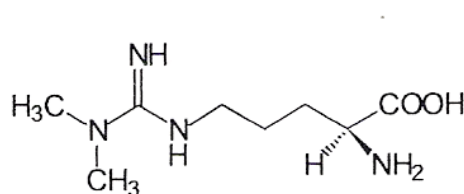


**Asymmetric dimethylarginine (ADMA) in serum and in plasma by Fluorimetry**  
**Code Z58010**

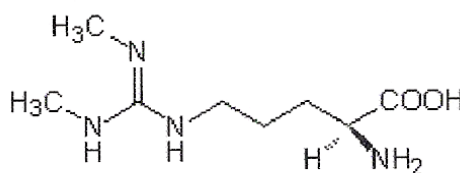
**INTRODUCTION**

Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of nitric oxide synthase (NOS). Elevated ADMA plasma levels have been reported in connection with diseases associated with an impaired endothelial L-arginine-NO pathway and endothelial dysfunction, atherosclerosis, hypercholesterolemia, chronic heart failure, diabetes mellitus and hypertension. These elevated levels, reducing the biosynthesis of nitric oxide, can impair blood flow, blood pressure, platelet adhesion and aggregation, leukocyte adhesion to vessel wall and increase endothelial oxidative stress.

ADMA is analysed in HPLC after plasma purification and derivatization.



(ADMA)



(SDMA)



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This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.

|       |                                               |         |
|-------|-----------------------------------------------|---------|
| N° 10 | ADMA in serum and/or in plasma by Fluorimetry | 09/2024 |
|-------|-----------------------------------------------|---------|

## **TECHNICAL FEATURES**

### **Principle of the Method :**

This method allows to analyse the asymmetric dimethylarginine (ADMA) with Clean-up columns procedure and following derivatization. Besides this method allows to analyse also the Arginine and symmetric dimethylarginine (SDMA).

After derivatization, 100 µl of solution is injected in HPLC system and analysed by Fluorescence.

**Recovery :** > 98 %

**Sensitivity :** < 0,05 µmol/l ADMA

**Linearity :** > 16,00 µmol/l ADMA

**Acceptable values in plasma:** < 0,38 – 1,3 µmol/l ADMA

*Ref. Analytical Biochemistry*

### **Components of the kit :**

All the components are ready-to-use. Stability: 3 years at 2 – 8 °C.

**Reagent A** – Conditioning Solution N° 1, 1 x 100 ml

**Reagent B** – Conditioning Solution N° 2, 1 x 100 ml

**Reagent C** – Conditioning Solution N° 3, 1 x 100 ml

**Reagent D** – Dilution Solution, 1 x 100 ml

**Reagent E** – Wash Solution N° 1, 1 x 100 ml

**Reagent F** – Wash Solution N° 2, 1 x 100 ml

**Reagent I** – Eluting Solution, 1 x 100 ml

**Reagent J** – Starter Solution, 1 x 5 ml

**Reagent L** – Derivatization Solution, 1 x 4,6 mg

(To reconstitute with 5 ml of Acetonitrile – not included in the kit)

**Reagent N** – Buffer Solution N° 1, 1 x 20 ml

**Reagent O** – Buffer Solution N° 2, 1 x 15 ml

**Reagent M** – Mobile Phase, 8 x 500 ml

**Reagent P** – Test solution ADMA, 1 x 5 ml

**See Warnings**

**Reagent Q** – Test solution SDMA, 1 x 5 ml

**See Warnings**

**Reagent R** – Test Solution Arginine, 1 x 5 ml

**See Warnings**

**Plasma Calibrator Iyophil., 1 x 5 ml**

**Code Z58016 (packed separately – see data sheets)**

**Clean-up Columns – 100 pcs**

### **Plasma Collection Procedure:**

Collect 2 ml of plasma in a suitable Heparin test tube. Delayed analyses require sample freezing at – 20 °C. Stable over 4 weeks.

