Labor Diagnostika Nord GmbH & Co. KG
Am Eichenhain 1, 48531 Nordhorn
Telefon: +49-5921-72728 101
Telefax: +49-5921-72728 188
Internet: http://www.ldn.de

Labor Diagnostika Nord GmbH & Co. KG

Instructions for use

Noradrenaline Research ELISA

Flexible test system for various biological sample types and volumes

Enzyme Immunoassay for the quantitative determination
of Noradrenaline (Norepinephrine)

REF **BA 10-5200**

96

+8 °C
+2

RUO

1. Principle of the test

Noradrenaline (norepinephrine) is extracted by using a cis-diol-specific affinity gel, acylated and then derivatized 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 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. Storage and stability

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date shown on the kit labels.

3. Contents of the kit

BA 1611	ACYL-BUFF	**Acylation Buffer**	1 x 20 mL	ready for use
BA 1613	ASSAY-BUFF	**Assay Buffer**	2 x 4 mL	ready for use, contains 1 M HCl
BA 1614	COENZYME	**Coenzyme**	2 x 0.75 mL	ready for use, S-adenosyl-L-methionine
BA 1615	ENZYME	**Enzyme**	4 x 1 mL	lyophilized, contains COMT
BA 1617	EXTRACT-BUFF	**Extraction Buffer**	2 x 4 mL	ready for use
BA 1618	EXTRACT-PLATE 48	**Extraction Plate**	2 x 48 wells	coated with boronate affinity gel
BA 1619	HCL	**Hydrochloric Acid**	1 x 20 mL	ready for use, yellow coloured, contains 0.025 M HCl
BA 3050	ADJUST-BUFF	**Adjustment Buffer**	1 x 4 mL	ready for use
BA 3075	ACYL-DILUENT	**Acylation Diluent**	1x 4 mL	ready for use
BA 5601	STANDARD A	**Standard A**	1 x 1 mL	ready for use
BA 5602	STANDARD B	**Standard B**	1 x 1 mL	ready for use
BA 5603	STANDARD C	**Standard C**	1 x 1 mL	ready for use
BA 5604	STANDARD D	**Standard D**	1 x 1 mL	ready for use
BA 5605	STANDARD E	**Standard E**	1 x 1 mL	ready for use
BA 5606	STANDARD F	**Standard F**	1 x 1 mL	ready for use
BA 5612	ACYL-CONC	**Acylation Concentrate**	1 x 0.25 mL	Concentrate. Has to be diluted prior to use.
BA 5651	CONTROL 1	**Control 1**	1 x 1 mL	ready for use
BA 5652	CONTROL 2	**Control 2**	1 x 1 mL	ready for use
BA 10-0025	WASH-CONC 25x	**Wash Buffer Concentrate**	2 x 20 mL	Concentrate. Dilute content with distilled water to a final volume of 500 mL
BA 10-0040	CONJUGATE	**Enzyme Conjugate**	1 x 11 mL	ready for use, anti-rabbit IgG conjugated with peroxidase
BA 10-0055	SUBSTRATE	**Substrate**	1 x 11 mL	ready for use, containing a solution of TMB
BA 10-0080	STOP-SOLN	**Stop Solution**	1 x 11 mL	ready for use, containing 0.25 M H_2SO_4
BA 10-0090	FOILS	**Adhesive Foil**	1 x 4	ready for use
BA 10-0231	NAD NMN	**Noradrenaline-Normetanephrine Microtiter Strips**	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, yellow coloured
BA 10-3032	96	**Microtiter Plate**	1 x 96 wells	12 strips, 8 wells each, break apart
BA 10-5210	NAD-AS	**Noradrenaline Antiserum**	1 x 6 mL	from rabbit, ready for use, yellow coloured, yellow screw cap

4. Additional materials and equipment required but not provided with the kit

- Calibrated variable precision micropipettes (e.g. 1-10 µL / 10-100 µL / 100-1000 µL)
- Microtiter plate washing device
- ELISA reader capable of reading absorbance at 450 nm (reference filter 620 – 650 nm)
- Shaker (shaking amplitude 3mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Distilled water
- Vortex mixer

5. Sample collection and storage

Storage: up to 6 hours at 2 – 8 °C; for longer periods (up to 6 months) at - 20°C or – 80 °C.
Advice for the preservation of the biological sample: to prevent catecholamine degradation add EDTA (final concentration 1mM) and sodium metabisulfite (final concentration 4 mM) to the sample.

6. Test procedure

Allow reagents and samples to reach room temperature. 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 500 mL. Store the diluted Wash Buffer Concentrate (Wash Buffer) at 2 – 8 °C. Shelf life: please refer to the expiry date indicated on the kit.

