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Instructions for use

Noradrenaline ELISA Fast Track

REF BA E-6200R

2°C–8°C

Σ 96

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

Noradrenaline ELISA Fast Track

1. Introduction

1.1 Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of noradrenaline (norepinephrine) in plasma and urine.

Noradrenaline (norepinephrine) is extracted by using a cis-diol-specific affinity gel, acylated and then converted enzymatically.
The competitive ELISA kit uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The derivatized standards, controls and samples and the solid phase bound analytes 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.

1.2 Background

In humans the catecholamines adrenaline (epinephrine), noradrenaline (norepinephrine) and dopamine are neurotransmitters of the sympathetic nervous system and are involved in many physiological processes. The sympathetic nervous system sets the body to a heightened state of alert, also called as the body's fight-or-flight response.
In the human body the catecholamines and their metabolites indicate the adaptation of the body to acute and chronic stress.

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 provided with the kit.
(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

Plasma
Samples containing precipitates or fibrin strands or which are haemolytic or lipemic might cause inaccurate results.

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 noradrenaline 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-0090 FOILS **Adhesive Foil** - Ready to use
Content: Adhesive Foils in a resealable pouch
Volume: 1 x 4 foils

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-0040 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-0231 NAD NMN **Noradrenaline Microtiter Strips**- Ready to use
Content: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable yellow pouch with desiccant

BA E-6210 NAD-AS **Noradrenaline Antiserum** - Ready to use
Content: Rabbit anti-noradrenaline antibody, yellow coloured
Volume: 1 x 6 ml/vial, yellow cap

BA R-0050 ADJUST-BUFF **Adjustment Buffer** - Ready to use

Content: TRIS buffer

Volume: 1 x 4 ml/vial, green cap

Standards and **Controls** - Ready to use

Cat. no.	Component	Colour / Cap	Concentration ng / ml NAD	Concentration nmol / l NAD	Volume / Vial
BA E-6601	STANDARD A	white	0	0	4 ml
BA E-6602	STANDARD B	light yellow	5	30	4 ml
BA E-6603	STANDARD C	orange	20	118	4 ml
BA E-6604	STANDARD D	dark blue	75	443	4 ml
BA E-6605	STANDARD E	light grey	250	1 478	4 ml
BA E-6606	STANDARD F	black	1 000	5 910	4 ml
BA E-6651	CONTROL 1	light green	Refer to QC report for expected value and acceptable range!		4 ml
BA E-6652	CONTROL 2	dark red			4 ml

Conversion: Noradrenaline (ng/ml) x 5.91 = Noradrenaline (nmol/l)

Content: Acidic buffer with non-mercury stabilizer, spiked with defined quantity of noradrenaline

BA R-6611 ACYL-BUFF **Acylation Buffer** - Ready to use

Content: Buffer with light alkaline pH for the acylation

Volume: 1 x 20 ml/vial, white cap

BA R-6612 ACYL-REAG **Acylation Reagent** - Ready to use

Content: Acylation reagent in DMF and DMSO

Volume: 1 x 3 ml/vial, light red cap

Hazards identification:

H360D May damage the unborn child.
H226 Flammable liquid and vapour.
H312 + H332 Harmful in contact with skin or if inhaled.
H319 Causes serious eye irritation.

BA R-6613 ASSAY-BUFF **Assay Buffer** - Ready to use

Content: 1M hydrochloric acid and a non-mercury preservative

Volume: 1 x 6 ml/vial, light grey cap

BA R-6614 COENZYME **Coenzyme** - Ready to use

Content: S-adenosyl-L-methionine

Volume: 1 x 4 ml/vial, purple cap

BA R-6615 ENZYME **Enzyme** - Lyophilized

Content: Catechol-O-methyltransferase

Volume: 2 vials, pink cap

BA R-6617 EXTRACT-BUFF **Extraction Buffer** - Ready to use

Content: Buffer containing carbonate

Volume: 1 x 6 ml/vial, brown cap

BA R-6618 EXTRACT-PLATE 48 **Extraction Plate -** Ready to use

Content: 2 x 48 well plates coated with boronate affinity gel in a resealable pouch

BA R-6619 HCL **Hydrochloric Acid** - Ready to use

Content: 0.025 M Hydrochloric Acid, yellow coloured

Volume: 1 x 20 ml/vial, dark green cap

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

- Calibrated precision pipettes to dispense volumes between 10 – 700 µl; 1 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
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)
- Vortex mixer

5. Sample collection and storage

Plasma
Whole blood should be collected into centrifuge tubes containing EDTA as anti-coagulant (Monovette™ or Vacuette™ for plasma) and centrifuged according to manufacturer's instructions immediately after collection. Haemolytic and lipemic samples should not be used for the assay.
Storage: up to 6 hours at 2 - 8 °C, for longer period (up to 6 month) at -20 °C.
Repeated freezing and thawing should be avoided.

