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. 1.0
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**
8 wells each, break apart precoated with ADMA

4.2 **Standards 1 - 6**
Each 4 ml, ready for use

<table>
<thead>
<tr>
<th>Standard</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>µmol/l</td>
<td>0</td>
<td>0.1</td>
<td>0.3</td>
<td>0.6</td>
<td>1.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

4.3 **Control 1 & 2**
Each 4 ml, ready for use
Range: see q.c. certificate

4.4 **Acylation Buffer**
3.5 ml, ready for use

4.5 **Acylation Reagent**
lyophilised, dissolve content in 2.8 ml Solvent before use; if required combine the contents of both vials (see also 6.4.)

4.6 **Antiserum**
5.5 ml, ready for use
Rabbit-anti-N-acyl-ADMA

4.7 **Enzyme Conjugate**
12 ml, ready for use
goat anti-rabbit-IgG-peroxidase

4.8 **Wash Buffer**
20 ml, concentrated
Dilute content with dist. water to 500 ml total volume.
4.9 **Substrate**
12 ml TMB solution, ready for use

4.10 **Stop Solution**
12 ml, ready for use
Contains 0.3 M sulphuric acid, not corrosive

4.11 **Reaction Plate**
for acylation

4.12 **Equalizing Reagent**
lyophilzed, dissolve content with 20.5 ml dist. water, dissolve carefully to minimize foam formation

4.13 **Solvent**
6 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)

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

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

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**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 500 ml. The diluted wash buffer has to be stored at 2 - 8 °C.

6.3. Equalizing Reagent EQUA-REAG

Dissolve the content with 20.5 ml dist. water, mix shortly and leave on a roll mixer or orbital shaker for 30 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 2.8 ml Solvent and shake for 5 minutes on an orbital shaker. After use the reagent has to be discarded. The Acylation Reagent has always to be prepared immediately before use. The second bottle allows a second 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.

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 multipette 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 25 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 multipette 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 90 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 50 µl for the ADMA-ELISA.

7. Test Procedure ELISA

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

7.1 Sample Incubation

- Pipette each 50 µl prepared Standards 1 to 6, 50 µl prepared controls and 50 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 15 –20 hours (overnight) at 2 – 8 °C.

7.2 Washing
Discard or aspirate the contents of the wells and wash thoroughly with each 250µl 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 60 minutes at room temperature on an orbital shaker.

7.4 **Washing**  
Repeat step 7.2.

7.5 **Substrate Incubation**  
Pipette each 100 µl Substrate into all wells and incubate for 20 to 30 minutes at room temperature on an orbital shaker.

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.

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). The concentration of the controls and samples can be read directly from this standard curve by using their average optical density.

**Typical standard curve:**

![Typical standard curve graph](image)
9. **Assay Characteristics**

**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.

**Sensitivity**

The sensitivity of the ADMA (Asymmetric Dimethylarginine) ELISA was found to be 0.05µmol/l.

**Correlation to LC-MS-MS**

The figure shows the correlation of the ADMA (Asymmetric Dimethylarginine) ELISA kit to the LC-MS-MS method. More data with higher number of samples are evaluated at the moment. Comparative investigations to HPLC without subsequent MS lead to inconsistent results.
Specificity (Cross Reactivity)

Structural related components were tested for possible interference with the antisera against ADMA used in the ADMA (Asymmetric Dimethylarginine) ELISA kit method. The tested compounds were Arginine, Monomethylarginine (NMMA) und SDMA.

<table>
<thead>
<tr>
<th>Substance</th>
<th>ED-50-Value (ng/ml)</th>
<th>Cross Reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADMA</td>
<td>126</td>
<td>100</td>
</tr>
<tr>
<td>Arginine</td>
<td>660,000</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>NMMA</td>
<td>12,200</td>
<td>1.0</td>
</tr>
<tr>
<td>SDMA</td>
<td>10,500</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Recovery

Increasing amounts of ADMA were added to a serum sample. Each spiked sample was assayed. The analytical recovery of ADMA was estimated at twelve different concentrations by using the theoretically expected and the actually measured values. The mean recovery from all concentrations was 97% (90 - 104%).

