



DRG[®] Normetanephrine Plasma ELISA (EIA-4319)



Revised 23 Dec. 2011 rm (Vers. 9.1)

USA: **RUO**

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

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

1 INTENDED USE AND PRINCIPLE OF THE TEST

Enzyme Immunoassay for measurement of free Normetanephrine in plasma.

First, the plasma proteins are removed by precipitation. After this Normetanephrine (Normetadrenaline) 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 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.).

It is recommended that each laboratory establishes its own reference intervals. The values reported in this test instruction are only indicative.

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.



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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 upon request.

2.5 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 °C - 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 CONTENTS OF THE KIT

REAC-TUBES	Reaction Tubes	2 x 50 tubes	ready for use
FOILS	Adhesive Foil	1 x 4	ready for use
WASH-CONC 50x	Wash Buffer Concentrate	1 x 20 mL	Concentrate. Dilute content with dist. water to a final volume of 1000 mL
CONJUGATE	Enzyme Conjugate	1 x 12 mL	ready for use, anti-rabbit IgG conjugated with peroxidase

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SUBSTRATE	Substrate	1 x 12 mL	ready for use, containing a solution of tetramethylbenzidine (TMB)
STOP-SOLN	Stop Solution	1 x 12 mL	ready for use, containing 0.25 M H ₂ SO ₄
NAD NMN	Noradrenaline-Normetanephrine Microtiter Strips	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, yellow coloured
NMN-AS	Normetanephrine Antiserum	1 x 6 mL	from rabbit, ready for use, yellow coloured, yellow screw cap
ASSAY-BUFF	Assay Buffer	1 x 12 mL	ready for use
EQUA-REAG	Equalizing Reagent	2 x 10 mL	lyophilized
STANDARD A	Standard A	1 x 12 mL	ready for use
STANDARD B	Standard B	1 x 4 mL	ready for use
STANDARD C	Standard C	1 x 4 mL	ready for use
STANDARD D	Standard D	1 x 4 mL	ready for use
STANDARD E	Standard E	1 x 4 mL	ready for use
STANDARD F	Standard F	1 x 4 mL	ready for use
ACYL-CONC	Acylation Concentrate	1 x 1.5 mL	concentrated
CONTROL 1	Control 1	1 x 4 mL	ready for use
CONTROL 2	Control 2	1 x 4 mL	ready for use



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- Calibrated variable precision micropipettes (e.g. 10-100 μ L / 100-1000 μ L)
- 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 °C - 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.*

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 2 °C - 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 μ L Acylation Reagent Concentrate to 3 mL distilled water and mix thoroughly.


Use immediately!

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 *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 **25 µL** of the clear supernatant for the Normetanephrine ELISA.

6.3 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.
The Acylation Solution is stable for only 3 minutes.
4. Incubate for **15 min** at **RT** (20 °C – 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 °C – 25 °C) on a **shaker** and incubate for **15 - 20 hours** (overnight) at **2 °C - 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 °C – 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 °C – 25 °C) on a shaker (approx. 600 rpm). *Avoid exposure to direct sun light!*
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.



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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
Conversion:	Normetanephrine (pg/mL) x 5.46 = Normetanephrine (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 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 °C – 25 °C.

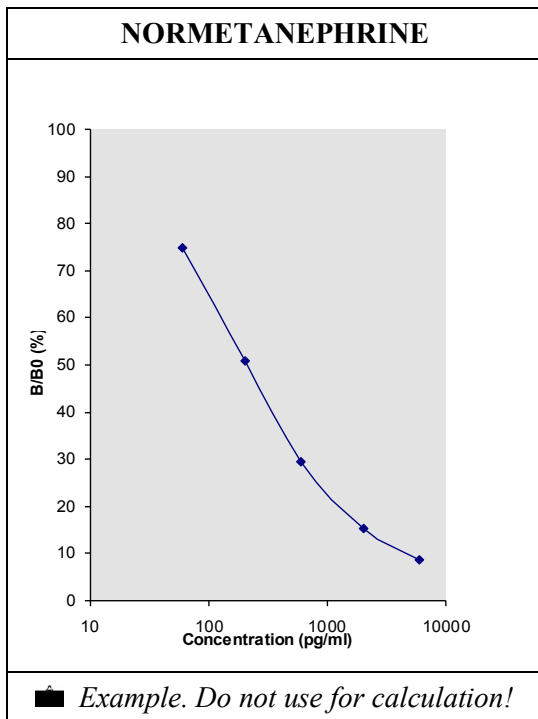
■ *In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm*



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7.3 Typical calibration curve



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