Instructions for Use

MetCombi Plasma ELISA

Enzyme immunoassay for the quantitative determination of free metanephrine and normetanephrine in human plasma.

REF **RE59202**

Σ **2 x 96**

2-8°C

EU: IVD CE U.S.: *For research use only. Not for use in diagnostic procedures.*

I B L I N T E R N A T I O N A L G M B H

Flughafenstrasse 52a
D-22335 Hamburg, Germany
Phone: +49 (0)40-53 28 91-0
Fax: +49 (0)40-53 28 91-11
IBL@IBL-International.com
www.IBL-International.com

1. INTENDED USE

Enzyme immunoassay for the quantitative determination of free metanephrine and normetanephrine in human plasma.

2. SUMMARY AND EXPLANATION

The catecholamines adrenalin, noradrenalin and dopamine are synthesized in the adrenal medulla, the sympathetic nervous system and in the brain. They influence virtually all tissues and are involved together with other hormonal and neuronal systems in the regulation of a wide variety of physiological processes.
As catecholamines and their metabolites metanephrine and normetanephrine are secreted in exessive amount in a number of diseases, they may be used for diagnostic purposes.
In this context, diagnosis as well as the follow-up of tumor diseases of the nervous system are of special importance. This applies primarily to the pheochromocytoma, but also the neuroblastoma and the ganglioneuroma.
Malignant growth is described in 10% of pheochromocytomas. Furthermore, an increase of catecholamines and their metabolites metanephrine and normetanephrine can be observed in the carcinoid.
Accumulating data shows that the metanephrines in plasma are the best marker for diagnosis and follow-up of pheochromocytoma.

3. TEST PRINCIPLE

After protein precipitation and acylation the metanephrines are measured by ELISA.
Acylated nor-/metanephrines from the sample and solid phase bound nor-/metanephrines compete for a fixed number of 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 nor-/metanephrine is detected by anti-rabbit IgG / peroxidase. The amount of antibody bound to the solid phase catecholamine is inversely proportional to the catecholamine concentration of the sample.

4. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
9. Avoid contact with Stop solution and Precipitating Reagent. It may cause irritations and burns.
10. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely. For this reason reagents should be treated as potential biohazards in use and for disposal.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sunlight. The storage and stability of specimens and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2-8 °C.

6. SPECIMEN COLLECTION AND STORAGE

⚠	The in-vivo catecholamine and metanephrines release is influenced by several foods and drugs. Vitamin B, coffee and bananas, alpha-methyldopa, MAO and COMT inhibitors as well as medications related to hypertension should be discontinued for at least 72 h prior to specimen collection.

Plasma (EDTA)

⚠	The blood sample should be stored at 2-8°C until centrifuged to separate the plasma within 2 h after blood collection.

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens.

Storage:	2-8°C	≤ -20°C (Aliquots)	≤ -70°C (Aliquots)	Keep away from heat or direct sunlight. Avoid repeated freeze-thaw cycles. Ship samples frozen.
Stability:	24 h	3 months	1 year	

7. MATERIALS SUPPLIED

⚠	The reagents provided with this kit are sufficient for up to 96 precipitations in single determination in the sample preparation: 88 patient samples, 6 standards and 2 controls. Duplicates for metanephrine and normetanephrine determination are recommended with a double acylation, sufficient for 40 patient samples, 6 standards and 2 controls. **Additional reagents are available upon request.**

