

Instructions for Use

IBL

Normetanephrine ELISA

Enzyme immunoassay for the in-vitro-diagnostic quantitative determination of normetanephrine in human urine.

REF **RE59171**

 **96**

   **2-8°C**

EU: **IVD**  U.S.: *For in-vitro diagnostic use only. 510(k) exempt.*

IBL IMMUNO-BIOLOGICAL LABORATORIES

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1. INTENDED USE

Enzyme immunoassay for the *in-vitro diagnostic* quantitative determination of normetanephrine in human urine.

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 increasing amounts 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.

3. TEST PRINCIPLE

The assay procedure follows the basic principle of competitive ELISA whereby there is competition between a biotinylated and a non-biotinylated antigen for a fixed number of antibody binding sites. The amount of biotinylated antigen bound to the antibody is inversely proportional to the analyte concentration of the sample. When the system is in equilibrium, the free biotinylated antigen is removed by a washing step and the antibody bound biotinylated antigen is determined by use of anti-biotin alkaline phosphatase as marker and p-nitrophenyl phosphate as substrate. Quantification of unknowns is achieved by comparing the enzymatic activity of unknowns with a response curve prepared by using known standards.

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. For further information (clinical background, test performance, automation protocols, alternative applications, literature, etc.) please refer to the IBL-Homepage.
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. Avoid contact with Stop solution. It may cause skin irritations and burns.

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 sun light. The storage and stability of specimen 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.

Urine

It is possible to use spontaneous as well as 24 h urine. The total volume of urine excreted during a 24 h period should be collected and mixed in a single bottle containing 10 - 15 mL of 6 N HCl as preservative. Determine total volume for calculation of results. **Mix and centrifuge samples before use in the assay.**

Storage:	≤ -20°C (Aliquots)	Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles.
Stability:	6 mon	

7. MATERIALS SUPPLIED

The reagents provided with this kit are sufficient for single determinations in the sample preparation (hydrolysis and acylation) and duplicates in the assay. Additional reagents are available upon request.

Quantity	Symbol	Component
1 x 50 mL	ASSAYBUF CONC	Assay Buffer, Concentrate (10x) Contains: phosphate buffer, BSA, 1 % NaN ₃ .
1 x 2 mL	ACYLREAG	Acylation Reagent Ready to use. Contains: dimethylformamide.
1 x 10 mL	INDICATORBUF	Indicator Buffer Purple colored. Ready to use. Contains: Tris buffer, phenol red (color change at pH < 7.5).
50 x	HYDRTUB	Hydrolyzation Tubes Disposable polystyrene tubes (uncoated).
1 x 20 mL	HCL	HCl Ready to use. 0.1 M HCl.
1 x 12x8	MTP	Microtiter Plate Break apart strips. Coated with anti-rabbit IgG (goat, polyclonal).
1 x 7 x 0.35 mL	CAL A-G	Standard A-G 0; 77; 192; 480; 1200; 3000; 7500 µg/L 0; 0.42; 1.05; 2.63; 6.56; 16.4; 41.0 µmol/L Ready to use. Contains: Normetanephrine, 0.1 M HCl.
1 x 2 x 0.5 mL	CONTROL 1+2	Control 1+2 Ready to use. Concentrations / acceptable ranges see vial labels.
3 x	BIOTIN LYO	Normetanephrine Biotin, lyophilized Contains: Normetanephrine Biotin, Tris buffer, < 0.1 % NaN ₃ (reconstituted).
1 x 5 mL	ANTISERUM	Normetanephrine Antiserum Blue colored. Ready to use. Contains: Antiserum (rabbit), phosphate buffer, 0.1 % NaN ₃ .
1 x 250 µL	ENZCONJ CONC	Enzyme Conjugate, Concentrate (100x) Contains: anti-Biotin antibodies, conjugated to alkaline phosphatase, Tris buffer, 0.01 % NaN ₃ .
9 x	PNPP SUBS	PNPP Substrate Tablets In one foil packet. Contains: p-nitrophenyl phosphate (PNPP).
1 x 27 mL	PNPP BUF	PNPP Substrate Buffer Ready to use. Contains: diethanolamine, water, 0.05 % NaN ₃ .
1 x 5 mL	PNPP STOP	PNPP Stop Solution Ready to use. Contains: 1 M NaOH, 0.25 M EDTA.
1 x 50 mL	WASHBUF CONC	Wash Buffer, Concentrate (10x) Contains: phosphate buffer, Tween, 0.2 % NaN ₃ .
3 x	FOIL	Adhesive Foil

8. MATERIALS REQUIRED BUT NOT SUPPLIED


1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV). Volumes: 10; 50; 100; 1000 µL
2. Disposable glass test tubes (12 x 75 mm)
3. Orbital shaker (200-900 rpm) (e.g. EAS 2/4, SLT)
4. Vortex mixer
5. Water bath, 90°C, 37°C
6. 8-Channel Micropipettor with reagent reservoirs
7. Wash bottle, automated or semi-automated microtiter plate washing system
8. Microtiter plate reader capable of reading absorbance at 405 nm (reference wavelength 600-650 nm)
9. Bidistilled or deionised water

10. Paper towels, pipette tips and timer

9. PROCEDURE NOTES


- 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.
- 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.
- Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each reagent, standard or specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
- It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
- Use a pipetting scheme to verify an appropriate plate layout.
- 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.
- 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 precisely with Wash Buffer, and that there are no residues in the wells.
- 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

	The contents of the kit for 96 determinations can be divided into 3 separate runs. The volumes stated below are for one run with 4 strips (32 determinations).
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If a larger number of strips is to be used, the volumes have to be changed accordingly.

