



**IMMUNOASSAYS AND SERVICES**

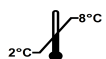
**BIOGENIC AMINES & NEUROSCIENCE | ENDOCRINOLOGY | FOOD SAFETY**

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**Instructions for use**  
**2-MET Plasma ELISA** **Fast Track**

**REF**

**BA E-8300R**



**RUO**

For research  
use only –  
Not for use  
in diagnostic  
procedures

## 2-MET Plasma ELISA Fast Track

### 1. Introduction

#### 1.1 Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of free Metanephrine and free Normetanephrine in plasma.

**Related Products:**

- Metanephrine Plasma ELISA Fast Track
- Normetanephrine Plasma ELISA Fast Track
- 2-MET Plasma RIA Fast Track

Metanephrine (Metadrenaline) and Normetanephrine (Normetadrenaline) are first extracted using an ion exchange matrix followed by an acylation process.

The subsequent competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated standards, controls and samples compete with the solid phase bound analytes for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm.

Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standards.



*The antibodies used in this test kit only recognise the biologically relevant L-forms of Metanephrines. Commercially available synthetic Normetanephrine or Metanephrine is always a mixture of the D- and L-form. The ratio between both forms differs widely from lot to lot. This has important implications if synthetic Metanephrines are used to enrich native samples. As only about 50% of the synthetic Metanephrines - the L-portion - will be detected by use of this kit, spiked samples will be underestimated. Therefore native samples containing solely the L-form should be used.*

#### 1.2 Background

Metanephrine and Normetanephrine are the metabolites of the catecholamines Epinephrine and Norepinephrine, respectively. Cells derived from neuroendocrine tumors (e.g. pheochromocytoma) are known to produce catecholamines, which are secreted episodically via vesicles into the blood stream. But beside this, a small portion of the catecholamines is metabolized inside the cells to the corresponding catecholamines metabolites – namely Metanephrine, Normetanephrine and 3-Methoxytyramine – which are secreted at low levels continuously into the blood stream.

## 2. Procedural cautions, guidelines, warnings and limitations

### 2.1 Procedural cautions, guidelines and warnings

- (1) This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- (2) Reagents of this kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- (3) The principles of Good Laboratory Practice (GLP) have to be followed.
- (4) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
- (5) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- (6) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
- (7) The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant and used in the frame provided. Microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up.
- (8) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
- (9) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- (10) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
- (11) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
- (12) A standard curve must be established for each run.

- (13) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
- (14) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
- (15) Avoid contact with Stop Solution containing 0.25 M H<sub>2</sub>SO<sub>4</sub>. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
- (16) TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them.
- (17) For information on hazardous substances included in the kit please refer to Safety Data Sheet (SDS). The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
- (18) Kit reagents must be regarded as hazardous waste and disposed according to national regulations.

## 2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.

### 2.2.1 Interfering substances

Samples containing precipitates or fibrin strands might cause inaccurate results. Hemolytic samples (up to 0.5 mg/ml hemoglobin), icteric samples (up to 25 mg/dl bilirubin) and lipemic samples (up to 1700 mg/dl triglycerides) have no influence on the assay results.

### 2.2.2 Drug interferences

Please refer to point "Sample collection and storage".

### 2.2.3 High-Dose-Hook effect

No hook effect was observed in this test.

## 3. Storage and stability

Store the unopened reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened, the reagents are stable for 1 month when stored at 2 - 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

## 4. Materials

### 4.1 Content of the kit

**BA D-0090**    FOILS                      **Adhesive Foil** - Ready to use

Content:        Adhesive Foils in a resealable pouch

Volume:        2 x 4 foils

**BA E-0030**    WASH-CONC 50x                      **Wash Buffer Concentrate** - Concentrated 50x

Content:        Buffer with a non-ionic detergent and physiological pH

Volume:        2 x 20 ml/vial, light purple cap

**BA E-0040**    CONJUGATE                              **Enzyme Conjugate** - Ready to use

Content:        Goat anti-rabbit immunoglobulins conjugated with peroxidase


Volume:        2 x 12 ml/vial, red cap

**BA E-0055**    SUBSTRATE                              **Substrate** - Ready to use

Content:        Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide

Volume:        2 x 12 ml/vial, black cap

**BA E-0080** STOP-SOLN **Stop Solution** - Ready to use

Content: 0.25 M sulfuric acid  
 Volume: 2 x 12 ml/vial, light grey cap  
 Hazards identification: 

H290 May be corrosive to metals.

