency.

### 7.2 Washing

Discard or aspirate the contents of the wells and wash thoroughly with each 300µl Wash Buffer (Shake shortly on an orbital shaker). Repeat the washing procedure 4 times. Remove residual liquid by tapping the inverted plate on clean absorbent paper.

### 7.3 Conjugate Incubation

Pipette each 100 µl enzyme conjugate into all wells. Incubate for 30 minutes at room temperature on an orbital shaker at medium frequency.

### 7.4 Washing

Repeat step 7.2.

### 7.5 Substrate Incubation

Pipette each 100 µl Substrate into all wells and incubate for 25 ± 5 minutes at room temperature on an orbital shaker with medium frequency.

### 7.6 Stopping

Pipette each 100 µl Stop Solution into all wells.

### 7.7 Reading

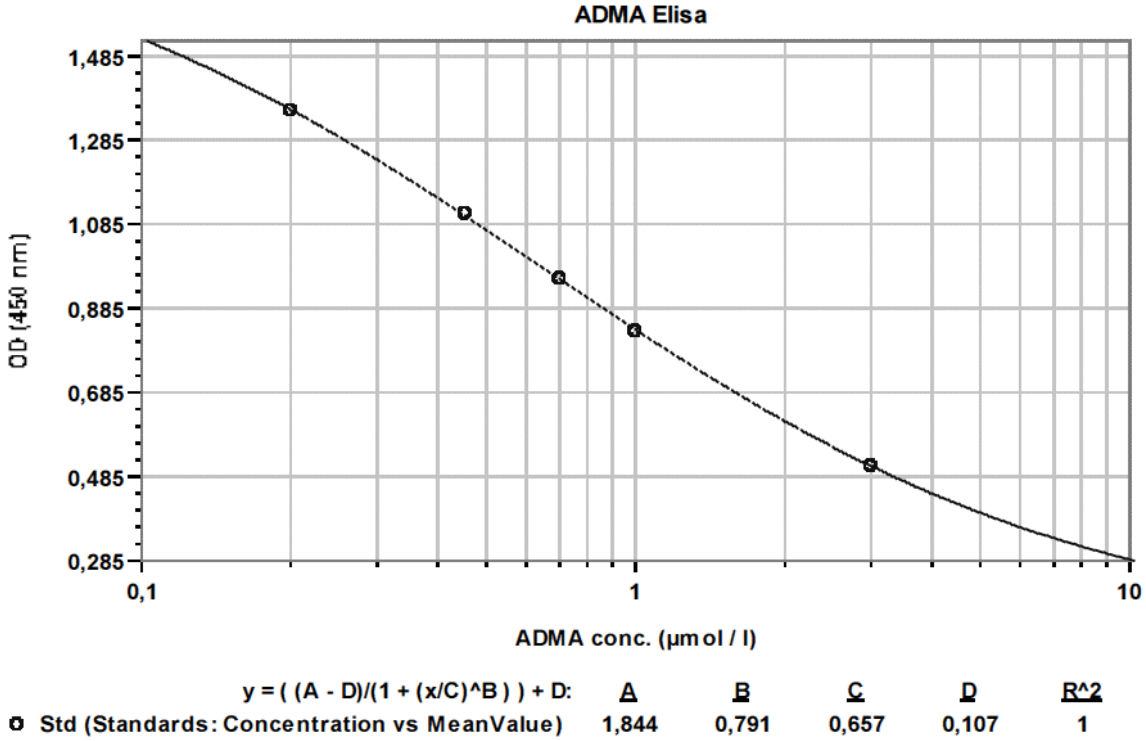
Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

## 8. Calculation of the Results

On a semilogarithmic graph paper the concentration of the standards (x-axis, logarithmic) are plotted against their corresponding optical density (y-axis, linear). Cubic spline, 4 parameter or similar iteration procedures are recommended for evaluation of the standard curve. The concentration of the controls and samples can be read directly from this standard curve by using their average optical density.

Conversion factor: 1 µmol ADMA/ 1=202 ng ADMA/ml

**Typical standard curve:**




# 9. Assay Characteristics

## 9.1 Expected Values

0.4 – 0.75 µmol/l (80 – 150 ng/ml)

The reference ranges given above should only be taken as a guideline. It is recommended that each laboratory should establish its own reference values.

## 9.2 Sensitivity

| Lower Limit of Detection | Calculation |
|---|---|
| 0.03 µmol / l | $OD_{Cal1}$ – 3 x SD |

## 9.3 Specificity (Cross Reactivity)

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

## 9.4 Recovery after Spiking

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

## 9.5 Linearity

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

## 9.6 Reproducibility

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

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

## Literature using this ADMA (Asymmetric Dimethylarginine) ELISA kit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## General Literature

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# Pipetting Scheme
# Sample Preparation

| | Standards | Control | Sample |
|---|---|---|---|
| Standard 1 - 6 | 20 | | |
| Control 1 & 2 | | 20 | |
| Sample | | | 20 |
| Acylation Buffer | 20 | 20 | 20 |
| Equalizing Reagent | 200 | 200 | 200 |

shake for 10 seconds

| | | | |
|---|---|---|---|
| freshly prepared Acylation Reagent µl | 50 | 50 | 50 |

Immediately incubate for 20 minutes at room temperature on an orbital shaker

Take each 25µl of the supernatant for the ELISA

# Pipetting Scheme ELISA

| | | Standard | Control | Sample |
|---|---|---|---|---|
| Standard 1 - 6 | µl | 25 | | |
| Control 1 & 2 | µl | | 25 | |
| Sample | µl | | | 25 |
| Antiserum | µl | 50 | 50 | 50 |

Cover with foil and incubate 90 minutes at room temperature on an orbital shaker.

wash 4 x with each 300 µl Wash Buffer

| | | | | |
|---|---|---|---|---|
| Enzyme Conjugat | µl | 100 | 100 | 100 |

shake for 30 minutes at room temperature

wash 4 x with each 300 µl Wash Buffer

| | | | | |
|---|---|---|---|---|
| Substrate | µl | 100 | 100 | 100 |

shake for 25± 5 minutes at room temperature

| | | | | |
|---|---|---|---|---|
| Stop Solution | µl | 100 | 100 | 100 |

read absorbance at 450 nm

## Warranty Information

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