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Instructions for use

Metanephrine Urine ELISA Fast Track

REF BA E-8400R

2°C–8°C

Σ 96

RUO For research use only – Not for use in diagnostic procedures

Metanephrine Urine ELISA Fast Track

1. Introduction

1.1 Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of Metanephrine in urine

During the sample preparation Metanephrine (Metadrenaline) is quantitatively acylated.
The subsequent competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated standards, controls and samples and the solid phase bound analyte compete for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm.
Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations.

⚠ *The anti-Metanephrine antibodies used in this test kit only recognise the biologically relevant L-forms of Metanephrine. Commercially available synthetic Metanephrine is always a mixture of the D- and L-forms. The ratio between both forms differs widely from lot to lot. This has important implications if synthetic Metanephrine is used to enrich native samples. As only about 50% of the synthetic Metanephrine – the L-portion – will be detected by use of this kit, spiked samples will be underestimated. Therefore native samples containing solely the L-form should be used.*

1.2 Background

Metanephrine and Normetanephrine are the metabolites of the catecholamines Epinephrine and Norepinephrine, respectively. They are metabolized to Vanillylmandelic acid or excreted with the urine.

As catecholamine secretion from neuroendocrine cells might show high variations, urine samples collected over a period of 24 hours are used to average these fluctuations.

2. Procedural cautions, guidelines, warnings and limitations

2.1 Procedural cautions, guidelines and warnings

(1) This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
(2) The principles of Good Laboratory Practice (GLP) have to be followed.
(3) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
(4) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
(5) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
(6) The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant and used in the frame provided.
(7) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
(8) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
(9) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
(10) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(11) A standard curve must be established for each run.
(12) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
(13) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
(14) Avoid contact with Stop Solution containing 0.25 M H_2SO_4. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
(15) TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them.
(16) For information on hazardous substances included in the kit please refer to Safety Data Sheet (SDS). The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
(17) Kit reagents must be regarded as hazardous waste and disposed according to national regulations.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.

2.2.1 Interfering substances

24-hour urine

Please note the sample preparation! If the percentage of the final concentration of acid is too high, this will lead to incorrect results for the urine samples.

2.2.2 Drug interferences

There are no known substances (drugs) which ingestion interferes with the measurement of Metanephrine level in the sample.

2.2.3 High-Dose-Hook effect

No hook effect was observed in this test.

3. Storage and stability

Store the unopened reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 – 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

4. Materials

4.1 Content of the kit

BA D-0023 REAC-TUBES **Reaction Tubes** - Ready to use

Content: Reaction Tubes in a resealable pouch

Volume: 2 x 50 tubes

BA E-0030 WASH-CONC 50x **Wash Buffer Concentrate -** Concentrated 50x

Content: Buffer with a non-ionic detergent and physiological pH

Volume: 1 x 20 ml/vial, light purple cap

BA E-0045 CONJUGATE **Enzyme Conjugate** - Ready to use

Content: Goat anti-rabbit immunoglobulins conjugated with peroxidase

Volume: 1 x 12 ml/vial, red cap

BA E-0055 SUBSTRATE **Substrate** - Ready to use

Content: Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide

Volume: 1 x 12 ml/vial, black cap

BA E-0080 STOP-SOLN **Stop Solution** - Ready to use

Content: 0.25 M sulfuric acid

Volume: 1 x 12 ml/vial, light grey cap

Hazards identification:

H290 May be corrosive to metals.

BA E-0131 TMT ADR MN **Metanephrine Microtiter Strips** - Ready to use

Content: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable blue pouch with desiccant

BA E-8410 MN-AS **Metanephrine Antiserum** - Ready to use

Content: Rabbit anti-Metanephrine antibody, blue coloured

Volume: 1 x 12 ml/vial, blue cap

BA R-0012 ACYL-CONC **Acylation Concentrate** - Concentrated

Content: Concentrated acylation reagent

Volume: 1 x 0.5 ml/vial, pink cap

Hazards identification:

H 314 Causes severe skin burns and eye damage.

BA R-8619 HCL **Hydrochloric Acid** - Ready to use

Content: 0.25 M hydrochloric acid, yellow coloured

Volume: 1 x 30 ml/vial, dark green cap

Standards and **Controls** - Ready to use

Cat. no.	Component	Colour/Cap	Concentration ng/ml (= µg/l) MN	Concentration nmol/l MN	Volume/ Vial
BA R-8601	STANDARD A	white	0	0	4 ml
BA R-8602	STANDARD B	light yellow	20	101	4 ml
BA R-8603	STANDARD C	orange	60	304	4 ml
BA R-8604	STANDARD D	dark blue	200	1 014	4 ml
BA R-8605	STANDARD E	light grey	600	3 042	4 ml
BA R-8606	STANDARD F	black	2 000	10 140	4 ml
BA R-8651	CONTROL 1	light green	Refer to QC-Report for expected value and acceptable range!		4 ml
BA R-8652	CONTROL 2	dark red			4 ml

