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Instructions for use

2-MET Plasma ELISA

REF

DEM-DEE8300



IVD



Metanephrine–Normetanephrine Plasma ELISA

1. Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of free Metanephrine and free Normetanephrine in plasma.

First, the plasma proteins are removed by precipitation. After this Metanephrine (Metadrenaline) and Normetanephrine (Normetadrenaline) are 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 analytes 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 standards.

■ The antisera used in this test kit only recognise the biologically relevant L-forms of metanephrines. Commercially available synthetic normetanephrine or 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 metanephrines are used to enrich native samples. As only about 50% of the synthetic metanephrines - 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.

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 - **except of the Acylation Concentrate** - at 2 - 8 °C until expiration date. The Acylation Concentrate should be stored at room temperature. 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 tubes	ready for use
BA D-0090	FOILS	Adhesive Foil	2 x 4	ready for use
BA E-0030	WASH-CONC 50x	Wash Buffer Concentrate	2 x 20 mL	Concentrate. Dilute content with dist. water to a final volume of 1000 mL
BA E-0040	CONJUGATE	Enzyme Conjugate	2 x 12 mL	ready for use, anti-rabbit IgG conjugated with peroxidase
BA E-0055	SUBSTRATE	Substrate	2 x 12 mL	ready for use, containing a solution of
BA E-0080	STOP-SOLN	Stop Solution	2 x 12 mL	ready for use, containing 0.25 M H ₂ SO ₄
BA E-0131	ADR MN	Adrenaline-Metanephrine Microtiter Strips	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, blue coloured
BA E-0231	NAD NMN	Noradrenaline-Normetanephrine Microtiter Strips	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, yellow coloured
BA E-8110	MN-AS	Metanephrine Antiserum	1 x 6 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA E-8210	NMN-AS	Normetanephrine Antiserum	1 x 6 mL	from rabbit, ready for use, yellow coloured, yellow screw cap
BA E-8313	ASSAY-BUFF	Assay Buffer	1 x 12 mL	ready for use
BA R-0028	EQUA-REAG	Equalizing Reagent	2 x 10 mL	lyophilized
BA R-8301	STANDARD A	Standard A	1 x 12 mL	ready for use
BA R-8302	STANDARD B	Standard B	1 x 4 mL	ready for use
BA R-8303	STANDARD C	Standard C	1 x 4 mL	ready for use
BA R-8304	STANDARD D	Standard D	1 x 4 mL	ready for use
BA R-8305	STANDARD E	Standard E	1 x 4 mL	ready for use
BA R-8306	STANDARD F	Standard F	1 x 4 mL	ready for use
BA R-8312	ACYL-CONC	Acylation Concentrate	1 x 1.5mL	concentrated
BA R-8351	CONTROL 1	Control 1	1 x 4 mL	ready for use
BA R-8352	CONTROL 2	Control 2	1 x 4 mL	ready for use

4.2 **Additional materials and equipment required but not provided with the kit**

- Calibrated variable precision micropipettes (e.g. 10-100 μL / 100-1000 μL)
- Microtiter plate washing device
- ELISA reader capable of reading absorbance at 450 nm
- Centrifuge capable of at least 3.000 x g
- Shaker (shaking amplitude 3mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Distilled water
- Vortex mixer

5. **Sample collection and storage**

EDTA- or citrate-plasma should be used.


Haemolytic and especially lipemic samples should not be used for the assay.

Storage: up to 6 hours at 2 - 8°C, for longer periods (up to 6 months) at - 20°C.

Repeated freezing and thawing should be avoided.

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.

 *The precipitation and acylation procedures are identical for both assays and have to be done only once. If samples have to be diluted the Equalizing Reagent (BA R-0028) has to be used.*

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

Equalizing Reagent

Reconstitute the Equalizing Reagent with 10 mL distilled water.


Reconstituted Equalizing Reagent which is not used immediately has to be stored in aliquots at -20 °C and may be thawed only once.

Acylation Solution


The Acylation Concentrate should be stored at room temperature.

Pipette 80 μL Acylation Reagent Concentrate to 3 mL distilled water and mix thoroughly.

Use immediately!