## **PREANALYTICAL PROCEDURE**

**STEP 1:** Pipette in a plastic or glass tube of 1,5 ml with cap:

- 200 µl of **Test solution (Reag. P, Q or R)**
- 200 µl of **Reagent N** – Buffer Solution N° 1
- 150 µl of **Reagent O** – Buffer Solution N° 2
- 30 µl of **Reagent J** – Starter Solution
- 30 µl of **Reagent L** – Derivatization Solution (after reconstitution)

**Vortex for 5 seconds**

**STEP 2:** Incubate at 20 °C (Room Temperature) for 30 minutes

**STEP 3:** Add 150 µl of **H<sub>2</sub>O HPLC grade**

### **INJECTION :**

- Inject 100 µl of this solution in the chromatographic system.

Verify that the Test Solution has retention time similar to fig. 1. If the Test is all right you can start with the analytical procedure; if not, check the functionality of the analytical system

***Important :*** Don't use this solution to calibrate!

## **CONDITIONING AND ACTIVATION OF CLEAN-UP COLUMNS**

Place Clean-up columns on plastic tube of 10 ml to collect eluate and then eliminate it.

- 1 Condition clean up columns by fluxing 1,0 ml of **Reagent A** – Conditioning Solution 1

**Discharge the percolated liquid**

- 2 Condition clean up columns by fluxing 1,0 ml of **Reagent B** – Conditioning Solution 2

**Discharge the percolated liquid**

- 3 Condition clean up columns by fluxing 1,0 ml of **Reagent C** – Conditioning Solution 3

**Discharge the percolated liquid**

## **ANALYTICAL PROCEDURE**

### **STEP 1 : Preparation of Samples and Calibrator**

- In a plastic tube of 10 ml dilute Chemical Standard Calibrator and Samples according to this scheme:

|                                      | <b>Calibrator</b> | <b>Samples</b> |
|--------------------------------------|-------------------|----------------|
| <b>Reagent D</b> – Dilution Solution | 1000 µl           | 1000 µl        |
| <b>Calibrator</b>                    | 400 µl            | /              |
| <b>Samples</b>                       | /                 | 400 µl         |

**Vortex for 5 seconds**

### **STEP 2 : Loading of Calibrator and Samples in Clean-up columns**

- Dispense **1,4 ml** (in 2 steps) of Calibrator and Samples previously prepared in the respective Clean-up columns.
- Let percolate solution through clean up column with a **flow of 1,0 ml/min** (drop by drop) also with the help of a moderate vacuum.

**Discharge the percolated liquid**

**STEP 3 : Cleaning of Samples through Clean up columns**

Pipette in Clean up columns in sequence:

- 1,0 ml of **Reagent E – Wash Solution N° 1**
- 1,0 ml of **Reagent F – Wash Solution N° 2**

**Discharge the percolated liquid**  
**Discharge the percolated liquid**  
**and let dry!**

**STEP 4 : Elution by Clean-up columns**

Place Clean-up columns in clean tubes. Pipette in Clean-up columns:

- 1,0 ml of **Reagent I – Eluting Solution** and collect eluate.

**Warning! Elute slowly for about 1 minute and let dry !**

**Vortex for 5 seconds**

**STEP 5 : Procedure of derivatization**

Pipette in a plastic or glass tube of 1,5 ml with cap :

- 200 µl of **ELUATE** from purification procedure
- 200 µl of **Reagent N – Buffer Solution N° 1**
- 150 µl of **Reagent O – Buffer Solution N° 2**
- 30 µl of **Reagent J – Starter Solution**
- 30 µl of **Reagent L – Derivatization Solution (after reconstitution)**

**Vortex for 5 seconds**

**STEP 6 :** Incubate at 20 °C for 30 minutes.

**STEP 7 :** Add 150 µl of **H<sub>2</sub>O HPLC grade**

**Vortex for 5 seconds**

**N.B.: at this step, the sample is stable 24 h at 2-8 °C**

**INJECTION :**

- Inject 100 µl of this solution in the chromatographic system.
- To determinate the Arginine, dilute the sample ( after **STEP 7** ) 1:20 with H<sub>2</sub>O HPLC grade and inject 100 µl of this solution in the chromatographic system.