Conversion: Metanephrine (ng/ml) x 5.07 = Metanephrine (nmol/l)

Content: Acidic buffer with non-mercury preservatives, spiked with defined quantity of Metanephrine

BA R-0075 ACYL-DILUENT **Acylation Diluent** - Ready to use

Content: Dimethylsulfoxide

Volume: 1 x 4 ml/vial, dark grey cap

BA R-8611 ACYL-BUFF **Acylation Buffer** - Ready to use

Content: TRIS buffer

Volume: 1 x 30 ml/vial, white cap

4.2 Additional materials and equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 10 – 600 µl; 1.2 – 3 ml
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 - 650 nm
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)
- Vortex mixer
- Temperature controlled water bath (90 °C) or similar heating device

⚠ The assay can be performed with or without shaking. If a microtiter plate shaker is used, it should have the following characteristics: shaking amplitude 3 mm; approx. 600 rpm

5. Sample collection and storage

Spontaneous or 24-hour urine, collected in a bottle containing 10 - 15 ml of 6 M HCl, should be used.
Determine the total volume of urine excreted during a period of 24 h for calculation of the results.
Storage: up to 5 days at 2 – 8 °C, for longer periods (up to 6 months) at -20 °C.
Repeated freezing and thawing should be avoided. Avoid exposure to direct sunlight.

6. Test procedure

Allow all reagents to reach room temperature and mix thoroughly by gentle inversion before use. Number the Reaction Tubes accordingly. Duplicate determinations are recommended. It is recommended to number the strips of the microwell plate before usage to avoid any mix-up.
The binding of the antibodies and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the absorption values may vary if a thermostat is not used. The higher the temperature, the higher the absorption values will be. The absorption values also depend on the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20 – 25 °C.

6.1 Preparation of reagents

Wash Buffer
Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 ml.
Storage: 1 month at 2 – 8 °C

Acylation Solution

⚠ *Before preparing the Acylation Solution make sure that the Acylation Diluent (BA R-0075) has reached room temperature (≥ 20°C) and forms a homogenous, crystal-free solution.*

Dilute the Acylation Concentrate (BA R-0012) 1 + 60 with Acylation-Diluent in a glass or polypropylene-vial.

Acylation Concentrate	10 µl	20 µl	25 µl	50 µl
Acylation-Diluent	600 µl	1.2 ml	1.5 ml	3 ml

⚠ *The Acylation Solution has to be prepared freshly prior to the assay (not longer than 60 minutes in advance). Discard after use!*

Metanephrine Microtiter Strips
In rare cases residues of the blocking and stabilizing reagent can be seen in the wells as small, white dots or lines. These residues do not influence the quality of the product.

6.2 Sample preparation and acylation

Hydrolysis

1.	Pipette **25 µl** of **standards**, **controls,** and **urine samples** into the respective **Reaction Tubes.**
2.	Add **250 µl Hydrochloric Acid** to all tubes.
3.	Mix thoroughly (vortex) and hydrolyze for **30 min** at **90 °C**.
4.	Cool down the tubes to room temperature.
⚠	***For the measurement of the free Metanephrine only, leave away step 3 and 4.***

Acylation

1.	Pipette **250 µl** of **Acylation Buffer** into all tubes.
2.	Add **25 µl** of **Acylation Solution** *(refer to 6.1)* to all tubes.
3.	Mix thoroughly (vortex) and acylate for **15 min** at **RT** (20 – 25 °C).
4.	Add **2.5 ml water** (deionized, distilled, or ultra-pure) to all tubes.
⚠	Take **25 µl** of the acylated **standards**, **controls and urine samples** for the **Metanephrine ELISA**.

6.3 Metanephrine ELISA

The usage of a shaker is not mandatory. The alternative protocol without shaker is highlighted in italic and shaded in grey.

1.	Pipette **25 µl** of the **acylated standards, controls and samples** into the appropriate wells of the **Metanephrine Microtiter Strips**.
2.	Pipette **100 µl** of the **Metanephrine Antiserum** into all wells.
3.	Incubate **30 min at RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). ***Without usage of a shaker:*** *shake* ***Metanephrine Microtiter Strips*** *shortly by hand and incubate for* ***1 h at RT*** *(20 – 25 °C).*
4.	Discard or aspirate the content of the wells. Wash the plate **3 x** by adding **300 µl** of **Wash Buffer**, **discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
5.	Pipette **100 µl** of the **Enzyme Conjugate** into all wells.
6.	Incubate for **15 min at RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). ***Without usage of a shaker:*** *incubate for* ***15 min at RT*** *(20 – 25 °C).*
7.	Discard or aspirate the content of the wells. Wash the plate **3 x** by adding **300 µl** of **Wash Buffer**, **discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
8.	Pipette **100 µl** of the **Substrate** into all wells.
9.	Incubate for **15 ± 2 min at RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). ⚠ ***Without usage of a shaker:*** *incubate for* ***15 min ± 2 at RT*** *(20 – 25 °C).* ***Avoid exposure to direct sunlight!***
10.	Add **100 µl** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
11.	**Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** (if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

Measuring range	**Metanephrine**
	10.5 – 2 000 ng/ml

The standard curve is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).
Use a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

⚠ *This assay is a competitive assay. This means: the OD-values are decreasing with increasing concentrations of the analyte. OD-values found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.*

The concentrations of the samples and controls can be read directly from the standard curve.

