



Instructions for Use


Nor-/ Metanephrin in Plasma ELISA


Enzymimmunoassay for the
Quantitative Determination of
Free Normetanephrine and Metanephrine in Plasma

CE

IVD

REF EA612/192

 2 x 96

 2 – 8 °C













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Table of Contents

1	Introduction and Principle of the Test.....	3
2	Warnings and Precautions	3
3	Storage and Stability	4
4	Contents of the Kit	4
5	Specimen Collection and Storage	6
6	Preparation of Reagents and Samples.....	7
7	Assay Procedure.....	9
8	Calculation of Results.....	11
9	Assay Characteristics.....	12
10	Changes to declare.....	13
11	Literature	14
	Pipetting Scheme - Sample Preparation	15
	Pipetting Scheme - ELISA.....	16

Symbols

	In Vitro Diagnostic Medical Device		EC Declaration of conformity
	Content		Expiry Date
	Lot Number		Store at
	Manufactured by		Sufficient for ... determinations
	Catalogue Number		Consult Instructions for Use

Hazard Pictograms



Warning



Danger

1 Introduction and Principle of the Test

Normetanephrine and metanephrine are physiologically formed from the catecholamines noradrenaline and adrenaline by the enzyme catechol-O-methyltransferase (COMT). Increased levels of normetanephrine and metanephrine can be found in patients suffering from pheochromocytoma, ganglioneuroma and other neurogenic tumors.

The assay kit provides materials for the quantitative measurement of free normetanephrine and metanephrine in human EDTA plasma. Normetanephrine and metanephrine are separated from plasma proteins through addition of precipitation reagents and then are quantitatively acylated to their N-acyl-derivates.

The competitive Nor-/ Metanephrine ELISA kit uses the microtitre plate format. Metanephrine and normetanephrine, respectively, are bound to the solid phase of the microtiter plate. 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-/metanephrines is detected by anti-rabbit IgG/peroxidase. The substrate TMB/peroxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase nor-/metanephrines is inversely proportional to the nor-/metanephrines concentration of the sample.

2 Warnings and Precautions

- For in vitro diagnostic use only. For professional use only.
- All reagents of human origin used in this kit are tested for HIV I/II antibodies, HCV and HBsAg and found to be negative. However, because no test method can offer complete assurance that infectious agents are absent, these reagents should be handled as potentially biohazardous materials.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.
- Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
- By handling reagents, controls and samples follow good laboratory practice and safety guidelines.
- Wear lab coats, disposable gloves and protective glasses.

- Some components of this kit are containing hazardous reagents. These components are marked with the adequate hazard label. Further information: See 4. Contents of the Kit and the safety data sheet.
- Avoid contact with reagents. It may causes eye and skin irritations and chemical burns.
- Chemicals and prepared or used reagents have to treated as hazardous waste according to national biohazard and safety guidelines or regulations.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled samples.






3 Storage and Stability

On arrival, store the kit at 2 - 8 °C. Once opened the kit is stable until its expiry date. For stability of the ready for use reagents: See vial labels. For stability and storage of prepared reagents refer to 6.1 Preparation of Reagents.

Allow all reagents to reach room temperature before use and refrigerate after use.

4 Contents of the Kit

Metanephrine-Microtiter Strips 8 wells each, break-apart Precoated with metanephrine, colour-coded blue	STRIPS-MN	12 strips
Normetanephrine-Microtiter Strips 8 wells each, break-apart Precoated with normetanephrine, colour-coded yellow	STRIPS-NMN	12 strips
Standards (1 - 6) Each 1.5 ml, lyophilised, concentrations: see QC certificate 2 x Standard 1 for dilution of high level samples	CAL 1 - 6	6 vials
Control 1 & 2 Each 1.5 ml, lyophilised, range: see QC certificate	CON 1 & 2	2 vials
Acylation Reagent 2.5 ml, lyophilised, dissolve with Solvent	ACYL-REAG	3 vials