Acylation Solution
The Acylation Concentrate has to be diluted 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!*

Enzyme Solution
Reconstitute the content of the vial labelled 'Enzyme' with 1 mL distilled water 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!*

6.2 Sample preparation

The Noradrenaline Research ELISA is a flexible test system for various biological sample types and volumes. It is not possible to give a general advice how to prepare the samples. However, the following basics should help the researcher to fit the protocol to his specific needs.

- Avoid excess of acid: excess of acid might exceed the buffer capacity of the extraction buffer. A pH > 7.0 during the extraction is mandatory.
- Prevent catecholamine degradation by adding preservatives to the sample (see 5. Sample collection and Storage).
- Avoid chaotropic chemicals like perchloric acid. The high salt content might reduce the recovery of Noradrenaline. If your samples already contain high amounts of perchloric acid, neutralize the sample prior to the extraction step.
- Tissue samples can be homogenised in 0.01 N HCl in the presence of EDTA and sodium metabisulfite. Under these conditions Noradrenaline is positively charged which reduces binding to proteins and optimizes solubility.
- Avoid samples that contain substances with a cis-diol structure. These will reduce the recovery of the Noradrenaline.
- It is advisable to perform a "Proof of Principle" to determine the recovery of the Noradrenaline in your samples. Prepare a stock solution of Noradrenaline. Add small amounts (to change the native sample matrix as less as possible) of the stock solutions to the sample matrix and check the recovery.
- The used sample volume determines the sensitivity of this test. Determine the sample volume needed to determine the Noradrenaline in your sample by testing different amounts of sample volume.

If you need any support in establishing a protocol for your specific purposes, do not hesitate to contact the manufacturer directly!

6.3 Extraction and acylation

The Research ELISA offers a flexible test system for various biological sample types and sizes. Step 1 of the extraction procedure depends on the sample volume:

- in case you have sample volumes between 1 – 100 µL follow **1.1**
- in case you have sample volumes between 100 – 500 µL follow **1.2**
- in case you have sample volumes between 500 – 750 µL follow **1.3**

⚠ ***Within a run it is only possible to measure samples with the same volume!***

1.	**1.1** **Sample volume 1 – 100 µL** Pipette into the respective wells of the Extraction Plate: **10 µL standards, 10 µL controls and 1 – 100 µL of the sample**. Fill up each well with distilled water to a **final volume** of 100 µl (e.g. 10 µl standard plus 90 µl dist. water).	**1.2** **Sample volume 100 – 500 µL** Pipette into the respective wells of the Extraction Plate: **10 µL standards, 10 µL controls and 100 – 500 µL of the sample**. Fill up each well with distilled water to a **final volume** of 500 µl (e.g. 10 µl standard plus 490 µl dist. water).	**1.3** **Sample volume 500 – 750 µL** Pipette into the respective wells of the Extraction Plate: **10 µL of Standards, 10 µL of controls and 500 – 750 µL of sample**. Fill up each well with distilled water to a **final volume** of 750 µl (e.g. 10 µl standard plus 740 µl dist. water).
2.	Pipette **50 µL of Assay Buffer** into all wells.		
3.	Pipette **50 µL of Extraction Buffer** into all wells		
4.	Cover the plate with adhesive foil. Incubate **60 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm).		
8.	Remove the foil and empty the plate. Blot dry by tapping the inverted plate on absorbent material.		
6.	Pipette **1 mL** of **Wash Buffer** into all wells. Cover the plate with adhesive foil.		
7.	Shake **5 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm).		
8.	Remove the foil and empty the plate. Blot dry by tapping the inverted plate on absorbent material.		
9.	**Wash one more time** as described (step 6, 7 and 8)!		
10.	Pipette **150 µL** of **Acylation Buffer** into all wells.		
11.	Pipette **25 µL** of **Acylation Solution** (refer to 6.1*)* into all wells.		
12.	Incubate **20 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).		
13.	Empty the plate and blot dry by tapping the inverted plate on absorbent material.		
14.	Pipette **1 mL** of **Wash Buffer** into all wells. Cover plate with adhesive foil.		
15.	Shake **5 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).		
16.	Remove the foil and empty the plate. Blot dry by tapping the inverted plate on absorbent material.		
17.	**Wash one more time** as described (step 14, 15, 16).		
18.	Pipette **100 µL** of **Hydrochloric Acid** into all wells.		
19. ⚠	Cover plate with adhesive foil. Incubate **10 min** at **RT** (20-25°C) on an o shaker (approx. 600 rpm). ***Do not decant the supernatant thereafter!*** **90 µL of the supernatant is needed for the subsequent enzymatic conversion**		

