SDMA (Symmetric Dimethylarginine) ELISA Assay Kit

Catalog Number: SDM31-K01
96 Wells
For Research Use Only (RUO). Not for use in clinical, diagnostic or therapeutic procedures.
v. 2.0 02.19.16
1. Introduction and Principle of the Test

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

Dosing of most drugs must be adapted in renal insufficiency, making accurate assessment of renal function an essential component of diagnostics in clinical medicine. Furthermore, even modest impairment of renal function has been recognized as a cardiovascular risk factor. As the most commonly used marker of renal excretory function, serum creatinine concentration, does not adequately respond to mild to moderate impairment of renal function, more sensitive markers for renal excretory function are urgently sought, especially in mild stages of renal impairment. SDMA is a methylated derivative of the amino acid L-arginine (symmetric dimethylarginine). SDMA is eliminated from the body exclusively by renal excretion; therefore SDMA plasma concentration is tightly related to renal function. Thus, quantification of plasma SDMA is an adequate means to assess renal function, as could be demonstrated in a series of recent clinical trials: In 18 clinical studies involving more than 2,100 samples systemic SDMA concentrations were highly correlated with inulin clearance as well as with various clearance estimates and better corresponded to mild renal function impairment than serum creatinine.

Thus, SDMA exhibits properties of a reliable marker of renal function. Furthermore, there is evidence showing that elevated SDMA levels, as they may occur in renal function impairment, may prospectively indicate future risk of cardiovascular disease and mortality independently of the level of renal impairment.

The competitive SDMA (Symmetric Dimethylarginine) ELISA Assay Kit uses the microtiter plate format. SDMA is bound to the solid phase of the microtiter plate. SDMA in the samples is acylated and competes with solid phase bound SDMA for a fixed number of rabbit anti-SDMA 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 SDMA 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 SDMA is inversely proportional to the SDMA concentration of the sample.
2. **Precautions**

- The SDMA (Symmetric 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 SDMA (Symmetric 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 SDMA (Symmetric Dimethylarginine) ELISA 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 of the SDMA (Symmetric Dimethylarginine) ELISA beyond the expiration date shown on the labels.
- Do not mix various lots of any SDMA (Symmetric 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 SDMA

4.2 **Standards 1 - 6**

   Each 4 ml, ready for use

   Concentrations:

<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>0.2</td>
<td>0.4</td>
<td>0.7</td>
<td>1.2</td>
<td>3.0</td>
</tr>
<tr>
<td>ng/ml</td>
<td>0</td>
<td>40</td>
<td>81</td>
<td>141</td>
<td>242</td>
<td>606</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**

   lyophilized, dissolve content in 3.0 ml Solvent before use
4.6 **Antiserum**  
7.0 ml, ready for use, yellow colored  
Rabbit-anti-N-acyl-SDMA  

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

4.8 **Wash Buffer**  
20 ml, 50x concentrated  
Dilute content with dist. water to 1000 ml total volume.  

4.9 **Substrate**  
13 ml TMB solution, ready for use  

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

4.11 **Reaction Plate**  
for acylation  

4.12 **Equalizing Reagent**  
Lyophilized, dissolve content with 21 ml dist. water,  
dissolve carefully to minimize foam formation  

4.13 **Solvent**  
5 ml contains 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**  
2 pieces  

**Additional materials and equipment required but not provided:**  
- Pipettes (20, 50, 100 and 200 µl)  
- Orbital shaker  
- Microplate washing device  
- Microplate photometer (450 nm)  
- Vortex mixer  
- Roll mixer
5. Sample Collection

5.1. Serum and Plasma

- The SDMA (Symmetric Dimethylarginine) ELISA can be performed with serum as well as with EDTA plasma samples.
- Hemolytic and lipemic samples should not be used.
- The samples can be stored up to 6 hours at 2 - 8 °C. For a longer storage (up to 18 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 1000 ml. The diluted wash buffer must be stored at 2 - 8 °C and is stable for 4 weeks. For longer storage, the diluted wash buffer has to be stored frozen at -20°C.

6.3. Equalizing Reagent **EQUA-REAG**

Dissolve the content with 21 ml dist. water, mix shortly and leave on a roll mixer for 20 minutes. Avoid excess formation of foam. The reconstituted Equalizing Reagent should be stored frozen at -20 °C and is stable until the expiry date.