Urine
Spontaneous urine or 24-hour urine, collected in a bottle containing 10 - 15 ml of 6 M HCl, can be used.
If 24-hour urine is used please record the total volume of the collected urine.
Storage: up to 48 hours at 2 - 8 °C, up to 24 hours at room temperature, for longer periods (up to 6 month) 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. 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 antiserum and the enzyme conjugate and the activity of the enzyme are temperature dependent, and the absorbance may vary if a thermostat is not used. The higher the temperature, the higher the absorbance will be. Varying incubation times will have a similar influence on the absorbance. 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*

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

Enzyme Solution
Reconstitute the content of the vial labelled 'Enzyme' with 1 ml water (deionized, distilled, or ultra-pure) and mix thoroughly. Add 0.3 ml of Coenzyme followed by 0.7 ml of Adjustment Buffer. The total volume of the Enzyme Solution is 2.0 ml.

⚠ *The Enzyme Solution has to be prepared freshly prior to the assay (not longer than 10 - 15 minutes in advance). Discard after use!*

Noradrenaline 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, extraction and acylation

1.	Pipette **10 µl** of **standards**, **controls**, **urine samples** and **300 µl** of **plasma samples** into the respective wells of the **Extraction Plate**.
2.	Add **250 µl** of **water** (deionized, distilled, or ultra-pure) to the wells with **standards, controls** and **urine samples.**
3.	Pipette **50 µl** of **Assay Buffer** into all wells.
4.	Pipette **50 µl** of **Extraction Buffer** into all wells.
5.	Cover plate with **Adhesive Foil** and incubate **30 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
6.	Remove the foil. Empty plate and blot dry by tapping the inverted plate on absorbent material.
7.	Pipette **1 ml** of **Wash Buffer** into all wells. Incubate the plate for **5 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). Empty plate and blot dry by tapping the inverted plate on absorbent material.
8.	Pipette another **1 ml** of **Wash Buffer** into all wells. Incubate the plate for **5 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). Empty plate and blot dry by tapping the inverted plate on absorbent material.
9.	Pipette **150 µl** of **Acylation Buffer** into all wells.
10.	Pipette **25 µl** of **Acylation Reagent** into all wells.
11.	Incubate **15 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
12.	Empty plate and blot dry by tapping the inverted plate on absorbent material.
13.	Pipette **1 ml** of **Wash Buffer** into all wells. Incubate the plate for **10 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). Empty plate and blot dry by tapping the inverted plate on absorbent material.
14.	Pipette **150 µl** of **Hydrochloric Acid** into all wells.
15.	Cover plate with **Adhesive Foil**. Incubate **10 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). Remove the foil and discard. ⚠ ***Do not decant the supernatant thereafter!*** The following volumes of the supernatant are needed for the subsequent ELISA:

Noradrenaline	**20 µl**

6.3 Noradrenaline ELISA

1.	Pipette **25 µl** of the **Enzyme Solution** (refer to 6.1*)* into all wells of the **Noradrenaline Microtiter Strips**.
2.	Pipette **20 µl** of the extracted **standards, controls and samples** into the appropriate wells.
3.	Incubate for **30 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
4.	Pipette **50 µl** of the **Noradrenaline Antiserum** into all wells and cover plate with **Adhesive Foil**.
5.	Incubate for **2 h** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
6.	Remove the foil. 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.
7.	Pipette **100 µl** of the **Enzyme Conjugate** into all wells.
8.	Incubate for **30 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
9.	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.
10.	Pipette **100 µl** of the **Substrate** into all wells and incubate for **25 ± 5 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). ⚠ ***Avoid exposure to direct sunlight!***
11.	Add **100 µl** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
12.	**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

		Noradrenaline
Measuring range	Urine	2.5 - 1000 ng/ml
	Plasma	93 – 33 333 pg/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.*

Urine samples and controls
The concentrations of the **urine samples** and the **Controls 1 and 2** can be read directly from the standard curve.

