DRG® Metanephrine (Urine) (EIA-2748)

RUO in the USA

Revised 7 Feb. 2011 rm (Vers. 5.1)

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

INTENDED USE

Enzyme immunoassay for determination of metanephrine in human urine.

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.

WARNINGS AND PRECAUTIONS

1. 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 DRG 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 upon request.
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.

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.

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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	

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 7 mL	ANTISERUM	**Metanephrine Antiserum** Green colored. Ready to use. Contains: Antiserum (rabbit), phosphate buffer, 0.1 % NaN_3.
3 x	BIOTIN LYO	**Metanephrine Biotin**, lyophilized Contains: Metanephrine Biotin, Tris buffer, < 0.1 % NaN_3 (reconstituted).
1 x 250 µL	ENZCONJ CONC	**Enzyme Conjugate**, Concentrate (100x) Contains: anti-Biotin antibodies, conjugated to 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). Additonal Hydrolysation Tubes are available upon request.
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.

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Quantity	Symbol	Component
1 x 10 mL	INDICATORBUF	**Indicator Buffer** Purple colored. Ready to use. Contains: Tris buffer, phenol red (color change at pH < 7.5).
1 x 50 mL	WASHBUF CONC	**Wash Buffer**, Concentrate (10x) Contains: Tris buffer, HCl, Tween, 0.2 % NaN_3.
1 x 12 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**

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

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. 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.

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.

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).
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 3000 µg/L.

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

Preparation of lyophilized or concentrated components

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

Dilute/ dissolve	Component		Diluent	Rela -tion	Remarks	Storage	Stabil ity
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	**Metanephrin e 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	1:10 1	Prepare freshly and use only once.	18-25°C	5 h

Hydrolyzation of Urine Samples, Standards and Controls for <u>total</u> Metanephrine (in Hydrolyzation Tubes)

⚠ The hydrolyzation step is necessary for the determination of total normetanephrine and total metanephrine. No hydrolyzation is required when assaying free 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.

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 15 min** at **Room temperature.**
7. Pipette **1 mL** of diluted **Assay Buffer** into each tube. Vortex.

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.

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.

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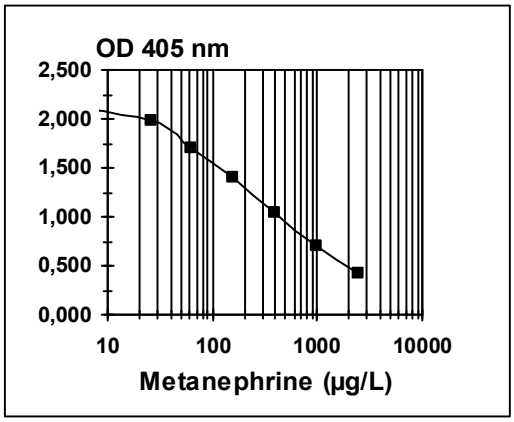
For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).
The concentration of the samples can be read directly from the standard curve.
In case of diluted samples the values have 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/24h = µg/L x L/24h
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	2.220	100
B	26	1.987	90
C	64	1.707	77
D	160	1.393	63
E	400	1.048	47
F	1000	0.702	32
G	2500	0.427	19



PRODUCT LITERATURE REFERENCES

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