**BA E-0131** ADR MN **Metanephrine Microtiter Strips** - Ready to use

Content: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable blue pouch with desiccant

**BA E-0231** NAD NMN **Normetanephrine Microtiter Strips** - Ready to use

Content: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable yellow pouch with desiccant

**BA E-8110** MN-AS **Metanephrine Antiserum** - Ready to use

Content: Rabbit anti- Metanephrine antibody, blue coloured  
 Volume: 1 x 6 ml/vial, blue cap

**BA E-8210** NMN-AS **Normetanephrine Antiserum** - Ready to use

Content: Rabbit anti- Normetanephrine antibody, yellow coloured  
 Volume: 1 x 6 ml/vial, yellow cap

**Standards and Controls** - Ready to use

Cat. no.	Component	Colour/Cap	Concentration pg/ml		Concentration pmol/l		Volume/ Vial
			<span style="border: 1px solid black; padding: 0 2px;">MN</span>	<span style="border: 1px solid black; padding: 0 2px;">NMN</span>	<span style="border: 1px solid black; padding: 0 2px;">MN</span>	<span style="border: 1px solid black; padding: 0 2px;">NMN</span>	
<b>BA E-8301</b>	<span style="border: 1px solid black; padding: 0 2px;">STANDARD</span> A	white	0	0	0	0	4 ml
<b>BA E-8302</b>	<span style="border: 1px solid black; padding: 0 2px;">STANDARD</span> B	light yellow	36	72	183	393	4 ml
<b>BA E-8303</b>	<span style="border: 1px solid black; padding: 0 2px;">STANDARD</span> C	orange	120	240	608	1 310	4 ml
<b>BA E-8304</b>	<span style="border: 1px solid black; padding: 0 2px;">STANDARD</span> D	dark blue	360	720	1 825	3 931	4 ml
<b>BA E-8305</b>	<span style="border: 1px solid black; padding: 0 2px;">STANDARD</span> E	light grey	1 200	2 400	6 084	13 104	4 ml
<b>BA E-8306</b>	<span style="border: 1px solid black; padding: 0 2px;">STANDARD</span> F	black	3 600	7 200	18 252	39 312	4 ml
<b>BA E-8351</b>	<span style="border: 1px solid black; padding: 0 2px;">CONTROL</span> 1	light green	Refer to QC-Report for expected value				4 ml
<b>BA E-8352</b>	<span style="border: 1px solid black; padding: 0 2px;">CONTROL</span> 2	dark red	and acceptable range!				4 ml

Conversion: Metanephrine (pg/ml) x 5.07 = Metanephrine (pmol/l)  
 Normetanephrine (pg/ml) x 5.46 = Normetanephrine (pmol/l)

Content: Acidic buffer with non-mercury stabilizer, spiked with a defined quantity of Metanephrine and Normetanephrine

**BA E-8327** ADJUST-BUFF **Adjustment Buffer** - Ready to use


Content: Tris-Buffer  
 Volume: 1 x 10 ml/vial, yellow cap

**BA R-8313** ASSAY-BUFF **Assay Buffer** - Ready to use

Content: 25% organic solvent  
 Volume: 1 x 30 ml/vial, orange cap

**BA R-8312** ACYL-CONC **Acylation Concentrate** - Concentrated

Content: Acylation reagent in DMSO  
 Volume: 1 x 1.5 ml/vial, dark grey cap

Hazards identification: 

H302 Harmful if swallowed.

- BA R-8318**    **EXTRACT-PLATE 96**    **Extraction Plate** - Ready to use  
 Content:        1 x 96 well plate, precoated with ion-exchanger in a resealable pouch
- BA R-8325**    **CLEAN-CONC 25x**    **Cleaning Concentrate** - Concentrated 25x  
 Content:        Buffer with sodium acetate  
 Volume:        1 x 20 ml/vial, brown cap
- BA R-8326**    **ELUTION-BUFF**        **Elution Buffer** - Ready to use  
 Content:        0.1 M Sodium hydroxide, dark purple coloured  
 Volume:        1 x 14 ml/vial, dark green cap
- BA R-8828**    **EQUA-REAG**         **Equalizing-Reagent** - Ready to use  
 Content:        Human serum, negative for HIV I/II, HBsAg and HCV  
 Volume:        1 x 14 ml/vial, white cap

#### 4.2 Additional materials and equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 20 - 350 µl; 3 ml
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 - 650 nm
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)
- Vortex mixer

#### 5. Sample collection and storage

Medications like Serotonin-noradrenaline reuptake inhibitors, tricyclic antidepressants, MAO inhibitors, antihypertensive drugs and L-DOPA can influence Metanephrine and Normetanephrine level. People who are taking such medication should consult with their doctor before specimen collection. Sympathomimetic agents, sport and smoking can also influence Metanephrine and Normetanephrine level. Alcohol and caffeinated drinks should be avoided the day before and including the day of sample collection.

