Instructions for Use

Metanephrine ELISA

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

REF **RE59181**

Σ **96**

2-8°C

EU: IVD CE

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1. INTENDED USE

Enzyme immunoassay for the *in-vitro diagnostic* quantitative determination of metanephrine 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 streptavidine 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.
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. 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 sunlight. The storage and stability of specimens and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the indicated expiry after the kit is broken. Make sure that the broken bag is tightly closed 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 sunlight. Avoid repeated freeze-thaw cycles.
Stability:	6 months	

7. MATERIALS SUPPLIED

⚠	The reagents provided with this kit are sufficient for up to 96 single determinations, or up to 48 duplicates, Metanephrine and Normetanephrine each.

Quantity	Symbol	Component
1 x 12 x 8	**MTP**	**Microtiter Plate** Break apart strips. Coated with anti-rabbit IgG (goat, polyclonal).
1 x 8 mL	**ANTISERUM**	**Metanephrine Antiserum** Green colored. Ready to use. Contains: Antiserum (rabbit), phosphate buffer, 0.1 % NaN_3.
1 x 8 mL	**BIOTIN**	**Metanephrine Biotin** Ready to use. Contains: Metanephrine Biotin, phosphate buffer, BSA, 0.1 % NaN_3.
1 x 400 µL	**ENZCONJ** **CONC**	**Enzyme Conjugate** Concentrate (50x) Contains: streptavidin alkaline phosphatase, Tris buffer, 0.01 % NaN_3.
1 x 7 x 0.35 mL	**CAL A-G**	**Standard A-G** 0; 26; 64; 160; 400; 1000; 2500 µg/L 0; 0.13; 0.33; 0.81; 2.03; 5.08; 12.7 µmol/L Ready to use. Contains: Metanephrine, 0.1 M HCl.
1 x 2 x 0.5 mL	**CONTROL 1+2**	**Control 1+2** Ready to use. Exact concentrations see vial labels or QC certficate.
1 x 2.25 mL	**ACYLREAG**	**Acylation Reagent** Ready to use. Contains: dimethylformamide.
2 x 50 x	**HYDRTUB**	**Hydrolyzation Tubes** Disposable polystyrene tubes (uncoated). Additional Hydrolysation tubes are available under REF KEZZ661.
1 x 20 mL	**HCL**	**HCl** Ready to use. 0.1 M HCl.
1 x 50 mL	**ASSAYBUF** **CONC**	**Assay Buffer** Concentrate (10x) Contains: phosphate buffer, BSA, 1 % NaN_3.
1 x 11 mL	**INDICATORBUF**	**Indicator Buffer** Purple colored. Ready to use. Contains: Tris buffer, phenol red (color change at pH < 7.5).
2 x 100 mL	**WASHBUF** **CONC**	**Wash Buffer** Concentrate (10x) Contains: Tris buffer, HCl, Tween, 0.2 % NaN_3.
1 x 13 mL	**PNPP SUBS**	**PNPP Substrate Solution** Ready to use. Contains: p-nitrophenyl phosphate (PNPP).
1 x 15 mL	**PNPP STOP**	**PNPP Stop Solution** Ready to use. Contains: 1 M NaOH, 0.25 M EDTA.
3 x	**FOIL**	**Adhesive Foil**

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 10; 50; 100; 1000 µL
2. Disposable glass 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
11. Waterproof marker for labelling the hydrolization tubes

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.
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. Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate 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.
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

- **For manual and automatic version**.

⚠	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).** **If the customer wants to reduce the number of standards from 7 to 6 he can omit Standard G. The reportable range will then be reduced to 1000 µg/L.**

If a larger number of strips is to be used, the volumes have to be changed accordingly.

10.1. Preparation of concentrated components

⚠	Do not mix up Metanephrine and Normetanephrine Enzyme Conjugate in case you use the Normetanephrine ELISA in parallel.

Dilute / dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
15 mL	**ASSAYBUF CONC**	ad 150 mL	bidist. water	1:10		2-8 °C	2 weeks
60 mL	**WASHBUF CONC**	ad 600 mL	bidist. water	1:10		2-8 °C	4 weeks
120 µL	**ENZCONJ CONC**	with 6 mL	diluted Assay Buffer	1:51	Prepare freshly and use only once.	18-25 °C	5 h

10.2. Hydrolyzation of Urine Samples, Standards and Controls for <u>total</u> Metanephrine (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.

