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Instructions for use

Metanephrine Plasma ELISA Fast Track

REF **BA E-8100**

2°C 8°C Σ 96 IVD CE

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Related Products:

- Normetanephrine Plasma ELISA Fast Track
- 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 in plasma. The determination of metanephrine helps in the detection of paragangliomas and pheochromocytomas.
Metanephrine (metadrenaline) is 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 resulting in a colour reaction. The reaction is monitored at a wavelength of 450 nm.
Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations. Manual processing of the ELISA is recommended. The use of automatic laboratory equipment is the responsibility of the user. This in-vitro diagnostic is for professional use only.

1.2 Clinical application

Metanephrine and normetanephrine are the metabolites of the catecholamines epinephrine and norepinephrine, respectively [1]. Cells derived from neuroendocrine tumors (e.g. pheochromocytoma and paraganglioma) are known to produce catecholamines, which are secreted episodically via vesicles into the blood stream [2, 3]. But beside this, a small portion of the catecholamines is metabolized inside the tumor cells to the corresponding catecholamines metabolites – namely metanephrine, normetanephrine (and 3-methoxytyramine in the case of dopamine) – which are secreted at low levels continuously into the blood stream [4, 5]. Recent studies and publications have shown that the quantification of these plasma free metanephrines and plasma free normetanephrines is the most accurate biochemical marker for the clinical diagnosis of pheochromocytoma and paraganglioma in patients [5-13]. Pheochromocytoma and paraganglioma are rare neuroendocrine tumors and occur with an estimated annual incidence of 1 – 8 cases per 1,000,000 [10, 14].
Therapeutic consequences should never be based on laboratory results alone, even if these results are assessed in accordance with the quality criteria of the method. Any laboratory result is only a part of the total clinical picture of the patient.
Only in cases where the laboratory results are in an acceptable agreement with the overall clinical picture of the patient, it can be used for therapeutic consequences.

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 must be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
(2) This assay was validated for a certain type of sample as indicated in Intended Use (please refer to Chapter 1). Any off-label use of this kit is in the responsibility of the user and the manufacturer cannot be held liable.
(3) The principles of Good Laboratory Practice (GLP) must be followed.
(4) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
(5) If serious incidents should occur in connection with this product, they should be reported to the manufacturer and the competent national authorities.
(6) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water. Avoid repeated freezing and thawing of reagents and specimens.
(7) The microplate contains snap-off strips. Unused wells must be stored at 2 – 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.
(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 for use 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 provided with the kit.
(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_2SO_4. 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. Rinse contaminated items before reuse.
(17) For information about 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 of according to national regulations.
(19) The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
(20) In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components must not be used for a test run. They must be stored properly until the manufacturer decides what to do with them. If it is decided that they are no longer suitable for measurements, they must be disposed of in accordance with national regulations.
(21) The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.
(22) 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.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.
Commercially available synthetic metanephrine is always a mixture of the D- and L-form. This has important implications if synthetic metanephrine is used to enrich native samples. The antibody used in this kit has a specific D- and L-form recognition rate. Please contact the manufacturer for details in case synthetic metanephrine was used to enrich native samples.

2.2.1 Interfering substances and proper handling of specimens

Samples containing precipitates or fibrin strands might cause inaccurate results.
Hemolytic samples (up to 0.5 mg/ml hemoglobin), icteric samples (up to 0.5 mg/ml bilirubin) and lipemic samples (up to 17 mg/ml triglycerides) have no influence on the assay results.
If the concentrations cannot be estimated and there are doubts as to whether the above limit values for hemolytic, icteric or lipemic samples are complied with, the samples should not be used in the assay.

2.2.2 Drug and food interferences

Medications like antihypertensive agents, antidepressants, antipsychotics, sympathomimetics and L-DOPA can influence plasma metanephrines levels. Caffeinated beverages, nicotine, and mood-enhancing drugs can also affect plasma metanephrines levels. In addition, stress and physical strain should be avoided shortly before sampling.

2.2.3 High-Dose-Hook effect

No hook effect was observed in this test.