##### **EDTA- or Heparin-Plasma**

Whole blood should be collected into centrifuge tubes (Monovette™ or Vacuette™) containing EDTA or heparin as anti-coagulant and centrifuged (according to manufacturer's instructions) immediately after collection.

Haemolytic, icteric and lipemic samples should not be used for the assay.

Storage: up to 6 hours at 2 - 8 °C, for longer period (up to 6 months) at -20 °C.


Repeated freezing and thawing should be avoided.

#### 6. Test procedure

The ELISA can be run using an overnight incubation without shaking (results within approx. 24 hours) or alternatively as a fast version with shortened antiserum incubation times with shaking (results within approx. 6 hours).

Allow all reagents to reach room temperature and mix thoroughly by gentle inversion before use. Number the Extraction Plate and microwell plates (microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up). Duplicate determinations are recommended.

The binding of the antibodies, the enzyme conjugates, and the activity of the enzyme used are temperature dependent, the absorption values may vary if a thermostat is not used. The higher the temperature, the higher the absorption values will be. The absorption values also depend on the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20 - 25 °C.

 *In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm.*

#### 6.1 Preparation of reagents

##### **Wash Buffer**

Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 ml.

Storage: 1 month at 2 - 8 °C

### Cleaning Buffer

Dilute the 20 ml Cleaning Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 500 ml.

Storage: 1 month at 2 – 8 °C

### Acylation Solution

As the Acylation Solution is only **stable for a maximum of 3 minutes**, it should not be prepared before starting the assay. Therefore its preparation is described in the protocol in chapter 6.3, step 3 and chapter 6.4, step 3.

Discard after use!

### Metanephrine Microtiter Strips and Normetanephrine Microtiter Strips

In rare cases residues of the blocking and stabilizing reagent can be seen in the wells as small, white dots or lines. These residues do not influence the quality of the product.

### Extraction Plate

In rare cases residues of the cation exchanger can be seen in the wells as small, black dots or lines. These residues do not influence the quality of the product.

## 6.2 Preparation of samples


 *The extraction procedure is the same for Metanephrine and Normetanephrine and has to be done only once.*

### Extraction

The following extraction procedure can be run with 200 µl or 250 µl of plasma sample.

The procedure for 250 µl plasma is highlighted in grey and italicised and may be used in case higher supernatant volumes for pipetting to the subsequent ELISA are preferred.

The ELISA procedure itself is not affected by this alternative protocol.

<b>1.</b> Pipette <b>20 µl</b> of <b>standards</b> and <b>controls</b> into the respective wells of the <b>Extraction Plate</b> . <b><i>Alternatively pipette 25 µl of standards and controls into the respective wells of the Extraction Plate.</i></b>		
<b>2.</b> Add <b>20 µl Standard A</b> to all wells containing <b>plasma samples</b> . <b><i>Alternatively add 25 µl Standard A to all wells containing plasma samples.</i></b>		
<b>3.</b> Add <b>200 µl</b> of <b>Equalizing Reagent</b> to the wells with <b>standards and controls</b> . <b><i>Alternatively add 250 µl of Equalizing Reagent to the wells with standards and controls.</i></b>		
<b>4.</b> Pipette <b>200 µl</b> of <b>plasma samples</b> to the respective wells. <b><i>Alternatively pipette 250 µl of plasma samples to the respective wells.</i></b>		
<b>5.</b> Incubate plate <b>for 2 hours</b> at <b>RT</b> (20 – 25 °C) on a <b>shaker</b> (approx. 600 rpm).		
<b>6.</b> Empty plate and blot dry by tapping the inverted plate on absorbent material.		
<b>7.</b> Pipette <b>250 µl</b> of <b>Assay Buffer</b> into all wells. Incubate the plate for <b>5 min</b> at <b>RT</b> (20 – 25 °C) on a <b>shaker</b> (approx. 600 rpm). Empty plate and blot dry by tapping the inverted plate on absorbent material.		
<b>8.</b> Wash the plate <b>3 x</b> by adding <b>350 µl</b> of <b>Cleaning Buffer</b> , <b>discarding</b> the content and <b>blotting dry each time</b> by tapping the inverted plate on absorbent material.		
<b>9.</b> Pipette <b>100 µl</b> of <b>Elution Buffer</b> into all wells. <b><i>Alternatively pipette 125 µl of Elution Buffer into all wells.</i></b> <i>Please note: the colour changes caused by the elution buffer can vary between standards and samples.</i>		
<b>10.</b> Cover plate with adhesive foil. Incubate <b>15 min</b> at <b>RT</b> (20 - 25 °C) on a <b>shaker</b> (approx. 600 rpm). Remove the foil.  <b><i>Do not decant the supernatant thereafter!</i></b> The following volumes of the supernatant are needed for the subsequent ELISA: <table border="1" data-bbox="188 1848 1295 1883"><tr><td><b>Metanephrine 50 µl</b></td><td><b>Normetanephrine 25 µl</b></td></tr></table>	<b>Metanephrine 50 µl</b>	<b>Normetanephrine 25 µl</b>
<b>Metanephrine 50 µl</b>	<b>Normetanephrine 25 µl</b>	