Acylation-Buffer 6 ml, ready for use, irritant	ACYL-BUFF		1 vial Warning
Metanephrine-Antiserum 0.45 ml, concentrated, colour-coded blue Rabbit-anti-N-acyl-metanephrine	AS-MN		1 vial
Normetanephrine-Antiserum 4 ml, ready for use, colour-coded yellow Rabbit-anti-N-acyl-normetanephrine	AS-NMN		1 vial
Enzyme Conjugate 13 ml, ready for use, anti-rabbit IgG-POD conjugate	CONJ		2 vials
Waschpuffer 20 ml, concentrated (50x)	WASH		2 vials
Substrate 13 ml TMB solution, ready for use	SUB		2 vials
Stop Solution 13 ml, ready for use, contains 0.3 M sulphuric acid	STOP		2 vials
Precipitation Tubes For precipitation	PRECI-TUBE		100 pieces
Precipitator 1 3.5 ml, ready for use, irritant	PRECI 1		1 vial Warning
Precipitator 2 3.5 ml, ready for use, irritant	PRECI 2		1 vial Warning
Solvent 5.5 ml, ready for use, contains acetone, irritant, highly flammable	SOLVENT	 	2 vials Warning Danger

Adhesive Foil**FOIL**

4 pieces

Ready for use

Additional materials and equipment required but not provided:

- Pipettes (25, 40, 50, 100 and 200 μ l)
- Multichannel pipette or Microplate washing device
- Eppendorf Multipipette (or similar devices)
- Distilled water
- Microplate photometer (450 nm)
- Orbital shaker
- Centrifuge (4,000 x g)
- Vortex mixer

5 Specimen Collection and Storage

EDTA plasma should be used.

Drugs, alcohol and tobacco as well as stress influence the catecholamine release. This may lead to false positive results for metanephrine and normetanephrine.

If clinically acceptable, medication (i.e. L-Dopa, alpha-blocker, antidepressants, MAO inhibitor, etc.) should be stopped five days before blood collection.

Patient should adhere to an at least four hours fasting; no tea, coffee, alcohol, nicotine and no strong physical activity.

It is recommended to let the patient rest for 20 to 30 minutes after the venipuncture and before collecting the blood sample.

The samples can be stored up to 6 hours at 2 – 8 °C. For a longer storage the samples must be frozen at -20 °C and are stable for at least 12 months.

Repeated freezing and thawing should be avoided.

6 Preparation of Reagents and Samples

6.1 Preparation of Reagents

6.1.1 Standards and Controls

Dissolve standards **CAL 1 – 6** and controls **CON 1 & 2** with 1.5 ml dist. water each, vortex shortly and leave on a roll mixer or similar shaker for minimum 20 minutes. Handle with care in order to minimize foam formation.

The reconstituted standards and controls should be stored frozen at 20 °C and are stable until expiry date printed on vial label.

6.1.2 Acylation Reagent

Dissolve the content of one bottle **ACYL-REAG** in 2.5 ml Solvent **SOLVENT** and shake for minimum 15 minutes on a roll mixer or similar shaker. The Acylation Reagent has always to be prepared immediately before use and is stable for at least 3 hours. The two additional bottles are allowing a second and a third run of the test. If the whole kit is to be used in one run it is recommended to pool the dissolved contents of two vials of Acylation Reagent. After use the reagent has to be discarded.

6.1.3 Metanephrine Antiserum

Dilute the concentrated Metanephrine Antiserum **AS-MN** 1 + 9 with dist. water before use. Diluted antiserum is stable for only one day. Therefore it is recommended to prepare the dilution freshly and only as much as necessary.

6.1.4 Wash Buffer

Dilute the content of **WASH** with dist. water to a total volume of 1,000 ml, mix shortly.

The diluted wash buffer has to be stored at 2 – 8 °C for a maximum period of 4 weeks. For longer storage the diluted wash buffer should be stored frozen at -20 °C and is stable until expiry date printed on vial label.

All other reagents are ready for use.

6.2 Preparation of Samples (Precipitation and Acylation)

Allow reagents and samples to reach room temperature. Determinations in duplicates are recommended. The preparation of the standards, controls and plasma samples is identical for both metanephrine and normetanephrine and has to be done only once.