6.4. Acylation Reagent **ACYL-REAG**

Dissolve the content of one bottle in 3 ml Solvent and shake for 10 minutes on an orbital shaker. After use the reagent has to be discarded. The Acylation Reagent is always to be prepared immediately before use and is stable for at least 3 hours. The other two bottles allows for a second and third run of the test. If the whole kit is used in one run, it is recommended to pool the dissolved contents of the vials of Acylation Reagent.
**Attention**

Please note that Solvent reacts with many plastic materials including plastic trays which are used as reservoir for multichannel pipettes. Solvent does not react with normal pipette tips and with glass devices. It is recommended to use a multipipette, fill it directly from the vial and add the Acylation Reagent to the wells.

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 200 µl Equalizing Reagent into all wells.

3. Mix the reaction plate for 10 seconds.

4. Prepare Acylation Reagent freshly and pipette 50 µl prepared Acylation Reagent each into all wells, mix **immediately**.

   **Attention**
   
   It is recommended to use a multipipette, fill it directly from the vial and add the Acylation Reagent to the wells. Color changes to violet.

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

**Take each 20 µl of the acylated samples for the SDMA (Symmetric Dimethylarginine) 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 20 µl prepared Standards 1 to 6, 20 µl prepared controls and 20 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 room temperature (20 – 25 °C) on an orbital shaker.

7.2 Washing
Discard or aspirate the contents of the wells and wash thoroughly with each 300 µl 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.

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.

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). 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.

Typical standard curve:

![Diagram showing a semilogarithmic graph with concentration of standards on the x-axis and optical density on the y-axis. The graph includes a typical standard curve with corresponding concentration levels marked.](image)
9. **Assay Characteristics**

**Expected Values**

0.3 – 0.75 µmol/l (60 – 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 SDMA (Symmetric Dimethylarginine) ELISA Assay Kit was found to be 0.03 µmol/l.

**Recovery**

<table>
<thead>
<tr>
<th></th>
<th>Range (µmol/l)</th>
<th>Mean (%)</th>
<th>Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA-Plasma</td>
<td>0.43 – 1.44</td>
<td>97</td>
<td>86 – 104</td>
</tr>
<tr>
<td>Serum</td>
<td>0.45 – 1.72</td>
<td>93</td>
<td>88 - 102</td>
</tr>
</tbody>
</table>

**Linearity**

<table>
<thead>
<tr>
<th></th>
<th>Range (µmol/l)</th>
<th>Highest Dil.</th>
<th>Mean (%)</th>
<th>Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA-Plasma</td>
<td>0.23 – 1.72</td>
<td>1 : 6 with Water</td>
<td>97</td>
<td>89 - 105</td>
</tr>
</tbody>
</table>

**Specificity (Cross Reactivity)**

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

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDMA</td>
<td>100</td>
</tr>
<tr>
<td>Arginine</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>NMMA</td>
<td>0.76</td>
</tr>
<tr>
<td>ADMA</td>
<td>0.74</td>
</tr>
<tr>
<td>Homoarginine</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Reproducibility

<table>
<thead>
<tr>
<th></th>
<th>Range (µmol/l)</th>
<th>Intra-Assay CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA-Plasma</td>
<td>0.52 – 0.82</td>
<td>4.9 – 6.2 %</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Range (µmol/l)</th>
<th>Intra-Assay CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA-Plasma</td>
<td>0.52 – 1.21</td>
<td>2.0 – 8.8 %</td>
</tr>
</tbody>
</table>

Method Comparison

<table>
<thead>
<tr>
<th></th>
<th>Method</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum / Plasma</td>
<td>LC/MS</td>
<td>Y = 0.96 x LC/MS + 0.05 R = 0.987; N = 32</td>
</tr>
</tbody>
</table>

9. Literature


### Pipetting Scheme
#### Sample Preparation

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

- Shake for 10 seconds

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

- Incubate for 20 minutes at room temperature on an orbital 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</td>
<td>20 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 1 &amp; 2</td>
<td></td>
<td>20 µl</td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td></td>
<td></td>
<td>20 µl</td>
</tr>
<tr>
<td>Antiserum</td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
</tbody>
</table>

- Shake on an orbital shaker for 90 minutes at room temperature while covered with adhesive foil
- Wash 4 x with each 300 µl Wash Buffer

| Enzyme Conjugate | 100 µl | 100 µl | 100 µl |

- Shake for 30 minutes at room temperature on an orbital shaker
- Wash 4 x with each 300 µl Wash Buffer

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

- Shake for 25 ± 5 minutes at room temperature on an orbital shaker

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

- Read absorbance at 450 nm
Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.