<table>
<thead>
<tr>
<th>Added µmol/L</th>
<th>Measured µmol/L</th>
<th>Expected µmol/L</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.43</td>
<td>0.53</td>
<td>104</td>
</tr>
<tr>
<td>0.1</td>
<td>0.55</td>
<td>0.62</td>
<td>98</td>
</tr>
<tr>
<td>0.19</td>
<td>0.61</td>
<td>0.71</td>
<td>94</td>
</tr>
<tr>
<td>0.28</td>
<td>0.79</td>
<td>0.80</td>
<td>99</td>
</tr>
<tr>
<td>0.45</td>
<td>0.83</td>
<td>0.88</td>
<td>97</td>
</tr>
<tr>
<td>0.73</td>
<td>1.08</td>
<td>1.16</td>
<td>93</td>
</tr>
<tr>
<td>0.97</td>
<td>1.33</td>
<td>1.40</td>
<td>95</td>
</tr>
<tr>
<td>1.20</td>
<td>1.60</td>
<td>1.63</td>
<td>98</td>
</tr>
<tr>
<td>1.42</td>
<td>1.67</td>
<td>1.85</td>
<td>90</td>
</tr>
<tr>
<td>1.92</td>
<td>2.24</td>
<td>2.35</td>
<td>95</td>
</tr>
<tr>
<td>2.33</td>
<td>2.76</td>
<td>2.76</td>
<td>100</td>
</tr>
<tr>
<td>2.80</td>
<td>3.32</td>
<td>3.23</td>
<td>103</td>
</tr>
</tbody>
</table>

mean value 97
Linearity

The linearity of the ADMA (Asymmetric Dimethylarginine) ELISA method was investigated using nine different dilutions of a serum sample. The mean linearity from all dilutions was 97% (88 - 112%)

<table>
<thead>
<tr>
<th>Dilution µmol/L</th>
<th>measured µmol/L</th>
<th>recalculated value µmol/L</th>
<th>recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>orig.</td>
<td>2.819</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 + 1</td>
<td>2.090</td>
<td>2.787</td>
<td>99</td>
</tr>
<tr>
<td>2 + 1</td>
<td>1.745</td>
<td>2.618</td>
<td>93</td>
</tr>
<tr>
<td>1 + 1</td>
<td>1.236</td>
<td>2.472</td>
<td>88</td>
</tr>
<tr>
<td>1 + 2</td>
<td>0.860</td>
<td>2.580</td>
<td>91</td>
</tr>
<tr>
<td>1 + 4</td>
<td>0.535</td>
<td>2.675</td>
<td>95</td>
</tr>
<tr>
<td>1 + 7</td>
<td>0.361</td>
<td>2.888</td>
<td>102</td>
</tr>
<tr>
<td>1 + 10</td>
<td>0.286</td>
<td>3.146</td>
<td>112</td>
</tr>
<tr>
<td>1 + 15</td>
<td>0.178</td>
<td>2.848</td>
<td>101</td>
</tr>
<tr>
<td>1 + 25</td>
<td>0.104</td>
<td>2.704</td>
<td>96</td>
</tr>
</tbody>
</table>

mean recovery 97

Reproducibility

The reproducibility of the ADMA (Asymmetric Dimethylarginine) ELISA kit method was investigated by determining the intra- and inter-assay-coefficients of variation (cv) by repeated measurements of different serum samples with different ADMA concentrations.

Intra-Assay Variation

<table>
<thead>
<tr>
<th>sample</th>
<th>n =</th>
<th>mean value</th>
<th>sd</th>
<th>cv (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>0.66</td>
<td>0.037</td>
<td>5.7</td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>1.01</td>
<td>0.066</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Inter-Assay Variation

<table>
<thead>
<tr>
<th>sample</th>
<th>n =</th>
<th>mean value</th>
<th>sd</th>
<th>cv (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28</td>
<td>0.63</td>
<td>0.07</td>
<td>10.3</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>1.01</td>
<td>0.10</td>
<td>9.8</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>1.38</td>
<td>0.13</td>
<td>9.4</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>2.26</td>
<td>0.19</td>
<td>8.3</td>
</tr>
</tbody>
</table>
9. Literature

Literature using this ADMA (Asymmetric Dimethylarginine) ELISA kit

Determination of ADMA using a novel ELISA assay.

Krempl TK, Kähler J, Maas R, Silberhorn L, Meinertz T, Böger RH.
Elevation of asymmetric dimethylarginine (ADMA) in patients with unstable angina and recurrent cardiovascular events.

Schulze F, Maas R, Freese R, Schwedhelm E, Silberhorn L, Böger RH.
Determination of a reference value for N,N-dimethyl-L-arginine in 500 subjects.

Asymmetric dimethylarginine and the risk of cardiovascular events and death in patients with coronary artery disease: results from the AtheroGene Study.
Circ. Res. 2005; 97 : e53-59

O’Dwyer MJ, Dempsey F, Crowley V, Kelleher D, McManus R, Ryan T.
Septic shock correlates with ADMA levels which may be influenced by a polymorphism in DDAH II: a prospective observational study.
Crit. Care 2006; 10; (5): R139

Asymmetrical dimethylarginine regulates endothelial function in methionine-induced but not in chronic homocystinemia in humans: effect of oxidative stress and proinflammatory cytokines