Quantity	Symbol	Component
1 x 12 x 8	MTP MN	**Microtiter Plate Metanephrine, (blue)** Break apart strips. Coated with: Metanephrine.
1 x 12 x 8	MTP NMN	**Microtiter Plate Normetanephrine, (yellow)** Break apart strips. Coated with: Normetanephrine.
2 x 1.5 mL	CAL A LYO	**Standard A,** lyophilized 0 pg/mL Contains: human plasma.
1 x 5 x 1.5 mL	CAL B-F LYO	**Standard B-F,** lyophilized Exact concentrations see vial labels or QC certficate. Contains: human plasma.
1 x 2 x 1.5 mL	CONTROL 1+2 LYO	**Control 1+2,** lyophilized Concentrations / acceptable ranges see QC certificate. Contains: human plasma.
1 x 2 x 3.5 mL	PREC REAG 1+2	**Precipitating Reagent 1 + 2** Ready to use.
3 x 2.5 mL	ACYL REAG LYO	**Acylation Reagent,** lyophilized
1 x 450 µL	ANTISERUM MN CONC	**Metanephrine Antiserum Concentrate (10x)** Blue colored. Contains: Antiserum (rabbit).
1 x 4 mL	ANTISERUM NMN	**Normetanephrine Antiserum** Ready to use. Yellow Colored. Contains: Antiserum (rabbit).
2 x 13 mL	ENZCONJ	**Enzyme Conjugate** Ready to use. Contains: anti-rabbit IgG POD Conjugate.
2 x 5.5 mL	SOLVENT	**Solvent** Ready to use. Contains: acetone.
1 x 6 mL	ACYL BUF	**Acylation Buffer** Ready to use. Contains: Tris-HCl-Buffer.
1 x 100x	PREC TUBES	**Precipitation Tubes**
2 x 20 mL	WASHBUF CONC	**Wash Buffer, Concentrate (50x)**
2 x 13 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains: 3,3',5,5'-Tetramethylbenzidine, H_2O_2.
2 x 13 mL	TMB STOP	**Stop Solution** **Ready to use.** Contains: 0.3 M H_2SO_4.
4 x	FOIL	**Adhesive Foil**

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 20; 25; 50; 100 and 200 µL.
2. Dynex DSX Processor and DSX tubes (automated version).
3. Centrifuge; ≥ 4000 x g
4. Orbital shaker (400-600 rpm)
5. Vortex mixer
6. Roller mixer
7. 8-Channel Micropipettor with reagent reservoirs
8. Wash bottle, automated or semi-automated microtiter plate washing system
9. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
10. Bidistilled or deionised water
11. Paper towels, pipette tips and timer

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout. A pipetting scheme covering both sample pretreatment and assay is available at the IBL-Homepage.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
7. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled evenly with Wash Buffer, and that there are no residues in the wells.
8. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

This enzyme immunoassay is evaluated for manual use and especially for automated use with the Dynex DSX ELISA processor for the determination of metanephrine and normetanephrine in plasma.
Therefore, the manual contains different working procedures.

⚠	The contents of the kit for 96 determinations can be divided into 3 separate runs. **The volumes stated below are for one run with 2 x 6 strips (2 x 48 determinations) Metanephrine and Normetanephrine.**
⚠	Additional Reagent and Solvent can be ordered separately from IBL under REF ACYL REAG: KEWP 751 or REF SOLVENT: KEWP 551.

10.1. Preparation of lyophilized or concentrated components

Dilute / dissolve	Component	with	Diluent	Remarks	Storage	Stability
6 vials	**CAL A-F**	1.5 mL	bidist. water	Vortex all vials and mix 20 min on a roller mixer.	≤ -20°C (Aliquots)	until Exp. date. Avoid repeated freeze-thaw cycles.
2 vials	**CONTROL 1+2**					
20 mL	**WASHBUF**	ad 1000 mL	bidist. water	**Relation: 1:50** Warm up at 37°C to dissolve crystals, if necessary.	2-8°C	4 weeks
200 µL	**ANTISERUM MN**	1800 µL	bidist. water	**Relation: 1:10** Mix carefully	18-25°C	1 day

⚠	**Acylation Reagent: Prepare freshly and use only once.**
⚠	Please note that solvent reacts with many plastic materials including plastic trays; solvent does not react with normal pipette tips and with glass devices. 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.

Dilute / dissolve	Component	with	Diluent	Remarks	Storage	Stability
1 vial	**ACYL REAG**	2.5 mL	**SOLVENT**	Mix 15 min on a roller mixer.	18-25°C	3 h

10.2. Precipitation and acylation in Precipitation Tubes

⚠	**Note automatic processing: For the preparation for the automated version pipette 200 µL standards and controls in 1.8 mL DSX tubes.** **Use the Precipitation Tubes included in the kit for samples only.**

⚠	Dilution of samples: Samples suspected to contain concentrations higher than the highest standard have to be diluted prior to precipitation step with standard A.