 *The Acylation Solution is only stable for a maximum of 3 minutes.*

6.2 **Precipitation**

- | | |
|---|---|
| 1. | Pipette 100 μL of standards , 100 μL of controls , and 500 μL of plasma samples into the respective Reaction Tubes . |
| 2. | Add 500 μL Equalizing Reagent to all tubes containing standards and controls. |
| 3. | Add 100 μL Standard A to all tubes containing plasma samples. |
| 4. | Mix Reaction Tubes thoroughly (vortex) and centrifuge for 15 minutes at 3,000 x g . |
|  | Take 75 μL of the clear supernatant for the Metanephrine ELISA and 25 μL of the clear supernatant for the Normetanephrine ELISA. |

6.3 Metanephrine ELISA

1.	Pipette 50 µL of Assay Buffer into the appropriate wells of the Metanephrine Microtiter Strips .
2.	Pipette 75 µL of the clear supernatant from the standards, controls and samples into the wells.
3.	Pipette 25 µL Acylation Solution (refer to 6.1) into all wells. <i>The Acylation Solution is stable for maximum of only 3 minutes.</i>
4.	Incubate for 15 min at RT (20-25°C) on a shaker (approx. 600 rpm).
5.	Pipette 50 µL of the Metanephrine Antiserum into all wells.
6.	Cover the plate with Adhesive Foil , shake for 1 min at RT (20-25°C) on a shaker and incubate for 15 - 20 hours (overnight) at 2-8°C .
7.	Remove the foil and discard. Discard or aspirate the contents of the wells and wash each well 4 times thoroughly with 300 µL Wash Buffer . Blot dry by tapping the inverted plate on absorbent material.
8.	Pipette 100 µL of the Enzyme Conjugate into all wells.
9.	Incubate for 30 min at RT (20-25°C) on a shaker (approx. 600 rpm).
10.	Discard or aspirate the contents of the wells and wash each well 4 times thoroughly with 300 µL Wash Buffer . Blot dry by tapping the inverted plate on absorbent material.
11.	Pipette 100 µL of the Substrate into all wells and incubate for 20-30 min at RT (20-25°C) on a shaker (approx. 600 rpm). <i>Avoid exposure to direct sun light!</i>
12.	Add 100 µL of the Stop Solution to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
13.	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.

6.4 Normetanephrine ELISA

1.	Pipette 50 µL of Assay Buffer into the appropriate wells of the Normetanephrine Microtiter Strips .
2.	Pipette 25 µL of the clear supernatant from the standards, controls and samples into the wells.
3.	Pipette 25 µL Acylation Solution (refer to 6.1) into all wells. <i>The Acylation Solution is stable for only 3 minutes.</i>
4.	Incubate for 15 min at RT (20-25°C) on a shaker (approx. 600 rpm).
5.	Pipette 50 µL of the Normetanephrine Antiserum into all wells.
6.	Cover the plate with Adhesive Foil , shake for 1 min at RT (20-25°C) on a shaker and incubate for 15 - 20 hours (overnight) at 2-8°C .
7.	Remove the foil and discard. Discard or aspirate the contents of the wells and wash each well 4 times thoroughly with 300 µL Wash Buffer . Blot dry by tapping the inverted plate on absorbent material.
8.	Pipette 100 µL of the Enzyme Conjugate into all wells.
9.	Incubate for 30 min at RT (20-25°C) on a shaker (approx. 600 rpm).
10.	Discard or aspirate the contents of the wells and wash each well 4 times thoroughly with 300 µL Wash Buffer . Blot dry by tapping the inverted plate on absorbent material.
11.	Pipette 100 µL of the Substrate into all wells and incubate for 20-30 min at RT (20-25°C) on a shaker (approx. 600 rpm). <i>Avoid exposure to direct sun light!</i>
12.	Add 100 µL of the Stop Solution to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
13.	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

Standard	Concentration of the standards					
	A	B	C	D	E	F
Normetanephrine (pg/mL)	0	48	160	480	1 600	4 800
Normetanephrine (pmol/L)	0	262	874	2 620	8 740	26 200
Metanephrine (pg/mL)	0	36	120	360	1 200	3 600
Metanephrine (pmol/L)	0	183	608	1 830	6 080	18 300
Conversion:	Normetanephrine (pg/mL) x 5.46 = Normetanephrine (pmol/L) Metanephrine (pg/mL) x 5.07 = Metanephrine (pmol/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.


Samples found with concentrations higher than the highest standard (Standard F) should be diluted accordingly with Equalizing Reagent (BA R-0028) and have to be re-assayed.