### 6.3 Metanephrine ELISA

1.	Pipette <b>25 µl</b> of <b>Adjustment Buffer</b> into all wells of the <b>Metanephrine Microtiter Strips</b> .
2.	Pipette <b>50 µl</b> of the extracted <b>standards, controls and samples</b> into the respective wells. <i>Please hold the Extraction Plate at a slight angle in order to facilitate this pipetting step.</i>
3.	Preparation of <b>Acylation Solution</b> : Pipette <b>80 µl Acylation Reagent Concentrate</b> (BA R-8312) to <b>3 ml water</b> (deionized, distilled, or ultra-pure) and mix thoroughly.
4.	Pipette <b>25 µl</b> of the freshly prepared <b>Acylation Solution</b> into all wells.
5.	Incubate for <b>15 min</b> at <b>RT</b> (20 – 25 °C) on a <b>shaker</b> (approx. 600 rpm).
6.	Pipette <b>50 µl</b> of the <b>Metanephrine Antiserum</b> into all wells.
7.	Cover the plate with <b>Adhesive Foil</b> , shake for <b>1 min</b> at <b>RT</b> (20 – 25 °C) on a <b>shaker</b> and incubate for <b>15 - 20 h</b> (overnight) at <b>2 – 8 °C</b> without shaking. <b>Alternatively incubate for 2 h at RT</b> (20 - 25 °C) on a <b>shaker</b> (approx. 600 rpm).
8.	Remove the foil. Discard or aspirate the contents of the wells. Wash the plate <b>4 x</b> by adding <b>300 µl</b> of <b>Wash Buffer</b> , <b>discarding</b> the content and <b>blotting dry each time</b> by tapping the inverted plate on absorbent material.
9.	Pipette <b>100 µl</b> of the <b>Enzyme Conjugate</b> into all wells.
10.	Incubate for <b>30 min</b> at <b>RT</b> (20 – 25 °C) on a <b>shaker</b> (approx. 600 rpm).
11.	Discard or aspirate the contents of the wells. Wash the plate <b>4 x</b> by adding <b>300 µl</b> of <b>Wash Buffer</b> , <b>discarding</b> the content and <b>blotting dry each time</b> by tapping the inverted plate on absorbent material.
12.	Pipette <b>100 µl</b> of the <b>Substrate</b> into all wells and incubate for <b>20 - 30 min</b> at <b>RT</b> (20 – 25 °C) on a <b>shaker</b> (approx. 600 rpm). <b>Avoid exposure to direct sunlight!</b>
13.	Add <b>100 µl</b> of the <b>Stop Solution</b> to all wells and shake the microtiter plate to ensure a homogeneous distribution of the solution.
14.	<b>Read</b> the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to <b>450 nm</b> (if available a reference wavelength between 620 nm and 650 nm is recommended).