3. Storage and stability

Store kit and reagents at 2 – 8 °C until expiration date. Do not use kit and components beyond the expiry date indicated on the kit labels. Once opened, the reagents are stable for 2 months when stored at 2 – 8 °C. Once the resealable pouch of the ELISA plate has been opened, care should be taken to close it tightly again including the desiccant.

4. Materials

4.1 Contents of the kit

BA D-0090	**FOILS**	**Adhesive Foil** – ready to use
Content:	Adhesive foils in a resealable pouch	
Number:	1 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:	1 x 20 ml/vial, purple cap	
BA E-0040	**CONJUGATE**	**Enzyme Conjugate** – ready to use
Content:	Goat anti-rabbit immunoglobulins conjugated with peroxidase	
Volume:	1 x 12 ml/vial, red cap	
Description:	Species is goat	
Hazard pictograms:	 GHS07	
Signal word:	Warning	

Hazardous ingredients:	2-methyl-2H-isothiazol-3-one
Hazard statements:	H317 May cause an allergic skin reaction.
Precautionary statements:	P280 Wear protective gloves. P302+P352 IF ON SKIN: Wash with plenty of water. P333+P313 If skin irritation or rash occurs: Get medical advice/attention. P501 Dispose of contents/container to an authorised waste collection point.

BA E-0055	**SUBSTRATE**	**Substrate** – ready to use
Content:	Chromogenic substrate containing 3,3',5,5'-tetramethylbenzidine, substrate buffer and hydrogen peroxide	
Volume:	1 x 12 ml/vial, black cap	

BA E-0080	**STOP-SOLN**	**Stop Solution** – ready to use
Content:	0.25 M sulfuric acid	
Volume:	1 x 12 ml/vial, grey cap	

BA E-0131	**LU** **ADR** **MN**	**Metanephrine Microtiter Strips** – ready to use
Content:	1 x 96 wells (12x8) antigen precoated microwell plate in a resealable blue pouch with desiccant	

BA E-8110	**MN-AS**	**Metanephrine Antiserum** – ready to use
Content:	Rabbit anti-metanephrine antibody in buffer with proteins and non-mercury preservative, blue coloured	
Volume:	1 x 6 ml/vial, blue cap	
Description:	Species of antibody is rabbit, species of protein in buffer is bovine	

BA E-8327	**ADJUST-BUFF**	**Adjustment Buffer** – ready to use
Content:	TRIS buffer	
Volume:	1 x 10 ml/vial, yellow cap	

BA R-8312	**ACYL-CONC**	**Acylation Concentrate** – concentrated
Content:	Acylation reagent in DMSO	
Volume:	1 x 1.5 ml/vial, white cap	
Hazard pictograms:	  GHS05 GHS08	
Signal word:	Danger	
Hazardous ingredients:	Succinic anhydride	
Hazard statements:	H314 Causes severe skin burns and eye damage. H317 May cause an allergic skin reaction. H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.	
Precautionary statements:	P260 Do not breathe mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection. P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304+P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing. P310 Immediately call a POISON CENTER/doctor. P501 Dispose of contents/container to an authorised waste collection point.	
EUH-statements:	EUH071 Corrosive to the respiratory tract.	

BA R-8313	**ASSAY-BUFF**	**Assay Buffer** – ready to use
Content:	25% organic solvent	
Volume:	1 x 30 ml/vial, orange cap	
Hazard pictograms:	 GHS02	

Signal word:	Warning
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, green cap
Hazard pictograms:	 GHS05
Signal word:	Danger
Hazard statements:	H314 Causes severe skin burns and eye damage.
Precautionary statements:	P280 Wear protective gloves, protective clothing, eye protection. P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310 Immediately call a doctor, a POISON CENTER. P501 Dispose of contents/container to an authorised waste collection point.
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
Description:	Species is human