1. Pipette **200 µl** each of **dissolved Standards** **CAL 1 – 6**, **Controls** **CON 1 & 2** and **Samples** into the respective marked Precipitation Tubes **PRECI-TUBE**.
2. Pipette **25 µl Precipitator 1** **PRECI 1** into each tube.
3. Pipette **25 µl Precipitator 2** **PRECI 2** into each tube.
4. Mix tubes thoroughly (vortex mixer).
5. Centrifuge tubes for 15 minutes with at least 4,000 x g, preferably with a swing-out rotor.
Attention: 4,000 x g is not identical to 4,000 x rpm (rounds per minute) and has to be adjusted for each centrifuge and rotor.
6. Pipette **50 µl Acylation Buffer** **ACYL-BUFF** into each tube and continue with step 7., immediately.
7. 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 evaporates quickly. Therefore, please do not use a tray with big surface together with a multichannel pipette for pipetting dissolved Acylation Reagent. Rather, use an Eppendorf multipipette (or similar device), fill the syringe directly from the vial with dissolved Acylation Reagent and pipette tube by tube.
Pipette **40 µl dissolved Acylation Reagent** **ACYL-REAG** into one tube and immediately vortex the tube softly at medium speed for 2 to 4 seconds and then continue with the next tube. Take care not to disturb the pellet at the bottom of the tube. Colour changes to red.
8. Centrifuge tubes for 15 minutes with at least 4,000 x g, preferably with a swing-out rotor.

Take 50 µl each for the Metanephrine and Normetanephrine ELISA.

7 Assay Procedure

7.1 Metanephrine ELISA

1. Pipette **50 µl each of acylated Standards, Controls and Samples** into the respective wells of the coated microtiter strips **STRIPS-MN** (blue).
2. Incubate for 1 hour at room temperature on an orbital shaker (medium shaking rate).
Do **not** cover the wells or the plate; leave the plate **open** on the shaker.
3. Pipette each **25 µl Metanephrine Antiserum AS-MN** into each well. Colour changes to blue.
4. Cover the plate with adhesive foil **FOIL** and incubate for 2 hours at room temperature (20 - 25 °C) on an orbital shaker (medium shaking rate).
5. Discard or aspirate the contents of the wells, add each **300 µl diluted Wash Buffer WASH**, again discard or aspirate the contents of the wells. Remove residual liquid by tapping the inverted plate on clean absorbent paper. Repeat the washing procedure 3 times.
Alternatively use a microplate washing device.
6. Pipette each **100 µl Enzyme Conjugate CONJ** into each well.
7. Incubate for 30 minutes at room temperature on an orbital shaker (medium shaking rate).
8. Washing: Repeat step 5.
9. Pipette each **100 µl Substrate SUB** into each well.
10. Incubate for 30 ± 5 minutes at room temperature (20 – 25 °C) on an orbital shaker (medium shaking rate).
11. Pipette **100 µl Stop Solution STOP** into each well, shake shortly.
12. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

7.2 Normetanephrine ELISA

1. Pipette **50 µl each of acylated Standards, Controls and Samples** into the respective wells of the coated microtiter strips **STRIPS-NMN** (yellow).
2. Incubate for 1 hour at room temperature on an orbital shaker (medium shaking rate).
Do **not** cover the wells or the plate; leave the plate **open** on the shaker.
3. Pipette each **25 µl Normetanephrine Antiserum** **AS-NMN** into each well. Colour changes to orange.
4. Cover the plate with adhesive foil **FOIL** and incubate for 2 hours at room temperature (20 – 25 °C) on an orbital shaker (medium shaking rate).
5. Discard or aspirate the contents of the wells, add each **300 µl diluted Wash Buffer** **WASH**, again discard or aspirate the contents of the wells. Remove residual liquid by tapping the inverted plate on clean absorbent paper. Repeat the washing procedure 3 times.
Alternatively use a microplate washing device.
6. Pipette each **100 µl Enzyme Conjugate** **CONJ** into each well.
7. Incubate for 30 minutes at room temperature on an orbital shaker (medium shaking rate).
8. Washing: Repeat step 5.
9. Pipette each **100 µl Substrate** **SUB** into each well.
10. Incubate for 30 ± 5 minutes at room temperature (20 – 25 °C) on an orbital shaker (medium shaking rate).
11. Pipette **100 µl Stop Solution** **STOP** into each well, shake shortly.
12. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

8 Calculation of Results

Concentrations of the standards: See QC certificate.