1.	Label the **Precipitation Tubes** and Pipette **200 µL** of each **Standard, Control and sample** into the respective tubes.
2.	Pipette **25 µL** of **precipitating reagent 1** into each tube.
3.	Pipette **25 µL** of **precipitating reagent 2** into each tube and **vortex** (5 – 10 sec.).
4.	**Centrifuge** the Precipitation Tubes for **15 min ≥ 4000 x g.**
5.	Pipette **50 µL Acylation Buffer** into each tube.
6.	Rather, use an Eppendorf multipette (or similar device), fill the syringe directly from the vial with dissolved Acylation Reagent and add well by well.
7.	Pipette **40 µL of freshly prepared Acylation Reagent** into each tube. **Vortex each tube immediately after pipetting** (2 - 4 sec.) Resuspension of the pellet is not necessary.
8.	**Centrifuge** the Precipitation Tubes for **15 min ≥ 4000 x g.**

11. TEST PROCEDURE

⚠	**The following procedure takes place in microtiter plate.** **Blue microtiter plate for metanephrine determination.** **Yellow microtiter plate for normetanephrine determination.**

11.1. Manual procedure short version

11.1.1. Metanephrine (blue microtiter plate) manual procedure short version

1.	Pipette **50 µL** of each acylated **Standard, Control** and **Patient sample** into the respective wells of the microtiter plate. **Do not cover the plate.**
2.	**Incubate 60 min** at room temperature **(18-25°C)** on an orbital shaker **(500 rpm).**
3.	Pipette **25 µL** of **diluted Metanephrine Antiserum** into each well. Color change to blue.
4.	Cover plate with adhesive foil. **Incubate 120 min** at room temperature **(18-25°C)** on an orbital shaker **(500 rpm).**
5.	Remove adhesive foil. Discard incubation solution. Wash plate **4 x** with **300 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
6.	Pipette **100 µL** of **Enzyme Conjugate** into each well. Cover plate with adhesive foil.
7.	**Incubate 30 min** at room temperature **(18-25°C)** on an orbital shaker **(500 rpm).**
8.	Remove adhesive foil. Discard incubation solution. Wash plate **4 x** with **300 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
9.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution.
10.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
11.	**Incubate 25-35 min** at room temperature **(18-25°C)** on an orbital shaker **(500 rpm)**.
12.	⚠ **The substrate reaction is time and temperature-dependent.** **Keep away from heat or direct sunlight.**
13.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate. Color change from blue to yellow.
14.	**Measure optical density** with a photometer **at 450 nm** (Reference-wavelength: 600-650 nm) within 15 min after pipetting of the Stop Solution.

11.1.2. Normetanephrine (yellow microtiter plate) manual procedure short version

1.	Pipette **50 µL** of each acylated **Standard, Control** and **Patient sample** into the respective wells of the microtiter plate. **Do not cover the plate.**
2.	**Incubate 60 min** at room temperature **(18-25°C)** on an orbital shaker **(500 rpm).**
3.	Pipette **25 µL** of **Normetanephrine Antiserum** into each well. Color change to orange.
4.	Cover plate with adhesive foil. **Incubate 120 min** at room temperature **(18-25°C)** on an orbital shaker **(500 rpm).**
5.	Remove adhesive foil. Discard incubation solution. Wash plate **4 x** with **300 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
6.	Pipette **100 µL** of **Enzyme Conjugate** into each well. Cover plate with adhesive foil.
7.	**Incubate 30 min** at room temperature **(18-25°C)** on an orbital shaker **(500 rpm).**
8.	Remove adhesive foil. Discard incubation solution. Wash plate **4 x** with **300 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
9.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution.
10.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
11.	**Incubate 25-35 min** at room temperature **(18-25°C)** on an orbital shaker **(500 rpm)**.
12.	⚠ **The substrate reaction is time and temperature-dependent.** **Keep away from heat or direct sunlight.**
13.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate. Color change from blue to yellow.
14.	**Measure optical density** with a photometer **at 450 nm** (Reference-wavelength: 600-650 nm) within 15 min after pipetting of the Stop Solution.

11.2. Alternative Version with overnight incubation

11.2.1. First Day: Manual procedure Metanephrine (blue microtiter plate)

1.	Pipette **50 µL** of each acylated **Standard, Control** and **Patient sample** into the respective wells of the microtiter plate. **Do not cover the plate.**
2.	**Incubate 60 min** at room temperature **(18-25°C)** on an orbital shaker **(500 rpm).**
3.	Cover plate with adhesive foil. **Incubate overnight (12-20 h) at 2-8 °C.**