10.1. Preparation of lyophilized or concentrated components

	Do not mix up Metanephrine and Normetanephrine Enzyme Conjugate in case you use the Metanephrine ELISA in parallel.
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Dilute/dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
15 mL	Assay Buffer	ad 150 mL	bidist. water	1:10		2-8°C	2 w
15 mL	Wash Buffer	ad 150 mL	bidist. water	1:10		2-8°C	4 w
1 vial	Normetanephrine Biotin	with 2 mL	diluted Assay Buffer		Prepare freshly and use only once. Let stand for 15 min. Mix without foaming.	≤ -20°C (Aliquots)	2 mon
60 µL	Enzyme Conjugate	with 6 mL	diluted Assay Buffer	1:101	Prepare freshly and use only once.	18-25°C	30 min
3	PNPP Substrate Tablets	with 8 mL	PNPP Substrate Buffer		Prepare freshly and use only once.	18-25°C	10 min

10.2. Hydrolyzation of Urine Samples, Standards and Controls for total Normetanephrine (in Hydrolyzation Tubes)

	The hydrolyzation step is necessary for the determination of <u>total</u> normetanephrine and total metanephrine. No hydrolyzation is required when assaying <u>free</u> normetanephrine and metanephrine. Samples suspected to contain concentrations higher than the highest standard have to be diluted with 0.1 M HCl before hydrolyzation step.
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The sample preparation may be carried out in two different ways. 50 µL urine are either hydrolyzed in the hydrolyzation tubes and thereafter transferred into glass tubes for acylation, or the complete sample preparation is carried out with 10 µL urine in the hydrolyzation tube (e. g. in case of pipetting robots).

10.2.1. Two step sample preparation

1.	Pipette 50 µL of each Standard A-G, Control and patient urine sample into the respective hydrolyzation tubes.
2.	Pipette 200 µL of HCl (0.1 M) into each tube.
3.	Close tubes. Hydrolyze 1 h at 90°C (check temperature with thermometer). Allow to cool down to room temperature afterwards. Vortex.
4.	Pipette 50 µL of each <u>hydrolyzed</u> Standard, Control and urine sample into the respective glass test tubes.
5.	Pipette 100 µL of Indicator Buffer into each tube.
6.	Pipette 20 µL of Acylation Reagent quickly into each tube. Vortex each tube immediately after pipetting.
7.	Cover tubes. Incubate 30 min at 37°C in a waterbath.
8.	Pipette 1 mL of diluted Assay Buffer into each tube. Vortex.

10.2.2. Entire sample preparation in the hydrolyzation tubes

1.	Pipette 10 µL of each Standard, Control and urine sample into the respective hydrolyzation tubes.
2.	Pipette 40 µL of 0.1 M HCl into each tube.
3.	Close tubes. Hydrolyze 1 h at 90°C (check temperature with thermometer). Allow to cool down to room temperature afterwards. Vortex.
4.	Pipette 100 µL of Indicator Buffer into each tube. Vortex.
5.	Pipette 20 µL of Acylation Reagent into each tube. Vortex each tube immediately after pipetting. Take care that addition of acylation reagent into the content of the tubes is complete.
6.	Close tubes. Incubate 30 min at 37°C in a waterbath.
7.	Pipette 1 mL of diluted Assay Buffer into each tube. Vortex.

11. TEST PROCEDURE

11.1. First Day (in microtiter plate)

1.	Pipette 20 µL of each <u>acylated</u> Standard, acylated Control and acylated patient sample into the respective wells of the microtiter plate.
2.	Pipette 50 µL of Normetanephine Biotin into each well.
3.	Pipette 50 µL of Normetanephine Antiserum into each well.
4.	Cover plate with adhesive foil. Shake plate carefully. Incubate overnight (14 - 20 h) at 2 - 8 °C.

11.2. Second Day

1.	Remove adhesive foil. Discard incubation solution. Wash plate 3 x with 250 µL of diluted Wash Buffer . Remove excess solution by tapping the inverted plate on a paper towel.
2.	Pipette 150 µL of freshly prepared Enzyme Conjugate into each well.
3.	Cover plate with new adhesive foil. Incubate 120 min at RT (18-25°C) on an orbital shaker (500 rpm).
4.	Approx. 10 min before end of incubation prepare PNPP Substrate Solution.
5.	Remove adhesive foil. Discard incubation solution. Wash plate 3 x with 250 µL of diluted Wash Buffer . Remove excess solution by tapping the inverted plate on a paper towel.
6.	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. Use positive displacement and avoid formation of air bubbles.
7.	Pipette 200 µL of freshly prepared PNPP Substrate Solution into each well.
8.	Incubate 20 min at RT (18-25°C) on an orbital shaker (500 rpm).
9.	Stop the substrate reaction by adding 50 µL of PNPP Stop Solution into each well. Briefly mix

	contents by gently shaking the plate.
10.	Measure optical density with a photometer at 405 nm (Reference-wavelength: 600-650 nm) within 60 min after pipetting of the Stop Solution.