Conversion:

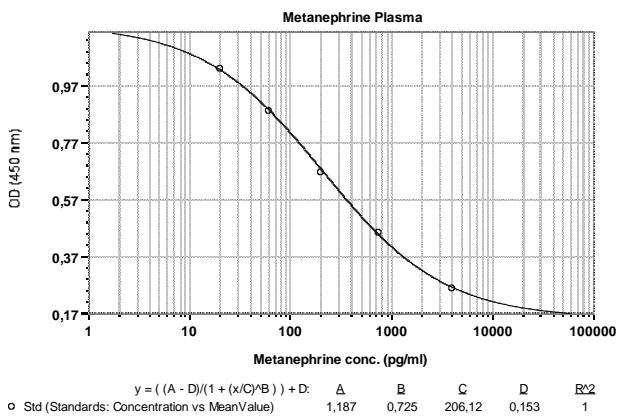
Metanephrine: 1 pg/ml = 5.07 pmol/l

Normetanephrine: 1 pg/ml = 5.46 pmol/l

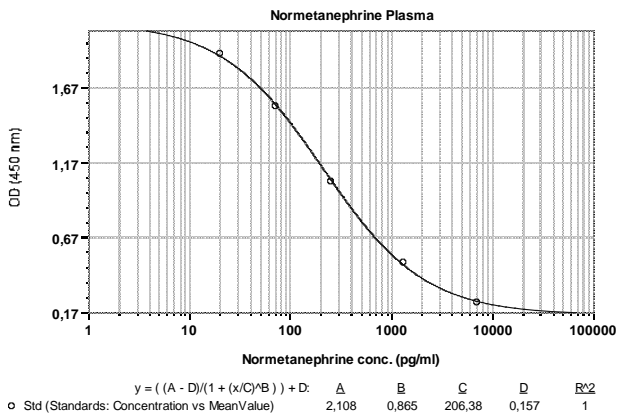
On a semilogarithmic graph paper the concentration of the standards (x-axis, logarithmic) are plotted against their corresponding optical density (y-axis, linear). Alternatively, the optical density of each standard and sample can be related to the optical density of the zero standard, expressed as the ratio OD/ODmax, and then plotted on the y-axis. A good fit is provided with 4 Parameter Logistic (alternatively Log-Logit or Cubic Spline). The concentration of the controls and samples can be read directly from the standard curve in pg/ml.

Below are listed typical examples of standard curves:

Metanephrine Plasma ELISA



Normetanephrine Plasma ELISA



Quality control: All kit controls must be found within the acceptable ranges as printed on the QC certificate. If the criteria are not met, the run is not valid and should be repeated.

9 Assay Characteristics

9.1 Normal Range

The reference ranges given below should only be taken as a guideline. It is recommended that each laboratory should establish its own normal values.

Metanephrine	Normetanephrine
< 90 pg/ml	< 190 pg/ml

9.2 Sensitivity

	Lower detection limit	Calculation
Metanephrine	< 7 pg/ml	OD _{Cal1} - 2xSD
Normetanephrine	< 7 pg/ml	OD _{Cal1} - 2xSD

9.3 Specificity (Cross Reactivity)

Substance	Metanephrine (%)	Normetanephrine (%)
Metanephrine	100	0.015
Normetanephrine	0.130	100
3-Methoxytyramine	0.003	0.076
Adrenaline	0.039	0.0003
Noradrenaline	0.0008	0.0030
Tyramine	0.0005	0.0043
Dopamine	< 0.0001	0.0006
Homovanillic acid	< 0.0001	< 0.0001
Vanillic mandelic acid	< 0.0001	< 0.0001
L-Dopa	< 0.0001	< 0.0001
L-Tyrosine	< 0.0001	< 0.0001