11.2.2. First Day: Manual procedure Normetanephrine (yellow microtiter plate)

1.	Pipette **50 µL** of each acylated **Standard, Control** and **Patient sample** into the respective wells of the microtiter plate. **Do not cover the plate.**
2.	**Incubate 60 min** at room temperature **(18-25°C)** on an orbital shaker **(500 rpm).**
3.	Cover plate with adhesive foil. **Incubate overnight (12-20 h) at 2-8 °C.**

11.2.3. Second Day: Manual procedure Metanephrine (blue microtiter plate)

1.	Remove adhesive foil. Pipette **25 µL** of **diluted Metanephrine Antiserum** into each well. Color change to blue.
2.	Cover plate with adhesive foil. **Incubate 120 min** at room temperature **(18-25°C)** on an orbital shaker **(500 rpm).**
3.	Remove adhesive foil. Discard incubation solution. Wash plate **4 x** with **300 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
4.	Pipette **100 µL** of **Enzyme Conjugate** into each well. Cover plate with adhesive foil.
5.	**Incubate 30 min** at room temperature **(18-25°C)** on an orbital shaker **(500 rpm).**
6.	Remove adhesive foil. Discard incubation solution. Wash plate **4 x** with **300 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
7.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution.
8.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
9.	**Incubate 25-35 min** at room temperature **(18-25°C)** on an orbital shaker **(500 rpm)**.
10.	⚠ **The substrate reaction is time and temperature-dependent.** **Keep away from heat or direct sunlight.**
11.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate. Color change from blue to yellow.
12.	**Measure optical density** with a photometer **at 450 nm** (Reference-wavelength: 600-650 nm) within 15 min after pipetting of the Stop Solution.

11.2.4. Second Day: Manual procedure Normetanephrine (yellow microtiter plate)

1.	Remove adhesive foil. Pipette **25 µL** of **Normetanephrine Antiserum** into each well. Color change to orange.
2.	Cover plate with adhesive foil. **Incubate 120 min** at room temperature **(18-25°C)** on an orbital shaker **(500 rpm).**
3.	Remove adhesive foil. Discard incubation solution. Wash plate **4 x** with **300 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
4.	Pipette **100 µL** of **Enzyme Conjugate** into each well. Cover plate with adhesive foil.
5.	**Incubate 30 min** at room temperature **(18-25°C)** on an orbital shaker **(500 rpm).**
6.	Remove adhesive foil. Discard incubation solution. Wash plate **4 x** with **300 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
7.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution.
8.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
9.	**Incubate 35-45 min** at room temperature **(18-25°C)** on an orbital shaker **(500 rpm)**.
10.	⚠ **The substrate reaction is time and temperature-dependent.** **Keep away from heat or direct sunlight.**
11.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate. Color change from blue to yellow.
12.	**Measure optical density** with a photometer **at 450 nm** (Reference-wavelength: 600-650 nm) within 15 min after pipetting of the Stop Solution.

11.3. Automated procedure

11.3.1. Automated procedure Metanephrine (blue microtiter plate)

1.	Pipette **50 µL** of each acylated **Standard, Control** and **sample** into the respective wells of the microtiter plate.
2.	**Incubate 3 minutes** (max. 5 minutes) **at 30°C on an orbital shaker** (medium shaking rate).
3.	**Incubate 60 minutes** (max. 70 minutes) **at RT** (room temperature)**.**
4.	Pipette **25 µL** of **diluted Metanephrine Antiserum** into each well.
5.	**Incubate 120 minutes** (max. 130 minutes) **at RT on an orbital shaker** (medium shaking rate).
6.	**Wash plate 5x with 300 µL of diluted Wash Buffer.** Aspirate excess solution.
7.	Pipette **100 µL** of **Enzyme Conjugate** into each well.
8.	**Incubate 30 minutes** (max. 32 minutes) **at RT on an orbital shaker** (medium shaking rate).
9.	**Wash plate 5x with 300 µL of diluted Wash Buffer**. Aspirate excess solution.
10.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
11.	**Incubate 20 minutes** (max. 25 minutes) **at RT on an orbital shaker** (medium shaking rate).
12.	Pipette **100 µL** of **TMB Stop Solution** into each well.
13.	**Measure optical density at 450 nm** (Reference-wavelength: 620 nm).