4.2 Calibration and Controls

Standards and Controls – ready to use

Cat. no.	Component	Colour/ Cap	Concentration [pg/ml] MN	Concentration [pmol/l] MN	Volume/ Vial
BA E-8301	**STANDARD A**	white	0	0	4 ml
BA E-8302	**STANDARD B**	yellow	36	183	4 ml
BA E-8303	**STANDARD C**	orange	120	608	4 ml
BA E-8304	**STANDARD D**	blue	360	1,825	4 ml
BA E-8305	**STANDARD E**	grey	1,200	6,084	4 ml
BA E-8306	**STANDARD F**	black	3,600	18,252	4 ml
BA E-8351	**CONTROL 1**	green	Refer to QC-Report for expected value and acceptable range.		4 ml
BA E-8352	**CONTROL 2**	red			4 ml

Conversion:	metanephrine [pg/ml] x 5.07 = metanephrine [pmol/l]
Content:	Acidic buffer with non-mercury stabilizer, spiked with a defined quantity of metanephrine.

4.3 Additional materials required but not provided in the kit

- Water (deionized, distilled, or ultra-pure)
- Absorbent material (paper towel)

4.4 Additional 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)
- Vortex mixer

5. Sample collection, handling and storage

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. When in doubt, it is recommended that hemolytic, icteric, and lipemic samples not be used in the assay (see 2.2.1).
Storage: up to 3 days at 2 – 8 °C, for longer period (up to 6 months) at -20 °C.
Repeated freezing and thawing should be avoided.

6. Test procedure

Allow all reagents and samples 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 antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent. The higher the temperature, the higher the absorption values will be. Varying incubation times will have similar influences on the absorbance. The optimal temperature during the enzyme immunoassay is between 20 – 25 °C.
If the product is prepared in parts, unused wells in Extraction Plates should be covered to avoid contamination. After preparation, the used wells must be labelled to prevent double use.
During the overnight incubation at 2 – 8 °C with the antiserum, the temperature should be uniform all over the ELISA plate to avoid any drift and edge-effect.

⚠ *The use of a microtiter plate shaker with the following specifications is mandatory: shaking amplitude 3 mm; approx. 600 rpm. Shaking with differing settings might influence the results.*

6.1 Preparation of reagents and further notes

Wash Buffer
Dilute the 20 ml Wash Buffer Concentrate **WASH-CONC** **50X** with water to a final volume of 1000 ml.
Storage: 2 months at 2 – 8 °C

Cleaning Buffer
Dilute the 20 ml Cleaning Concentrate **CLEAN-CONC** **25X** with water to a final volume of 500 ml.
Storage: 2 months 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. Discard after use!

Metanephrine 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 – 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.

1.	Pipette **20 µl** of **standards** and **controls** into the respective wells of the **EXTRACT-PLATE 96**.
	Alternatively *pipette* ***25 µl*** *of* ***standards*** *and* ***controls.***
2.	Add **20 µl STANDARD A** to all wells intended for the **plasma samples**.
	Alternatively *add* ***25 µl*** **STANDARD A**.
3.	Add **200 µl** of **EQUA-REAG** to the wells with **standards and controls.**
	Alternatively *add* ***250 µl*** *of* **EQUA-REAG**.
4.	Pipette **200 µl** of **plasma samples** to the respective wells.
	Alternatively *pipette* ***250 µl*** *of* ***plasma samples.***
5.	Incubate plate **for 2 h** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
6.	Empty plate and blot dry by tapping the inverted plate on absorbent material.
7.	Pipette **250 µl** of **ASSAY-BUFF** into all wells. Incubate the plate for **5 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). Empty plate and blot dry by tapping the inverted plate on absorbent material.
8.	Wash the plate **3 times** by adding **350 µl** of **Cleaning Buffer**, **discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
9.	Pipette **100 µl** of **ELUTION-BUFF** into all wells.
	Alternatively *pipette* ***125 µl*** *of* **ELUTION-BUFF**.
	Please note: The colour changes caused by the elution buffer can vary between standards and samples.
10.	Cover plate with **FOILS**. Incubate **15 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
⚠	Remove the **FOILS**.
	Do not decant the supernatant thereafter!
	The following volume of the supernatant is needed for the subsequent ELISA:
	Metanephrine 50 µl