Calculate the 24 h excretion for each urine sample: **µg/24h = µg/l x l/24h**

Plasma samples
The read concentrations of the **plasma samples** have to be **divided by 30.**

Conversion
Noradrenaline (ng/ml) x 5.91 = Noradrenaline (nmol/l)

Expected reference values
It is strongly recommended that each laboratory should determine its own normal and abnormal values.

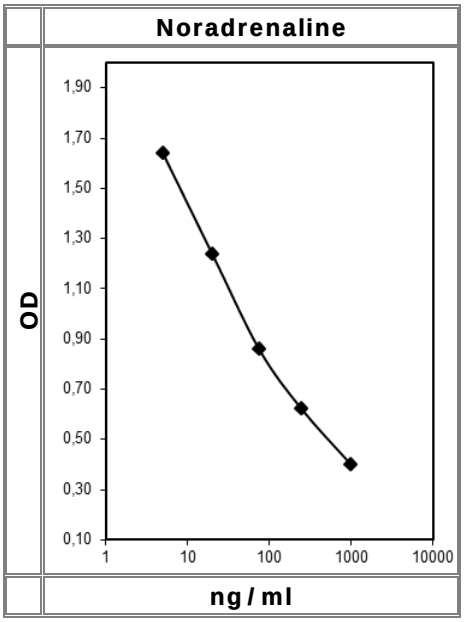
	Noradrenaline
24-hour urine	< 90 µg/day (535 nmol/day)
Plasma	< 600 pg/ml

7.1 Quality control

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

7.2 Typical standard curve

⚠ *Example, do not use for calculation!*



8. Assay characteristics

			Noradrenaline
Analytical Sensitivity	**LOD**	Urine (ng/ml)	1.7
		Plasma (pg/ml)	36
	LOQ	Urine (ng/ml)	2.5
		Plasma (pg/ml)	93

	Substance	Cross Reactivity (%)
		Noradrenaline
Analytical Specificity (Cross Reactivity)	Derivatized Adrenaline	0.08
	Derivatized Noradrenaline	100
	Derivatized Dopamine	0.03
	Metanephrine	< 0.01
	Normetanephrine	0.16
	3-Methoxytyramine	< 0.01
	3-Methoxy-4-hydroxyphenylglycol	< 0.01
	Tyramine	< 0.01
	Phenylalanine, Caffeinic acid, L-Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid	< 0.01

Precision							
Intra-Assay Urine (n = 60)				**Intra-Assay Plasma (n = 60)**			
	Sample	**Range (ng/ml)**	**CV (%)**		**Sample**	**Range (pg/ml)**	**CV (%)**
Noradrenaline	1	26.1 ± 3.6	13.8	Noradrenaline	1	510 ± 65	12.8
	2	97 ± 12.8	13.4		2	1358 ± 194	14.3
	3	267 ± 35	13.1		3	3363 ± 374	11.1
Inter-Assay Urine (n = 33)				**Inter-Assay Plasma (n = 18)**			
	Sample	Range (ng/ml)	CV (%)		Sample	Range (pg/ml)	CV (%)
Noradrenaline	1	19.5 ± 3.9	20.0	Noradrenaline	1	445 ± 40.9	9.2
	2	80.6 ± 10.6	13.2		2	1232 ± 134	10.9
	3	226 ± 39.5	17.4		3	3283 ± 302	9.2

			Serial dilution up to	Range (%)	Mean (%)
Linearity	Noradrenaline	Urine	1:512	100 - 127	112
		Plasma	1:512	102 - 125	112

			Mean (%)	Range (%)	Range
Recovery	Noradrenaline	Urine	103	91 - 113	58.6 – 260 ng/ml
		Plasma	87	75 - 107	51 – 14 251 pg/ml

9. References/Literature

(1) Kim et al. Vitamin C prevents stress-induced damage on the heart caused by the death of cardiomyocytes, through the down-regulation of the excessive production of catecholamine, TNF-α, and ROS production in GULO(-I-) Vit C-Insufficient mice. Free Radical Biology and Medicine, 65:573-583 (2013)

(2) Bada et al. Peripheral vasodilatation determines cardiac output in exercising humans: insight from atrial pacing. The Journal of Physiology, 590(8):2051-2060 (2012)

(3) Parks et al. Employment and work schedule are related to telomere length in women. Occupational & Environmental Medicine 68(8):582-589 (2011)

⚠ ***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!