Wang TZ, Chen WJ, Cheng WC, Lin JW, Chen MF, Lee YT.
Relation of improvement in endothelium-dependent flowmediated vasodilation after Rosiglitazone to changes in asymmetric dimethylarginine, endothelin-1, and C-reactive protein in nondiabetic patients with the metabolic syndrome
Am. J. Cardiol. 2006; 9: 1057-1062

Wanby P, Nilsson I, Brudin L, Nyhammar I, Gustafsson I, Carlsson M.
Increased plasma levels of asymmetric dimethylarginine in patients with carotid stenosis: no evidence for the role of the common FABBP2 A54T gene polymorphism
Acta Neurol. Scand. 2007; 115: 90-96

Konishi H, Sydow K, Cooke JP.
Dimethylarginine dimethylaminohydrolase promotes endothelial repair after vascular injury

Asymmetric dimethyl-arginine and coronary artery calcification in young adults entering middle age: the CARDIA Study
Melikian N, Wheatcroft SB, Ogah OS, Murphy C, Chowienczyk PJ, Wierzbicki AS, Sanders TA, Jiang B, Duncan ER, Shah AM, Kearney MT.  
**Asymmetric dimethylarginine and reduced nitric oxide bioavailability in young Black African men**  
Hypertension 2007; 49: 873-877

Horowitz JD, Heresztyn T.  
**An overview of plasma concentrations of asymmetric dimethylarginine (ADMA) in health and disease and in clinical studies: Methodological considerations.**  

Korish AA, Arafah MM.  
**Catechin combined with vitamins C and E ameliorates insulin resistance (IR) and atherosclerotic changes in aged rats with chronic renal failure (CRF)**  

Charitidou C, Farmakiotis D, Zournatzii V, Pidionia I, Pegiou T, Karamanis N, Hatzistilianou M, Katsikis I, Panidis D.  
**The administration of estrogens, combined with anti-androgens, has beneficial effects on the hormonal features and asymmetric dimethyl-arginine levels, in women with the polycystic ovary syndrome**  
Atherosclerosis 2007; in press

**General Literature**

Vallance P, Leone A, Calver A, Collier J, Moncada S.  
**Accumulation of an endogenous inhibitor of NO synthesis in chronic renal failure**  
Lancet 1992; 339: 572 - 575

**Relationship between insulin resistance and an endogenous nitric oxide synthase inhibitor**  

**Asymmetric dimethylarginine (ADMA): An endogenous inhibitor of nitric oxide synthase predicts mortality in end-stage renal disease (ESRD)**  
Lancet 2001; 358: 2113-2117

Nijveldt RJ, Teerlink T, Van der Hoven B, Siroen MP, Kuik DJ, Rauwerda JA, van Leeuwen PA.  
**Asymmetrical dimethylarginine (ADMA) in critically ill patients: high plasma ADMA concentration is an independent risk factor of ICU mortality**  

Savvidou MD, Hingorani AD, Tsikas D, Frolich JC, Vallance P, Nicolaides KH.  
**Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia**  
Lancet 2003; 361: 1511-1517

Böger RH.  
**The emerging role of asymmetric dimethylarginine as a novel cardiovascular risk factor**  

Lu TM, Ding YA, Lin SJ, Lee WS, Tai HC.  
**Plasma levels of asymmetrical dimethylarginine and adverse cardiovascular events after percutaneous coronary intervention.**  
Eur Heart J. 2003; 24: 1912-1919
### Pipetting Scheme

#### Sample Preparation

<table>
<thead>
<tr>
<th>Standard 1 - 6</th>
<th>µl</th>
<th>Standards</th>
<th>Control</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1 &amp; 2</td>
<td>µl</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Sample</td>
<td>µl</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Acylation Buffer</td>
<td>µl</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Equalizing Reagent</td>
<td>µl</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

shake for 10 seconds

<table>
<thead>
<tr>
<th>freshly prepared Acylation Reagent</th>
<th>µl</th>
<th>Standards</th>
<th>Control</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

incubate for 90 minutes at room temperature on an orbital shaker
do not cover wells or plate, leave the plate open on the shaker
Pipetting Scheme ELISA

<table>
<thead>
<tr>
<th></th>
<th>Standard</th>
<th>Control</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 1 - 6 µl</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 1 &amp; 2 µl</td>
<td></td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Sample µl</td>
<td></td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>Antiserum µl</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

shake shortly on an orbital shaker

incubate 15 – 20 hours (overnight) at 2 - 8 °C

wash 4 x with each 250 µl Wash Buffer

| Enzyme Conjugat µl | 100 | 100 | 100 |

shake for 60 minutes at room temperature

wash 4 x with each 250 µl Wash Buffer

| Substrate µl | 100 | 100 | 100 |

shake for 20 - 30 minutes at room temperature

| Stop Solution µl | 100 | 100 | 100 |

read absorbance at 450 nm

Warranty Information

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