6.3 Metanephrine ELISA

1.	Pipette **25 µl** of **ADJUST-BUFF** into all wells of the **Metanephrine Microtiter Strips III ADR MN**.
2.	Pipette **50 µl** of the extracted **standards, controls and samples** into the respective wells. *Please hold the Extraction Plate at a slight angle in order to facilitate this pipetting step.*
3.	Preparation of **Acylation Solution**: Pipette **80 µl ACYL-CONC** to **3 ml water** and mix thoroughly.
4.	Pipette **25 µl** of the freshly prepared **Acylation Solution** into all wells.
5.	Incubate for **15 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
6.	Pipette **50 µl** of the **Metanephrine Antiserum MN-AS** into all wells.
7.	Cover the plate with **FOILS**, shake for **1 min** at **RT** (20 – 25 °C) on a **shaker** and incubate for **15 – 20 h** (overnight) at **2 – 8 °C** without shaking.
8.	Remove the foil. Discard or aspirate the contents of the wells. Wash the plate **4 times** by adding **300 µl** of **Wash Buffer, discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
9.	Pipette **100 µl** of the **CONJUGATE** into all wells.
10.	Incubate for **30 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
11.	Discard or aspirate the contents of the wells. Wash the plate **4 times** by adding **300 µl** of **Wash Buffer, discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
12.	Pipette **100 µl** of the **SUBSTRATE** into all wells and incubate for **20 – 30 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). ***Avoid exposure to direct sunlight!***
13.	Add **100 µl** of the **STOP-SOLN** to all wells and shake the microtiter plate shortly.
14.	**Read** the absorbance of the solution in the wells within 10 min, using a microplate reader set to **450 nm** (if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

Measuring range	Metanephrine
	15.1 – 3,600 pg/ml

The standard curve, which can be used to determine the concentration of the unknown samples, 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) using a concentration of 0.001 pg/ml for Standard A (this alignment is mandatory because of the logarithmic presentation of the data). Use non-linear regression for curve fitting (e.g. 4-parameter, marquardt).

⚠ *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 **EQUA-REAG** and must be re-assayed. For the calculation of the concentrations this dilution factor has to be taken into account.

Conversion:

metanephrine [pg/ml] x 5.07 = metanephrine [pmol/l]

7.1 Expected reference value

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 [2] with blood samples taken in the sitting position.

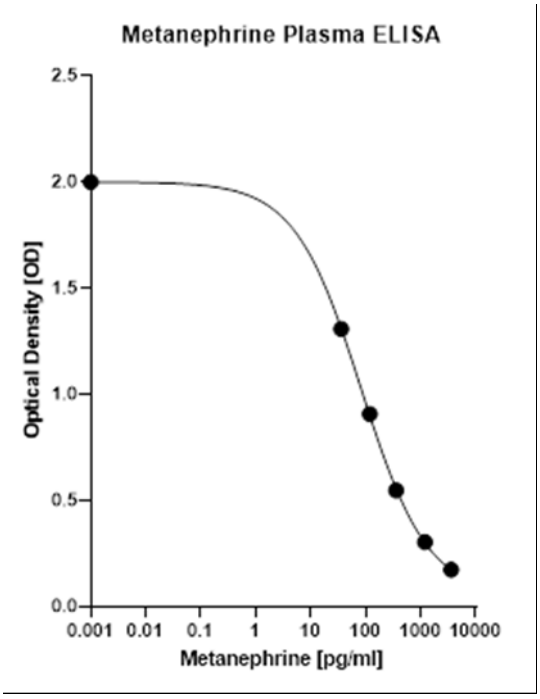
	Metanephrine
expected reference value (ULN)	< 100 pg/ml
typical pathological range	up to 3,534 pg/ml

For the interpretation of the results, a grey area has to be considered. This grey area does not depend on the methodology used and is reflected in a slight to mediate increase in metanephrine and normetanephrine up to 4 times the upper cut-off [15]. Approx. 20% of the tumors are found in this grey area, especially in the case of the Hereditary Syndrome, incidental tumors and in sporadic cases of pheochromocytomas with a diameter less than 1 cm.

