DIAsource ImmunoAssays S.A.
Rue de l' Industrie 8,
1400 Nivelles
Belgium

Instructions for use

Metanephrine Urine ELISA Fast Track

REF KAPL10-0500

96

+2 +8 °C

IVD

CE

Metanephrine Urine ELISA

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 kit 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 antiserum binding sites. After the system is in equilibrium, free antigen and free antigen-antiserum 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.

2. Advice on handling the test

2.1 Reliability of the test results

In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, RILIBÄK, etc.). Special attention must be paid to control checks for precision and correctness during the test; the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions.
It is recommended that each laboratory establishes its own reference intervals. The values reported in this test instruction are only indicative.
The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.

2.2 Complaints

In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

2.3 Warranty

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

2.4 Disposal

Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.
The appropriate safety data sheets of the individual products are available on the homepage. The safety data sheets correspond to the standard: ISO 11014-1.

2.5 Interference

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

2.6 Precautions

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation.
All reagents of this testkit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures.
All reagents, however, should be treated as potential biohazards in use and for disposal.

3. Storage and stability

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Do not mix various lots of any kit component within an individual assay.

4.1 Contents of the kit

BA D-0023	REAC-TUBES	**Reaction Tubes**	2 x 50	ready for use
BA E-0030	WASH-CONC 50x	**Wash Buffer Concentrate**	1 x 20 mL	concentrate, dilute content with dist. water to a final volume of 1000 mL
BA E-0045	CONJUGATE	**Enzyme Conjugate**	1 x 12 mL	ready for use, anti-rabbit IgG conjugated with peroxidase
BA E-0055	SUBSTRATE	**Substrate**	1 x 12 mL	ready for use, containing a solution of tetramethylbenzidine (TMB)
BA E-0080	STOP-SOLN	**Stop Solution**	1 x 12 mL	ready for use, containing 0.25 M H_2SO_4
BA E-0131	TM ARD MN	**Adrenaline-Metanephrine Microtiter Strips**	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, blue coloured
BA E-8410	MN-AS	**Metanephrine Antiserum**	1 x 12 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA R-0012	ACYL-CONC	**Acylation Concentrate**	1 x 0.5 mL	Concentrate. Has to be diluted prior to use.
BA R-0075	ACYL-DILUENT	**Acylation Diluent**	1 x 4 mL	ready for use
BA R-8601	STANDARD A	**Standard A**	1 x 2 mL	ready for use
BA R-8602	STANDARD B	**Standard B**	1 x 2 mL	ready for use
BA R-8603	STANDARD C	**Standard C**	1 x 2 mL	ready for use
BA R-8604	STANDARD D	**Standard D**	1 x 2 mL	ready for use
BA R-8605	STANDARD E	**Standard E**	1 x 2 mL	ready for use
BA R-8606	STANDARD F	**Standard F**	1 x 2 mL	ready for use
BA R-8619	HCL	**Hydrochloric Acid**	1 x 30 mL	ready for use, contains 0.25 M HCl
BA R-8651	CONTROL 1	**Control 1**	1 x 2 mL	ready for use
BA R-8652	CONTROL 2	**Control 2**	1 x 2 mL	ready for use
BA R-8611	ACYL-BUFF	**Acylation Buffer**	1 x 30 mL	ready for use

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

- Calibrated variable precision micropipettes (e.g. 10-100 µL / 100-1.000µL)
- Microtiter plate washing device
- ELISA reader capable of reading absorbance at 450 nm and 620 or 650 nm
- Centrifuge capable of at least 3.000 x g
- Absorbent material (paper towel)
- Distilled water, Vortex mixer
- Temperature controlled water bath (90°C) or similar heating device

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

6.1 Preparation of reagents

Wash Buffer

Dilute the 20 mL Wash Buffer Concentrate with distilled water to a final volume of 1000 mL.
Storage: up to 6 months 4–8°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!*

6.2 Sample preparation and acylation

Hydrolysis

1.	Pipette **25 µL** of **standards**, **25 µL** of **controls,** and **25 µL** of **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** to all tubes.
3.	Mix thoroughly (vortex) and acylate for **15 minutes** at **RT** (20-25°C).
4.	Add **2.5 mL dist. water** 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 hour at RT*** *(20-25°C).*
4.	Discard or aspirate the contents of the wells and **wash** each well **3 times** thoroughly with **300 µL Washbuffer**. Blot dry 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 contents of the wells and **wash** each well **3 times** thoroughly with **300 µL Washbuffer**. Blot dry 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).*
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** and a reference wavelength between 620 nm and 650 nm.

7. Calculation of results

	Concentration of the standards					
Standard	**A**	**B**	**C**	**D**	**E**	**F**
Metanephrine (ng/mL)	0	15	45	150	600	2 400
Metanephrine (nmol/L)	0	76	228	761	3 042	12 168
Conversion:	Metanephrine (ng/mL) x 5.07 = Metanephrine (nmol/L)					

The calibration 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).

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

7.1 Quality control

It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC Report.

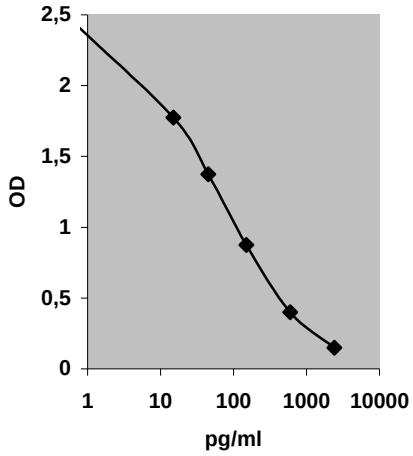
7.2 Calibration

The binding of the antisera and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the extinction values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. The extinction values also depend on the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20-25°C.

⚠ *In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm*

7.3 Typical calibration curve

METANEPHRINE



⚠ *Example. Do not use for calculation!*

8. Assay characteristics

Expected Reference Values		**Metanephrine**
	Urine	< 350 µg/day

Analytical Sensitivity (Limit of Detection)		**Metanephrine**
	Urine	13 ng/mL

Analytical Specificity (Cross Reactivity)	**Substance**	**Cross Reactivity (%)**
		Metanephrine
	Derivatized Metanephrine	100
	Derivatized Normetanephrine	0.15
	Derivatized 3-methoxytyramine	< 0.001
	Adrenaline	3.3
	Noradrenaline	< 0.001
	Dopamine	< 0.001
	Vanillic mandelic acid, L-Dopa, Homovanillic acid, L-Tyrosin, Tyramin	< 0.001

Precision

Intra-Assay				**Inter-Assay**			
	Sample	Range (ng/mL)	CV (%)		Sample	Range (ng/mL)	CV (%)
Metanephrine	1	69 ± 8.6	12.6	Metanephrine	1	102 ± 15.4	7.7
	2	446 ± 23	5.2		2	448 ± 40	8.9

			Range	Serial dilution up to	Mean (%)
Linearity	Metanephrine	Urine	40 – 1.600 ng/mL	1:16	98

			Mean (%)	Range (%)	% Recovery after spiking
Recovery	Metanephrine	Urine	105	98 – 119	

Method Comparison versus HPLC*	Metanephrine	Urine	HPLC = 0.9 RIA – 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.

⚠ ***For actual literature, information about clinical significance or any other information please contact your local supplier.***

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content		CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!