### **ADMA in serum and plasma - Warnings**

#### **REAGENT P : TEST SOLUTION ADMA**

|      |          |
|------|----------|
| ADMA | 5 µmol/l |
|------|----------|

#### **REAGENT Q : TEST SOLUTION SDMA**

|      |          |
|------|----------|
| SDMA | 5 µmol/l |
|------|----------|

#### **REAGENT R : TEST SOLUTION ARGININE**

|          |          |
|----------|----------|
| ARGININE | 5 µmol/l |
|----------|----------|

#### **PARAMETERS OF FLUORIMETRIC DETECTOR**

|                  |        |
|------------------|--------|
| λ of EXCITATION  | 420 nm |
| λ of EMISSION    | 483 nm |
| VOLTAGE          | Medium |
| INTEGRATION TIME | 5 sec  |

#### **HPLC COLUMN PROTECTION**

To save the analytical Phenyl column SPHERISORB 250 mm x 4,6 mm, 5 µm, the use of Guard Column Cartridge Holder/PRECOLUMN CARTRIDGE, cod. ZPSS830008 and of PRECOLUMN PREFILTERS (1 x 10 pcs.) cod. ZPSS830078 is obligatory.

#### **HPLC COLUMN CONDITIONING**

Install a new analytical Phenyl column (SPHERISORB 250 mm x 4,6 mm, 5 µ). Disconnect the detector and flux 30 ml of H<sub>2</sub>O : Acetonitrile ( 50 : 50 v/v ) solution and subsequently 30 ml of H<sub>2</sub>O per HPLC, set flow at 1,0 ml / min. **Don't recycle the washing solutions.** Filter the mobile phase with a vacuum system and a suitable filter of 0,22 µ. Condition the column with the mobile phase at a flow of 1,0 ml / min and discharge the first 30 ml. Condition further on the column for 30 min also at recycling phase. Finally inject the derivatized Chemical Standard and verify the quality of the HPLC run.

**It is NOT possible to make analysis at recycling phase.**

#### **COLUMN CLEANING**

Disconnect the detector. Flux 30 ml of water and discharge. Flux a solution of H<sub>2</sub>O HPLC grade : Acetonitrile (40 : 60 v/v) for 30 min. Discharge. A new use of the column needs a flow of 30 ml of H<sub>2</sub>O before a new conditioning with the mobile phase.

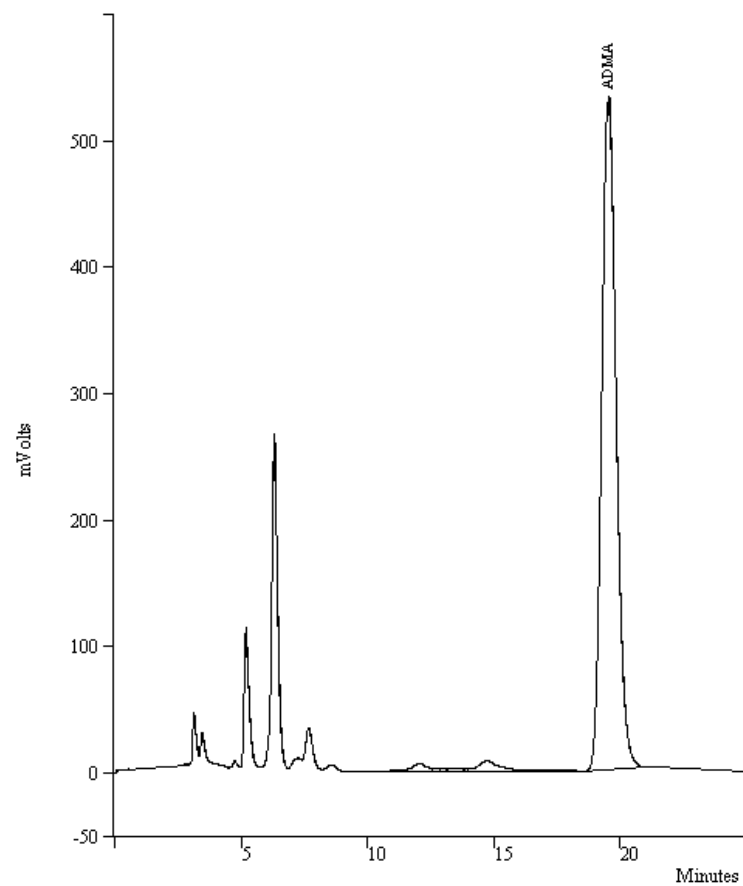
#### **HPLC PARAMETERS**

|          |               |
|----------|---------------|
| LOOP     | 100 µl        |
| FLOW     | 1,0 ml/min.   |
| PRESSURE | About 120 bar |