10.2.1. Sample preparation in the hydrolyzation tubes

1.	Pipette **10 µL** of each **Standard, Control and urine sample** into the respectively labelled 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 15 min** at **RT (18-25 °C).**
7.	Pipette **1 mL** of diluted **Assay Buffer** into each tube. Vortex.

11. TEST PROCEDURE

- **In microtiter plate for manual and automated version**

1.	Pipette **50 µL** of each acylated **Standard**, acylated **Control** and acylated **patient sample** into the respective wells of the microtiter plate.
2.	Pipette **50 µL** of **Metanephrine Biotin** into each well.
3.	Pipette **50 µL** of **Metanephrine Antiserum** into each well.
4.	Cover plate with adhesive foil. Shake plate carefully. **Incubate 1 h** at **RT on an orbital shaker (500 rpm).**
5.	Remove adhesive foil. Discard incubation solution. Wash plate with an automate **6 x** with **250 µL** of diluted **Wash Buffer (3 x manually)**. Remove excess solution by tapping the inverted plate on a paper towel.
6.	Pipette **150 µL** of freshly prepared **Enzyme Conjugate** into each well.
7.	Cover plate with new adhesive foil. **Incubate 30 min** at **RT (18-25 °C)** on an orbital shaker (500 rpm).
8.	Remove adhesive foil. Discard incubation solution. Wash plate with an automate **6 x** with **250 µL** of diluted **Wash Buffer (3 x manually).** 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. Use positive displacement and avoid formation of air bubbles.
10.	Pipette **100 µL** of **PNPP Substrate Solution** into each well.
11.	**Incubate 40 min** at **RT (18-25 °C)** on an orbital shaker (500 rpm).
12.	Stop the substrate reaction by adding **100 µL** of **PNPP Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
13.	**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 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 labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials.

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.

Remarks for participants in the Quality Control Assessment Schemes

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

If the organizers use native patient samples with elevated metanephrine concentrations, this problem does not occur. The reason for this is that the antibody used in the kit recognizes the biologically active form of metanephrine. 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 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.

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: µg/24 h = µg/L x L/24 h

Conversion:

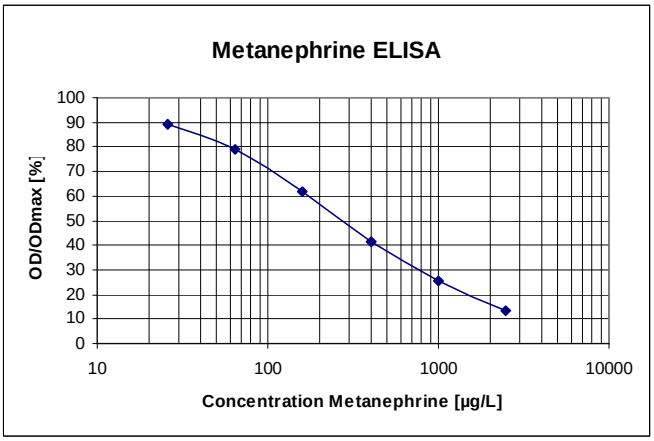
1 ng/mL = 1 µg/L

Metanephrine (µg/L) x 5.07 x 10^{-3} = µmol/L

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	Metanephrine (µg/L)	OD_{Mean}	OD/OD_{max} (%)
A	0.0	1.867	100
B	26	1.670	89
C	64	1.473	79
D	160	1.153	62
E	400	0.776	42
F	1000	0.473	25
G	2500	0.245	13



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: 134 µg/d Range: 25 – 312 µg/d (95 % percentile)

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

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

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)	Cross-reactivity of other substances tested < 0.2 %
	Adrenalin	0.1	
	Normetanephrine	0.2	
	Caffeic acid	0.1	
	(±)-Synephrine	0.8	
Analytical Sensitivity (Limit of Detection)	12 µg/L	Mean signal (Zero-Standard) - 2SD	
Precision	Range (µg/L)	CV (%)	
Intra-Assay	116-1477	8.8-5.0	
Inter-Assay	111-1559	7.3–11.3	
Linearity	Range (µg/L)	Serial dilution up to	Range (%)
	57-1533	1:8	85-125
Recovery	Mean (%)	Range (%)	% Recovery after spiking
	96	86-120	
Method Comparison versus commercial ELISA	y (IBL RE59181) = 0.89 (Other Assay) + 50.40		r^2 = 0.93; r = 0.96; n = 75
Method Comparison manual versus Automated version	manual version = 1.05 (DSX) + 13.27		r^2 = 0.99; r = 0.99; n = 13

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

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.

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