### 6.4 Normetanephrine ELISA

1.	Pipette <b>25 µl</b> of <b>Adjustment Buffer</b> into all wells of the <b>Normetanephrine Microtiter Strips</b> .
2.	Pipette <b>25 µl</b> of the extracted <b>standards, controls and samples</b> into the respective wells. <i>Please hold the Extraction Plate at a slight angle in order to facilitate this pipetting step.</i>
3.	Preparation of <b>Acylation Solution</b> : Pipette <b>80 µl Acylation Reagent Concentrate</b> (BA R-8312) to <b>3 ml water</b> (deionized, distilled, or ultra-pure) and mix thoroughly.
4.	Pipette <b>25 µl</b> of the freshly prepared <b>Acylation Solution</b> into all wells.
5.	Incubate for <b>15 min</b> at <b>RT</b> (20 – 25 °C) on a <b>shaker</b> (approx. 600 rpm).
6.	Pipette <b>50 µl</b> of the <b>Normetanephrine Antiserum</b> into all wells.
7.	Cover the plate with <b>Adhesive Foil</b> , shake for <b>1 min</b> at <b>RT</b> (20 – 25 °C) on a <b>shaker</b> and incubate for <b>15 - 20 h</b> (overnight) at <b>2 – 8 °C</b> without shaking. <b>Alternatively incubate for 2 h at RT</b> (20 - 25 °C) on a <b>shaker</b> (approx. 600 rpm).
8.	Remove the foil. Discard or aspirate the contents of the wells. Wash the plate <b>4 x</b> by adding <b>300 µl</b> of <b>Wash Buffer</b> , <b>discarding</b> the content and <b>blotting dry each time</b> by tapping the inverted plate on absorbent material.
9.	Pipette <b>100 µl</b> of the <b>Enzyme Conjugate</b> into all wells.
10.	Incubate for <b>30 min</b> at <b>RT</b> (20 – 25 °C) on a <b>shaker</b> (approx. 600 rpm).
11.	Discard or aspirate the contents of the wells. Wash the plate <b>4 x</b> by adding <b>300 µl</b> of <b>Wash Buffer</b> , <b>discarding</b> the content and <b>blotting dry each time</b> by tapping the inverted plate on absorbent material.
12.	Pipette <b>100 µl</b> of the <b>Substrate</b> into all wells and incubate for <b>20 - 30 min</b> at <b>RT</b> (20 – 25 °C) on a <b>shaker</b> (approx. 600 rpm). <b>Avoid exposure to direct sunlight!</b>
13.	Add <b>100 µl</b> of the <b>Stop Solution</b> to all wells and shake the microtiter plate to ensure a homogeneous distribution of the solution.
14.	<b>Read</b> the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to <b>450 nm</b> (if available a reference wavelength between 620 nm and 650 nm is recommended).

**7. Calculation of results**

<b>Measuring range (overnight ELISA)</b>	<b>Metanephrine</b>	<b>Normetanephrine</b>
	15.1 – 3 600 pg/ml	22.8 – 7 200 pg/ml

The standard curve is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).  
Use a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

⚠ *This assay is a competitive assay. This means: the OD-values are decreasing with increasing concentrations of the analyte. OD-values found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.*

The concentrations of the **samples** and **controls** can be read directly from the standard curve.

Samples found with concentrations higher than the highest standard (Standard F) should be diluted accordingly with the included Equalizing Reagent and have to be re-assayed.

**Conversion**

Metanephrine (pg/ml) × 5.07 = Metanephrine (pmol/l)

Normetanephrine (pg/ml) × 5.46 = Normetanephrine (pmol/l)

**Expected reference values**

It is strongly recommended that each laboratory should determine its own reference values.  
The expected reference values indicated below are based on method comparison studies to LC-MS/MS <sup>(1)</sup> with blood samples taken in the sitting position.

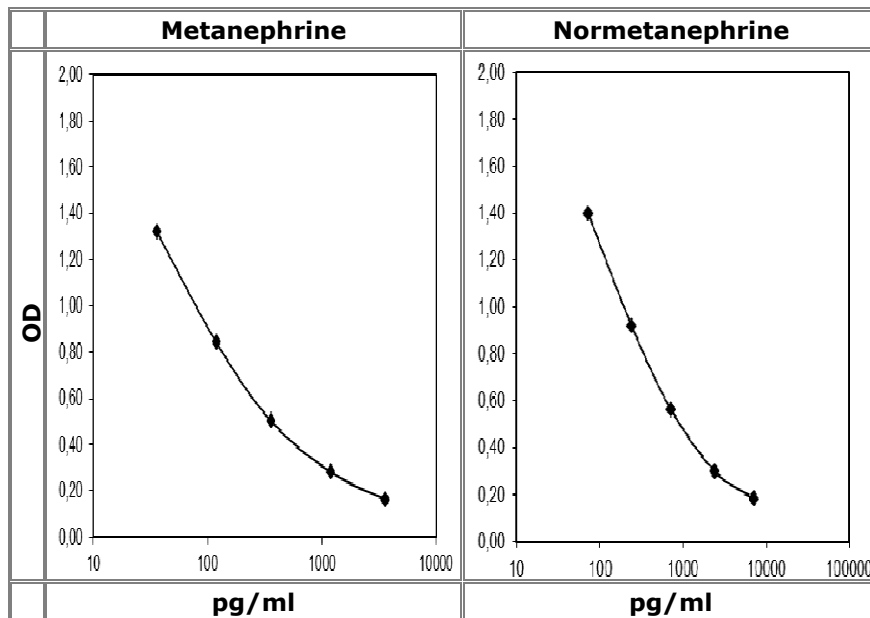
<b>Metanephrine</b>	<b>Normetanephrine</b>
< 65 pg/ml	< 196 pg/ml