11.3.2. Automated procedure Normetanephrine (yellow microtiter plate)

1.	Pipette **50 µL** of each acylated **Standard, Control** and **sample** into the respective wells of the microtiter plate.
2.	**Incubate 3 minutes** (max. 5 minutes) **at 30°C on an orbital shaker** (medium shaking rate).
3.	**Incubate 60 minutes** (max. 70 minutes) **at RT** (room temperature).
4.	Pipette **25 µL** of **Normetanephrine Antiserum** into each well.
5.	**Incubate 120 minutes** (max. 130 minutes) **at RT on an orbital shaker** (medium shaking rate).
6.	**Wash plate 5x with 300 µL of diluted Wash Buffer.** Aspirate excess solution.
7.	Pipette **100 µL** of **Enzyme Conjugate** into each well.
8.	**Incubate 30 minutes** (max. 32 minutes) **at RT on an orbital shaker** (medium shaking rate).
9.	**Wash plate 5x with 300 µL of diluted Wash Buffer.** Aspirate excess solution.
10.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
11.	**Incubate 20 minutes** (max. 25 minutes) **at RT on an orbital shaker** (medium shaking rate).
12.	Pipette **100 µL** of **TMB Stop Solution** into each well.
13.	**Measure optical density at 450 nm** (Reference-wavelength: 620 nm).

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or comparable standards /laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the vial labels. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.
In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

13. CALCULATION OF RESULTS

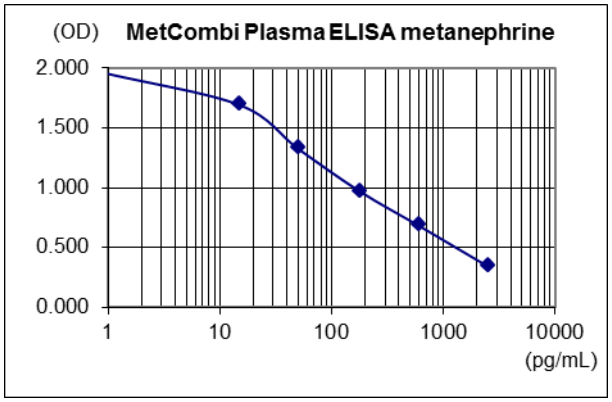
The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logistics or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentration of the samples can be read directly from the standard curve.

In case of diluted samples the values have to be multiplied with the corresponding dilution factor.

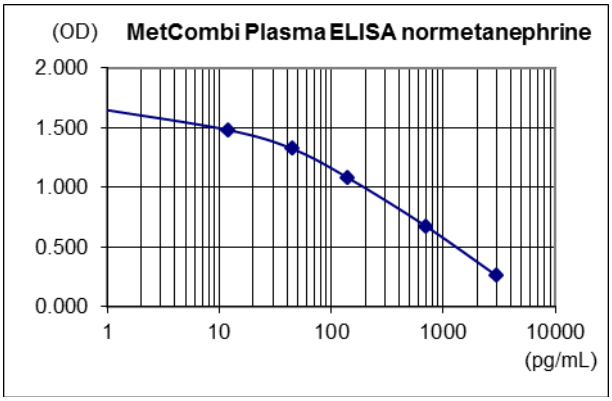
Conversion:
Metanephrine (pg/mL) x 5.07 = pmol/L
Normetanephrine (pg/mL) x 5.46 = pmol/L



Typical Calibration Curve Metanephrine

(Example. Do not use for calculation!)

Standard	pg/mL	OD_{Mean}
A	0	1.724
B	15	1.475
C	50	1.284
D	180	0.947
E	600	0.602
F	2500	0.288



Typical Calibration Curve Normetanephrine

(Example. Do not use for calculation!)

Standard	pg/mL	OD_{Mean}
A	0	1.654
B	12	1.481
C	45	1.322
D	140	1.081
E	700	0.674
F	3000	0.258

14. EXPECTED VALUES

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

Apparently healthy subjects show the following values:	Plasma		It is recommended that each laboratory establishes its own range of normal values.
	(pg/mL)	(nmol/L)	
Metanephrine	< 90	< 0.459	
Normetanephrine	< 190	< 1.037	

15. LIMITATIONS OF THE PROCEDURE

Specimen collection and storage have a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.
For cross-reactivities, see PERFORMANCE.