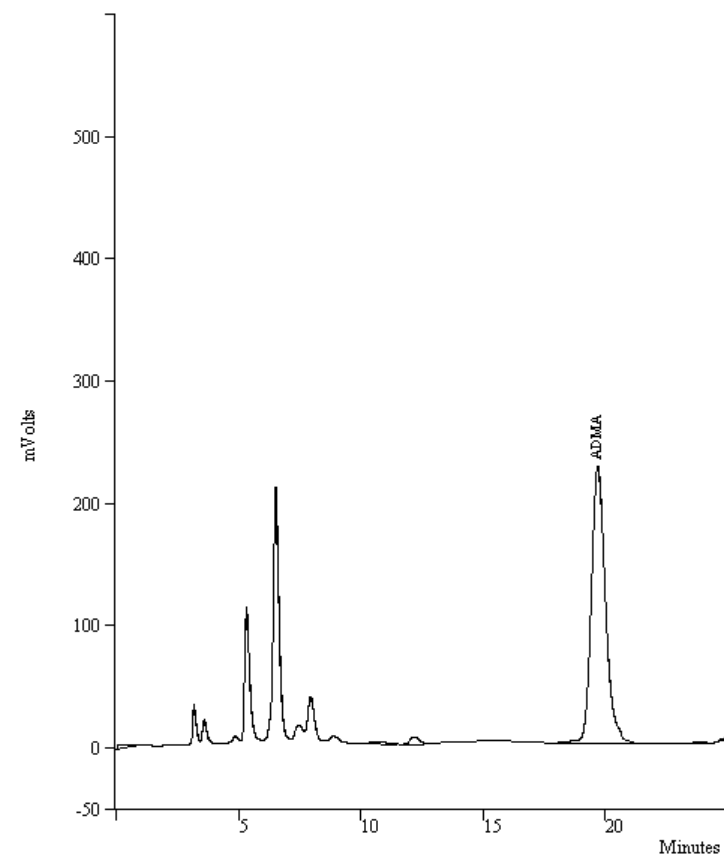
#### **ACCESSORIES AND CONSUMABLES**

| CODE       | DESCRIPTION                                                     | PACKAGING    |
|------------|-----------------------------------------------------------------|--------------|
| Z58016     | Calibrator lyophil. in plasma for ADMA, SDMA, Arginine          | 4 x 5 ml     |
| Z58019     | Control lyophil. in plasma for ADMA, SDMA, Arginine, Levels 1,2 | 2 x 5 x 5 ml |
| ZPSS830815 | Phenyl Spherisorb (250 x 4,6mm -5 um) Analytical Column         | 1 Pc         |
| ZPSS830008 | In Line Guard Cart Holder kit                                   | 1 Pc         |
| ZPSS830078 | Precolumn phenyl prefilters Spher (4,6 mm x 1 cm – 5 µ) Guard   | 1 x 3 Pcs    |
| S29057U    | Standard glass vials of 2 ml with screw cap                     | 1 x 100 Pcs  |