7.1 Quality control

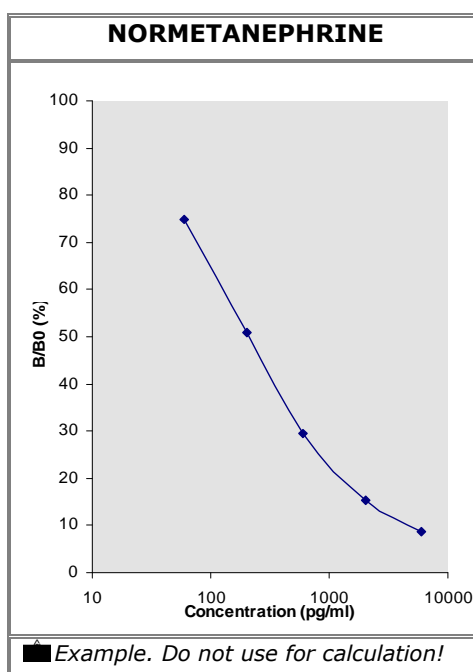
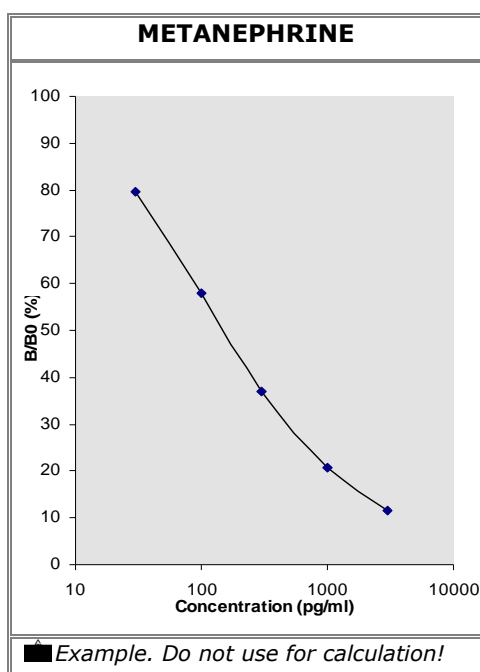
It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC Report.

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 curves



8. Assay characteristics

Expected Reference Values		Metanephrine	Normetanephrine
	Plasma	< 90 pg/mL	< 180 pg/mL

Analytical Sensitivity (Limit of Detection)		Metanephrine	Normetanephrine
	Plasma	17 pg/mL	23 pg/mL

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)	
		Metanephrine	Normetanephrine
	Derivatized Metanephrine	100	0.08
Derivatized Normetanephrine	0.04	100	
3-Methoxytyramin.HCl	< 0.001	1,74	
Adrenaline	< 0.001	< 0.001	
Noradrenaline	< 0.001	< 0.001	
Dopamin.HCl	< 0.001	< 0.001	
VMS	< 0.001	< 0.001	
HMVS	< 0.001	< 0.001	
L-DOPA	< 0.001	< 0.001	
L-Tyrosin	< 0.001	< 0.001	
Tyramine.HCl	< 0.001	< 0.001	
Normetanephrine	< 0.001	< 0.001	
Acetaminophen	< 0.001	< 0.001	

Precision							
Intra-Assay				Inter-Assay			
	Sample	Range (pg/mL)	CV (%)		Sample	Range (pg/mL)	CV (%)
Metanephrine	1	155 ± 17	11	Metanephrine	1	133 ± 13	10
	2	245 ± 28	11		2	257 ± 23	8.9
	3	523 ± 38	7.3		3	528 ± 50	9.5
Normetanephrine	1	167 ± 12	7.3	Normetanephrine	1	161 ± 18	11
	2	373 ± 29	7.8		2	370 ± 19	5.1
	3	832 ± 60	7.2		3	844 ± 51	6.0







Linearity			Range	Serial dilution up to	Range (%)
	Metanephrine	Plasma	41 - 2100	1: 65	109
	Normetanephrine	Plasma	58 - 5800	1: 129	109

Recovery			Mean (%)	Range (%)	% Recovery after spiking
	Metanephrine	Plasma	97	85 - 111	
	Normetanephrine	Plasma	92	80 - 108	

Method Comparison: ELISA vs. LC-MS/MS	Metanephrine	Plasma	LC-MS/MS = 1.1x - 19.4	r = 0.98; n = 59
	Normetanephrine	Plasma	LC-MS/MS = 1.2x + 10.5	r = 0.99; n = 59

For updated 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	IVD	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!

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