**7.1 Quality control**

The confidence limits of the kit controls are indicated in the QC-Report.

**7.2 Typical standard curves**

⚠ *Examples, do not use for calculation!*





## 8. Assay characteristics (overnight ELISA)

Analytical Sensitivity		Metanephrine	Normetanephrine
	LOD (pg/ml)	14.9	17.9
	LOQ (pg/ml)	15.1	22.8

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)	
		Metanephrine	Normetanephrine
	Derivatized Metanephrine	100	0.72
Derivatized Normetanephrine	0.05	100	
3-Methoxytyramin	< 0.01	6.5*	
Adrenaline	< 0.01	< 0.01	
Noradrenaline	< 0.01	< 0.01	
Dopamin	< 0.01	< 0.01	
Vanillic mandelic acid	< 0.01	< 0.01	
Homovanillic acid	< 0.01	< 0.01	
L-DOPA	< 0.01	< 0.01	
L-Tyrosin	< 0.01	< 0.01	
Tyramine	< 0.01	< 0.01	
Normetanephrine	< 0.01	< 0.01	
Acetaminophen	< 0.01	< 0.01	

\*Normetanephrine concentrations are not influenced by 3-Methoxytyramine in case of normal 3-Methoxytyramine concentrations. Only very high 3-Methoxytyramine concentrations found in rare cases of exclusively dopamine secreting tumours can cause false positive results.

Precision							
Intra-Assay				Inter-Assay			
	Sample	Mean (pg/ml)	CV (%)		Sample	Mean (pg/ml)	CV (%)
Metanephrine	1	66.3	11.4	Metanephrine	1	67.8	17.6
	2	122	13.5		2	134	12.7
	3	308	10.6		3	319	11.0
	4	783	9.2		4	847	7.5
Normetanephrine	1	149	9.5	Normetanephrine	1	156	10.6
	2	282	9.1		2	287	5.0
	3	734	8.2		3	769	5.1
	4	1 956	10.5		4	1 949	5.9

Linearity		Serial dilution up to	Mean (%)	Range (%)
	Metanephrine	1:64	107	101 - 124
	Normetanephrine	1:64	98	92 - 102

Recovery		Mean (%)	Range (%)
	Metanephrine	88	80 - 99
	Normetanephrine	109	105 - 114







Method Comparison: ELISA vs. LC-MS/MS <sup>(1)</sup>	Metanephrine	$y=0.91x + 1.8; r^2 = 0.96; n = 46$
	Normetanephrine	$y=0.93x + 13; r^2 = 0.99; n = 48$

## 9. References/Literature

- (1) De Jong et al. Plasma free metanephrine measurement using automated online solid phase extraction HPLC-Tandem mass spectrometry. Clin Chem, 53(9): 1684-1693 (2007)
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- (4) Berkel et al. Diagnosis of endocrine disease: Biochemical diagnosis of phaeochromocytoma and paraganglioma. Eur J Endocrinol, 170: R109-R119
- (5) Manz et al. Development of enantioselective immunoassays for free plasma metanephrines. Ann.N.Y.Acad.Sci., 1018:582-587 (2004)
- (6) De Jong et al. Dietary Influences on Plasma and Urinary Metanephrines: Implications for Diagnosis of Catecholamine-Producing Tumors. J Clin Endocrinol Metab, 94(8):2841-2849 (2009)
- (7) Deutschbein et al. Influences of Various Confounding Variable and Storage Conditions on Metanephrine and Normetanephrine Levels in Plasma. Clin Endocrinol, 72(2):153-160 (2010)
- (8) Eisenhofer et al. Biochemical Diagnosis of Pheochromocytoma: How to Distinguish True- from False-Positive Test Results. The Journal of Clinical Endocrinology & Metabolism, 88(6):2656-2666 (2003)

**⚠ For updated literature or any other information please contact your local supplier.**

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