6.4 Enzymatic Conversion

1.	Pipette **90 µL** of the **extracted standards, controls** and **samples** into the respective wells of the **Microtiter Plate.**
2.	Add **25 µL** of **Enzyme Solution** (refer to 6.1*)* to all wells.
3.	Cover plate with **Adhesive Foil**. Incubate **1 min** at **RT (20-25°C)** on a shaker to mix.
4.	Incubate for **2 hours** at **37°C.** The following volumes of the supernatants are needed for the subsequent ELISA: **Noradrenaline 100 µL**

6.5 Noradrenaline ELISA

1.	Pipette **100 µL** of **standards, controls** and **samples** from the **Enzyme Plate** (refer to 6.4) into the respective pre-coated **Noradrenaline Microtiter Strips.**
2.	Pipette **50 µL** of the respective **Noradrenaline Antiserum** into all wells.
3.	Cover the plate with **Adhesive Foil**. Incubate for **1 min** at **RT** (20-25°C) on a **shaker**.
4.	Incubate for **15 – 20 hours** (overnight) at **2 – 8 °C.**
5.	Remove the foil and 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.
6.	Pipette **100 µL** of **Enzyme Conjugate** into all wells.
7.	Cover the plate with **Adhesive Foil** and incubate **30 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm).
8.	Remove the foil and 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.
9.	Pipette **100 µL** of **Substrate** into all wells.
10. ⚠	Incubate **20-30 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm). ***Avoid exposure to direct sun light!***
11.	Pipette **100 µL** of **Stop Solution** into all wells.
12.	**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

The calibration curve from which the concentrations in the samples can be read off, is obtained by plotting the absorbance readings (calculate the mean absorbance) measured for the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).
The use of a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima) is recommended.

The standards refer to:

	Concentration of the standards (ng/mL)					
Standard	**A**	**B**	**C**	**D**	**E**	**F**
Noradrenaline	0	0.45	1.5	4.5	15	45

⚠ **The concentrations of the samples taken from the standard curve have to be multiplied by a correction factor.**

$$\textbf{Correction factor} = \frac{\textbf{10 µL (volume of standards extracted)}}{\textbf{sample volume (µL) extracted}}$$

Example: 750µL of the sample is extracted and the concentration taken from the standard curve is 0.45 ng/mL Noradrenaline.
Correction factor = 10/750 = 0.013
Concentration of the sample = 0.45 ng/mL x 0.013 = 0.006ng/mL = 6 pg/mL Noradrenaline

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*

8. Assay characteristics

	Substance	Cross Reactivity (%)
		Noradrenaline
Analytical Specificity (Cross Reactivity)	Derivatized Adrenaline	0.14
	Derivatized Noradrenaline	100
	Derivatized Dopamine	0.2
	Metanephrine	< 0.003
	Normetanephrine	0.48
	3-Methoxytyramine	< 0.003
	3-Methoxy-4-hydroxyphenylglycol	0.01
	Tyramine	< 0.003
	Phenylalanine, Caffeinic acid, L-Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid	< 0.003

Analytical Sensitivity (Limit of Detection)	**Noradrenaline**
	0,2 ng/mL x C*

C* = Correction factor (refer to 7.)

Precision				
Intra-Assay Human EDTA-Plasma				
	Sample	Mean ± 3 SD (pg/mL)	SD (pg/mL)	CV (%)
Noradrenaline	high	1377.4 ± 483.6	161.2	11.7
	medium	502.6 ± 126.9	42.3	8.4
	low	32.7 ± 15.3	5.1	15.6
Intra-Assay Cell Culture Medium (RPMI)				
	Sample	Mean ± 3 SD (pg/mL)	SD (pg/mL)	CV (%)
Noradrenaline	high	2027.8 ± 712.5	237.5	11.7
	medium	716.5 ± 179.7	59.9	8.4
	low	46.0 ± 16.8	5.6	12.2

Recovery	Mean (%)	Range (%)	SD (%)	CV (%)
Noradrenaline				
Human EDTA-Plasma	116.5	104.8 – 125.6	8.0	6.9
Cell Culture Medium	96.7	70.6 – 124.7	17.1	17.7

9. Advice on handling the test

9.1 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.

9.2 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 complaint form and return it completely filled in to the manufacturer.

9.3 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.

9.4 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.

9.5 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.

9.6 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.

⚠ ***Actual literature, information about clinical significance or any other information about the test are available on the homepage or contact the manufacturer directly.***

Symbols:

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