9.4 Recovery

	Range (pg/ml)	Mean (%)	Range (%)
Metanephrine	20 - 900	94	82 - 117
Normetanephrine	34 - 1633	96	90 - 108

9.5 Linearity (Dilution with Standard 1)

	Range (pg/ml)	Highest Dilution	Mean (%)	Range (%)
Metanephrine	43 - 886	1 : 20	103	96 - 112
Normetanephrine	70 - 1613	1 : 20	93	86 - 105

9.6 Precision

	Range (pg/ml)	Intra-Assay-CV	Range (pg/ml)	Inter-Assay-CV
Metanephrine	157 – 403	7.9 – 7.8 %	118 – 276	8.8 – 8.6 %
Normetanephrine	193 – 757	8.4 – 4.1 %	246 – 551	9.3 – 9.2 %

9.7 Method Comparison

	Method	Correlation
Metanephrine	LC/MS	$Y = 1.04 \times \text{LC/MS} - 23$; $R = 0.991$; $N = 32$
Normetanephrine	LC/MS	$Y = 0.99 \times \text{LC/MS} - 8$; $R = 0.984$; $N = 32$

9.8 Calibration

The assay is calibrated by addition of defined stock solutions. The accuracy of the method was verified by comparing normal ranges (see 9.1) and other methods (see 9.7).

9.9 Limitations of Procedure

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire.

Samples showing concentrations above the highest standard have to be diluted with Standard 1 and reassayed.

9.10 Interfering Substances

Hemolytic, lipemic and icteric samples should not be used.

10 Changes to declare

Version _5: IFU has been re-formatted. The manufacturer and distributor information have been changed. Component names as printed on labels were included in sections 6, 7 and pipetting schemes to provide greater clarity. Pipetting scheme has been revised. No changes have been made to components or execution of protocols themselves.

11 Literature

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- Lenders, J.; Keiser, H.; Goldstein, D.; et al. (1995):
Plasma Metanephrines in the Diagnosis of Pheochromocytoma
Annals of Internal Medicine • Volume 123 • Number 2

Pipetting Scheme - Sample Preparation

For Metanephrine and Normetanephrine together in one PRECI-TUBE:

		Standards	Controls	Samples
PRECI-TUBES:				
CAL 1 - 6	μl	200		
CON 1 & 2	μl		200	
EDTA-Plasma	μl			200
PRECI 1	μl	25	25	25
PRECI 2	μl	25	25	25

Vortex thoroughly

Centrifuge for 15 minutes with at least 4,000 x g

ACYL-BUFF	μl	50	50	50
ACYL-REAG	μl	40	40	40

Immediately, after pipetting ACYL-REAG into tube, vortex softly at medium speed for 2 to 4 seconds, before continuing with the next tube.

Take care not to disturb the pellet at the bottom of the tube.

Centrifuge for 15 minutes with at least 4,000 x g

Take 50 μl each for the Metanephrine and Normetanephrine ELISAs

Pipetting Scheme - ELISA

For Metanephrine and Normetanephrine in separate microtiterplates:

	Metanephrin (blue) STRIPS-MN			Normetanephrin (yellow) STRIPS-NMN		
	Acyl. Stand.	Acyl. Contr.	Acyl. Samples	Acyl. Stand.	Acyl. Contr.	Acyl. Samples
Transfer from PRECI-TUBEs into STRIPS	50	50	50	50	50	50

Shake for 1 hour at room temperature, do not cover plates

AS-MN	μl	25	25	25	-	-	-
AS-NMN	μl	-	-	-	25	25	25

Cover plate with FOIL

Shake for 2 hours at room temperature

4 x washing (approx. 300 μl WASH per Well)

CONJ	μl	100	100	100	100	100	100
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Shake for 30 minutes at room temperature

4 x washing (approx. 300 μl WASH per Well)

SUB	μl	100	100	100	100	100	100
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Shake for 30 ± 5 minutes at room temperature

STOP	μl	100	100	100	100	100	100
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Reading of absorbance at 450 nm