The amount of analyte excreted per day (µg/day) is calculated according to:

concentration of the sample (in µg/l) x volume of urine excreted per day (in l/day)

Example
The concentration of the sample read from the curve is 125 µg/l. The amount of urine collected during 24 hours is 1.3 l. Then the amount of analyte excreted during one day would be:

125 µg/l x 1.3 l/day = 162.5 µg/day

Samples found with concentrations higher than the highest standard (Standard F) should be diluted accordingly with Standard A and have to be re-assayed.

Conversion
Metanephrine (ng/ml) x 5.07 = Metanephrine (nmol/l)

Expected reference value
It is strongly recommended that each laboratory should determine its own reference value.

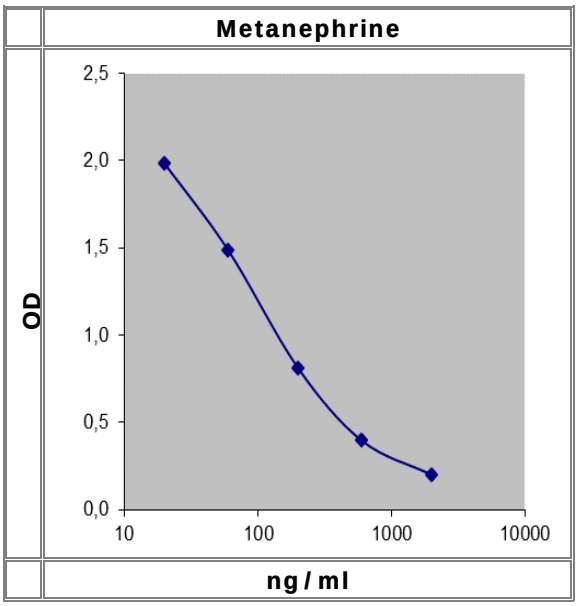
	Metanephrine
24-hour urine	< 350 µg/day

7.1 Quality control

The confidence limits of the kit controls are indicated on the QC-Report.

7.2 Typical standard curve

⚠ *Example, do not use for calculation!*



8. Assay characteristics

		Metanephrine
Analytical Sensitivity	LOD	8.6 ng/ml
	LOB	6.0 ng/ml
	LOQ	10.5 ng/ml

	Substance	**Cross Reactivity (%)**
		Metanephrine
Analytical Specificity (Cross Reactivity)	Derivatized Metanephrine	100
	Derivatized Normetanephrine	0.15
	Derivatized 3-methoxytyramine	< 0.01
	Adrenaline	3.3
	Noradrenaline	< 0.01
	Dopamine	< 0.01
	Vanillic mandelic acid, L-Dopa, Homovanillic acid, L-Tyrosin, Tyramin	< 0.01

Precision							
Intra-Assay				**Inter-Assay**			
	Sample	Range (ng/ml)	CV (%)		Sample	Range (ng/ml)	CV (%)
Metanephrine	1	34.4 ± 3.1	9	Metanephrine	1	32.8 ± 5.4	16
	2	59.8 ± 5.1	9		2	57.2 ± 9.0	16
	3	141 ± 13.8	10		3	144 ± 25.0	17
	4	575 ± 71.4	12		4	394 ± 64.1	16

		Range (ng/ml)	Serial dilution up to	Mean (%)
Linearity	Metanephrine	46.2 – 204	1:64	102

		Mean (%)	Range (%)	Range (ng/ml)
Recovery	Metanephrine	97	85 – 113	20.2 – 1484

Method Comparison versus HPLC*	Metanephrine	HPLC = 0.9 ELISA – 0.8	r = 0.99; n = 40
*The concentrations were assessed using both the ELISA and the HPLC method (external QC samples from UK NEQAS). The correlation between ELISA and HPLC is excellent. Please take in mind, that the UK control values are the mean of about 40 different HPLC users, and contain always one pathological sample per sending.			

9. References/Literature

(1) Parrott et al. Urinary corticosterone and normetanephrine levels after voluntary wheel and forced treadmill running in the db/db mouse. Journal of Diabetes Mellitus, 1(4):71-78 (2011)

(2) Petramala et al. Multiple Catecholamine-Secreting Paragangliomas: Diagnosis after Hemorrhagic Stroke in a Young Woman. Endocrine Practice, 14(3):340-346 (2008)

(3) Sato et al. Central control of bone remodeling by neuromedin U. Nature Medicine, 13:1234-1240 (2007)

⚠ ***For updated literature or any other information please contact your local supplier.***

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number	RUO	For research use only!