In case of a result in the grey area, it is recommended to collect a new sample together with an anamnesis concerning especially influences like the medication and age of the patient. If the sample continues to be found in the grey area, a clonidine suppression test may be considered [16, 17].

7.2 Typical standard curve

⚠ *Example: Do not use for calculation!*



8. Control samples

It is recommended to use control samples according to national regulations. Use controls at both normal and pathological levels. Commercially obtained control samples should be treated like unknown samples. Control samples

should fall within established confidence limits (please refer to *"Limitations"* chapter 2.2). The confidence limits of the kit controls are indicated on the QC-Report.

9. Assay characteristics

9.1 Performance data

Analytical Sensitivity	
	Metanephrine
Limit of Blank (LOB)	9.9 pg/ml
Limit of Detection (LOD)	14.9 pg/ml
Limit of Quantification (LOQ)	15.1 pg/ml

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

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

Lot-to-Lot			
	Sample	Mean ± SD [pg/ml]	CV [%]
Metanephrine (n = 6)	1	97.7 ± 16.5	16.9
	2	870 ± 117	13.5

Recovery was determined according to the CLSI standard EP 34 1st ed.

Recovery			
	Range [pg/ml]	Mean [%]	Range [%]
Metanephrine	20.9 – 1,291	102	90 – 109

Linearity			
	Serial dilution up to	Mean [%]	Range [%]
Metanephrine	1:64	107	101 – 124

Method Comparison: ELISA vs. LC-MS/MS [13]	
Metanephrine	$y = 0.91x + 1.8$; $r^2 = 0.96$; n = 46

Diagnostic Performance [2]*				
	Diagnostic Specificity [%]	Diagnostic Sensitivity [%]	Positive Predictive Value (PPV) [%]	Negative Predictive Value (NPV) [%]
Metanephrine	96	45	79	84
	Positive Likelihood Ratio (LR+)		Negative Likelihood Ratio (LR-)	
	11.25		0.57	

* The determination of both metanephrine and normetanephrine, using the 2-MET Plasma ELISA Fast Track BA E-8300, results in a better diagnostic performance (diagnostic sensitivity 100% and diagnostic specificity 96%).

9.2 Metrological Traceability

The values assigned to the standards and controls of the Metanephrine Plasma ELISA Fast Track are traceable to SI Units by weighing with quality-controlled analyte.

Standards and Controls	
	Uncertainty [%]
Metanephrine	2.7

Metanephrine Plasma ELISA Fast Track	
	Expanded Uncertainty [%] k = 2*
Metanephrine	26.0

* This defines an interval about the measured result that will include the true value with a probability of 95%.

10. References/Literature

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For updated literature or any other information please contact your local supplier.
The summary of safety and performance according to article 29 of regulation (EU) 2017/746 can be downloaded from the website www.ldn.de.

11. Changes

Version	Release Date	Chapter	Change
20.0	2022-03-25	All	- The alternative version, 2 h at RT incubation with antiserum, was removed - The IFU was revised according to the IVDR regulations (EU) 2017/746 - Sample stability (chapter 5) changed - Expected reference value (ULN) changed (chapter 7.1) - Typical pathological range was added (Chapter 7.1) - LOB, Lot to Lot and diagnostic performance were added to the assay characteristics (chapter 9.1) - Metrological traceability was added (chapter 9.2) - References/Literature was updated (chapter 10)
21.0	2023-09-18	2.1/9.2 4.1 7.1 9.1 10	- Editorial changes - Hazard labelling updated according to SDS - Hint added regarding clonidine suppression test - Recovery updated - References updated
22.0	2024-12-19	4.1 7 9.2	- Hazard labelling BA E-0040 updated according to SDS - Note added to the dilution factor in the calculation - Metrological Traceability updated

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Use-by date		Batch code		For in-vitro diagnostic use only!
	Consult instructions for use		Content		CE marking of conformity
	Caution		Catalogue number		Distributor
	Date of manufacture				