# ADMA in serum and/or in plasma ( Reference Chromatograms )

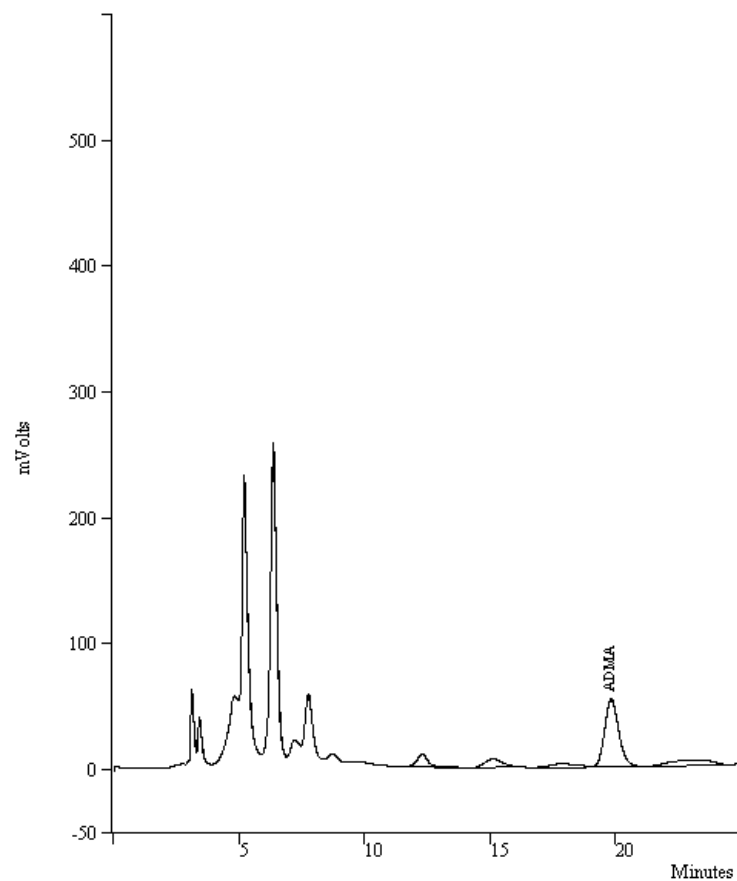


|               |                      |
|---------------|----------------------|
| <b>Fig. 1</b> | <b>Test Solution</b> |
|               | R.T. 19.5 ADMA       |

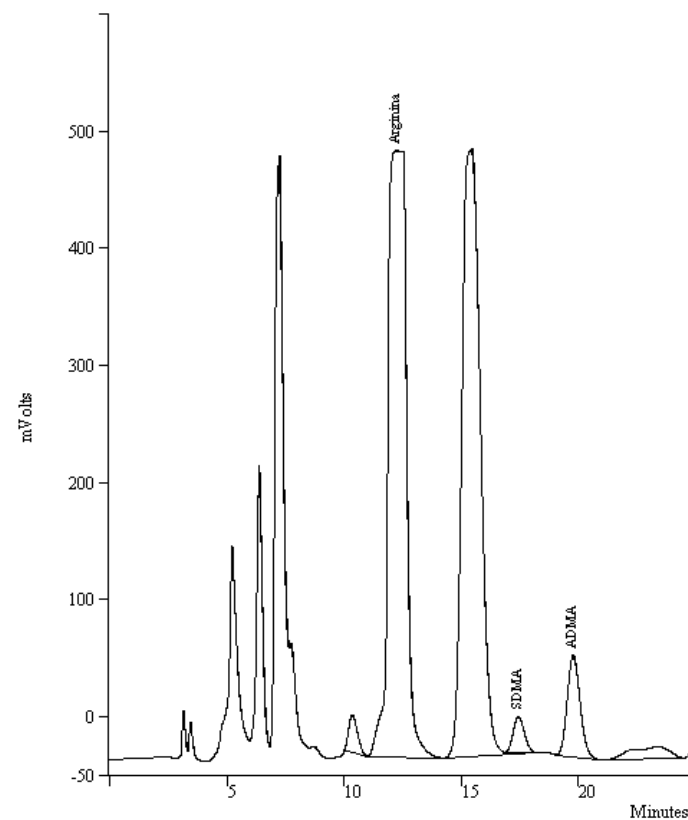


|               |                           |
|---------------|---------------------------|
| <b>Fig. 2</b> | <b>Chemical Standard</b>  |
|               | R.T. 19.6 ADMA 5,0 µmol/l |

# ADMA in serum and/or in plasma ( Reference Chromatograms )



|               |                                      |
|---------------|--------------------------------------|
| <b>Fig. 3</b> | <b>Calibration Standard</b>          |
|               | R.T. 19.8 ADMA 1,0 $\mu\text{mol/l}$ |



|               |                                       |
|---------------|---------------------------------------|
| <b>Fig. 4</b> | <b>Enriched Plasma Sample</b>         |
|               | R.T. 19.7 ADMA 1,38 $\mu\text{mol/l}$ |