16. PERFORMANCE

<table>
<tr><td rowspan="12">Analytical Specificity (Cross Reactivity)</td><td></td><td colspan="2">Cross Reactivity (%)</td><td rowspan="12" colspan="2">Cross-reactivity of other substances tested < 0.001 %</td></tr>
<tr><td>Substance</td><td>Metanephrine</td><td>Normetanephrine</td></tr>
<tr><td>Metanephrine</td><td>100</td><td>0.015</td></tr>
<tr><td>Normetanephrine</td><td>0.130</td><td>100</td></tr>
<tr><td>3-Methoxytyramine</td><td>0.003</td><td>0.076</td></tr>
<tr><td>Adrenalin</td><td>0.039</td><td>0.0003</td></tr>
<tr><td>Noradrenalin</td><td>0.0008</td><td>0.0030</td></tr>
<tr><td>Tyramine</td><td>0.0005</td><td>0.0043</td></tr>
<tr><td>Dopamine</td><td><0.0001</td><td>0.0006</td></tr>
<tr><td>Homovanillic acid</td><td><0.0001</td><td><0.0001</td></tr>
<tr><td>Vanillic mandelic acid</td><td><0.0001</td><td><0.0001</td></tr>
<tr><td>L-DOPA</td><td><0.0001</td><td><0.0001</td></tr>
<tr><td>L-Tyrosine</td><td><0.0001</td><td><0.0001</td></tr>
<tr><td rowspan="2">Analytical Sensitivity (Limit of Detection)</td><td>Metanephrine</td><td>7 pg/mL</td><td colspan="3" rowspan="2">Mean signal (Zero-Standard) - 2SD</td></tr>
<tr><td>Normetanephrine</td><td>7 pg/mL</td></tr>
<tr><td>Precision</td><td></td><td>Range (pg/mL)</td><td>CV (%)</td><td colspan="2" rowspan="5"></td></tr>
<tr><td rowspan="2">Intra-Assay</td><td>Metanephrine</td><td>157-403</td><td>7.9–7.8</td></tr>
<tr><td>Normetanephrine</td><td>193–757</td><td>8.4–4.1</td></tr>
<tr><td rowspan="2">Inter-Assay</td><td>Metanephrine</td><td>118-276</td><td>8.8–8.6</td></tr>
<tr><td>Normetanephrine</td><td>246-551</td><td>9.3–9.2</td></tr>
<tr><td rowspan="3">Linearity</td><td></td><td>Range (pg/mL)</td><td>Serial dilution up to</td><td>Mean (%)</td><td>Range (%)</td></tr>
<tr><td>Metanephrine</td><td>43–886</td><td>1:20</td><td>103</td><td>96–112</td></tr>
<tr><td>Normetanephrine</td><td>70–1613</td><td>1:20</td><td>93</td><td>86–105</td></tr>
<tr><td rowspan="3">Recovery after spiking</td><td></td><td>Range (pg/mL)</td><td>Mean (%)</td><td colspan="2">Range (%)</td></tr>
<tr><td>Metanephrine</td><td>20–900</td><td>94</td><td colspan="2">82 – 117</td></tr>
<tr><td>Normetanephrine</td><td>34–1633</td><td>96</td><td colspan="2">90 - 108</td></tr>
<tr><td rowspan="2">Method Comparison versus LC/MS</td><td>Metanephrine</td><td colspan="2">IBL-ELISA = 1.04 x [LC/MS] - 23</td><td colspan="2">r = 0.990 ; n = 32</td></tr>
<tr><td>Normetanephrine</td><td colspan="2">IBL-ELISA = 0.99 x [LC/MS] - 8</td><td colspan="2">r = 0.984 ; n = 32</td></tr>
<tr><td rowspan="2">Method Comparison
Overnight versus short version</td><td>Metanephrine</td><td colspan="2">short version = 1.089 x - 2.054</td><td colspan="2">r = 0.993 ; n = 138</td></tr>
<tr><td>Normetanephrine</td><td colspan="2">short version = 0.926x +11.78</td><td colspan="2">r = 0.978 ; n = 138</td></tr>
</table>

17. PRODUCT LITERATURE REFERENCES

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Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

IBL AFFILIATES WORLDWIDE

IBL International GmbH Flughafenstr. 52A, 22335 Hamburg, Germany	Tel.: E-MAIL: WEB:	+ 49 (0) 40 532891 -0 Fax: -11 IBL@IBL-International.com http://www.IBL-International.com
IBL International Corp. 194 Wildcat Road, Toronto, Ontario M3J 2N5, Canada	Tel.: E-MAIL: WEB:	+1 (416) 645 -1703 Fax: -1704 Sales@IBL-International.com http://www.IBL-International.com

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