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 other applicable standards/laws. 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.

It is recommended to participate at appropriate quality assessment trials.

Remarks for participants in the Quality Control Assessment Schemes

IBL-Hamburg is regularly taking part with the Normetanephrine immunoassay in Quality Control Assessment Schemes. According to the organizers of the QC schemes they are using racemic (+/-) normetanephrine for spiking of their specimen and not the biologically active form of normetanephrine. Consequently, when measuring spiked samples in the high concentration range, the customer will find about 30-40% lower results with the normetanephrine immunoassay compared with HPLC.

If the organizers use native patient samples with elevated normetanephrine concentrations, this problem does not occur. The reason for this is that the antibody used in the kit recognizes the biologically active form of normetanephrine. Therefore, please check the preparation of specimen used in the QC scheme when interpreting your results.

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 Logisitcs 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 from the standard curve.

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

Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

Calculate the 24 h excretion for each urine sample: $\mu\text{g}/24\text{h} = \mu\text{g}/\text{L} \times \text{L}/24\text{h}$

Conversion:

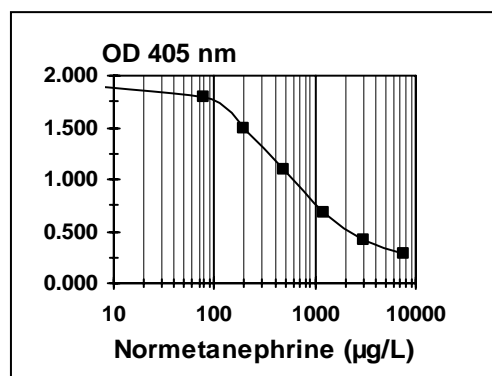
1 ng/mL = 1 $\mu\text{g}/\text{L}$

Normetanephrine ($\mu\text{g}/\text{L}$) $\times 5.46 \times 10^{-3} = \mu\text{mol}/\text{L}$

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	Normetanephrine ($\mu\text{g}/\text{L}$)	Mean OD	OD/OD _{max} (%)
A	0.0	2.095	100.0
B	77	1.795	85.7
C	192	1.495	71.4
D	480	1.097	52.3
E	1200	0.688	32.8
F	3000	0.422	20.1
G	7500	0.284	13.5



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:

Mean: 207 $\mu\text{g}/\text{d}$ Range: 30 - 440 $\mu\text{g}/\text{d}$ (95 % percentile)

It is recommended that each laboratory establishes its own range of normal values.

15. LIMITATIONS OF THE PROCEDURE

Specimen collection has a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.

For cross-reactivities, see PERFORMANCE.

16. PERFORMANCE

Analytical Specificity (Cross Reactivity)	Substance		Cross Reactivity (%)	Cross-reactivity of other substances tested < 0.05 %
	Metanephrine		0.6	
	3-Methoxy-Thyramine		0.4	
	4-OH-3-Methoxy-Phenylglycol		0.15	
Analytical Sensitivity (Limit of Detection)	12 µg/L	Mean signal (Zero-Standard) - 2SD		
Precision	Range (µg/L)	CV (%)		
	Intra-Assay	55 - 3444	2.8 - 10.6	
	Inter-Assay	70 - 1350	5.0 - 12.3	
Linearity	Range (µg/L)	Serial dilution up to	Range (%)	
	467 - 4891	1:32	80 - 113	
Recovery	Mean (%)	Range (%)	% Recovery after spiking	
	97	89 - 114		
Method Comparison versus GCMS	GCMS = 1.14 x IBL-Assay + 5.08		r = 0.94; n = 47	

17. PRODUCT LITERATURE REFERENCES

1. Creces J., Appleton Ch.: Catecholamines and their Metabolites: Evaluation of a commercial ELISA. Clin. Biochem., QML Pathology, Brisbane QLD (2004)
2. Wassell J et al. Freedom from drug interference in new immunoassays for urinary catecholamines and metanephrines. Clin Chem 45:12 2216-2223 (1999)
Address: Wassell Julie, Wythenshawe hospital, Manchester, UK.
3. Wolthers BG, Kema IP, Volmer M, Wesemann R, Westermann J and Manz B. Evaluation of urinary metanephrine and normetanephrine enzyme immunoassay (ELISA) kits by comparison with isotope dilution mass spectrometry. Clin. Chem., 43: 114-120 (1997).
Address: Bert G. Wolthers, Central Laboratory for Clinical Chemistry, University Hospital, P.O. Box 30.001, 9700 RB Groningen, The Netherlands

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθμός-Κατ.:
	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:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
	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: / Fabricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
<p>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.</p>	

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