Labor Diagnostika Nord GmbH & Co. KG
Am Eichenhain 1, 48531 Nordhorn
Telefon: +49-5921-8197 0
Telefax: +49-5921-8197 222
e-mail: info@ldn.de
Internet: http://www.ldn.de

Instructions for use

3-CAT Research ELISA ™

REF **BA E-5600**

3x96

+2 +8 °C

RUO For Research use only- Not for use in diagnostic procedures

Adrenaline – Noradrenaline - Dopamine Research ELISA

1. Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of Adrenaline (Epinephrine), Noradrenaline (Norepinephrine) and Dopamine. Flexible test system for various biological sample types and volumes.
Adrenaline (Epinephrine), Noradrenaline (Norepinephrine) and Dopamine are 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 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 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-0032	TU 96	**Microtiter Plate**	1 x 96 wells	12 strips, 8 wells each, break apart
BA D-0090	FOILS	**Adhesive Foil**	3 x 4	ready for use
BA E-0030	WASH-CONC 50x	**Wash Buffer Concentrate**	3 x 20 mL	Concentrate. Dilute content with dist. water to a final volume of 1000 mL
BA E-0040	CONJUGATE	**Enzyme Conjugate**	3 x 12 mL	ready for use, anti-rabbit IgG conjugated with peroxidase
BA E-0055	SUBSTRATE	**Substrate**	3 x 12 mL	ready for use, containing a solution of TMB
BA E-0080	STOP-SOLN	**Stop Solution**	3 x 12 mL	ready for use, containing 0.25 M H_2SO_4
BA E-0131	TU ARD MN	**Adrenaline-Metanephrine Microtiter Strips**	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, blue coloured
BA E-0231	TU NAD NMN	**Noradrenaline-Normetanephrine Microtiter Strips**	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, yellow coloured
BA E-0331	TU DOP	**Dopamine Microtiter Strips**	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, green coloured
BA E-5110	ADR-AS	**Adrenaline Antiserum**	1 x 6 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA E-5210	NAD-AS	**Noradrenaline Antiserum**	1 x 6 mL	from rabbit, ready for use, yellow coloured, yellow screw cap
BA E-5310	DOP-AS	**Dopamine Antiserum**	1 x 6 mL	from rabbit, ready for use, green coloured, green screw cap
BA R-0050	ADJUST-BUFF	**Adjustment Buffer**	1 x 4 mL	ready for use
BA R-4617	TE-BUFF	**TE Buffer**	1 x 4 mL	ready for use
BA R-5601	STANDARD A	**Standard A**	1 x 4 mL	ready for use
BA R-5602	STANDARD B	**Standard B**	1 x 4 mL	ready for use
BA R-5603	STANDARD C	**Standard C**	1 x 4 mL	ready for use
BA R-5604	STANDARD D	**Standard D**	1 x 4 mL	ready for use
BA R-5605	STANDARD E	**Standard E**	1 x 4 mL	ready for use
BA R-5606	STANDARD F	**Standard F**	1 x 4 mL	ready for use
BA R-5651	CONTROL 1	**Control 1**	1 x 4 mL	ready for use
BA R-5652	CONTROL 2	**Control 2**	1 x 4 mL	ready for use
BA R-6611	ACYL-BUFF	**Acylation Buffer**	1 x 20 mL	ready for use
BA R-6612	ACYL-REAG	**Acylation Reagent**	1 x 3 mL	ready for use
BA R-6614	COENZYME	**Coenzyme**	1 x 4 mL	ready for use, S-adenosyl-L-methionine
BA R-6615	ENZYME	**Enzyme**	4 x 1 mL	lyophilized, contains the enzyme COMT
BA R-6618	EXTRACT-PLATE 48	**Extraction Plate**	2 x 48 wells	coated with boronate affinity gel
BA R-6619	HCL	**Hydrochloric Acid**	1 x 20 mL	ready for use, yellow coloured, contains 0.025 M HCl

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

- Calibrated variable precision micropipettes (e.g. 1-10 µL / 10-100 µL / 100-1000µL)
- 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 measurements 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 2–8°C

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 Catecholamine 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 Adrenaline, Noradrenaline and Dopamine. If your samples already contain high amounts of perchloric acid, neutralize them 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, Adrenaline, Noradrenaline and Dopamine are 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 catecholamines.
- It is advisable to perform a "Proof of Principle" to determine the recovery of the catecholamines in your samples. Prepare a stock solution of Adrenaline, Noradrenaline and Dopamine. 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 the test. Determine the sample volume needed to determine the catecholamines 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 or your local distributor directly!

6.3 Extraction and acylation

The 3-CAT Research ELISA offers a flexible test system for various biological sample types and volumes. 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: **30 µL standards, 30 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. 30 µl standard plus 70 µl dist. water).	**1.2** **Sample volume 100 – 500 µL** Pipette into the respective wells of the Extraction Plate: **30 µL standards, 30 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. 30 µl standard plus 470 µl dist. water).	**1.3** **Sample volume 500 – 750 µL** Pipette into the respective wells of the Extraction Plate: **30 µL of Standards, 30 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. 30 µl standard plus 720 µl dist. water).
2.	Pipette **25 µL** of **TE Buffer** into all wells		
3.	Cover the plate with adhesive foil. Shake **60 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm).		
4.	Remove the foil and empty the plate. Blot dry by tapping the inverted plate on absorbent material.		
5.	Pipette **1 mL** of **Wash Buffer** into all wells.		
6.	Shake **5 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm).		
7.	Blot dry by tapping the inverted plate on absorbent material.		
8.	**Wash one more time** as described (step 5, 6 and 7)!		
9.	Pipette **150 µL** of **Acylation Buffer** into all wells.		
10.	Pipette **25 µL** of **Acylation Reagent** into all wells.		
11.	Shake **20 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).		
12.	Empty the plate and blot dry by tapping the inverted plate on absorbent material.		
13.	Pipette **1 mL** of **Wash Buffer** into all wells.		
14.	Shake **5 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).		
15.	Blot dry by tapping the inverted plate on absorbent material.		
16.	**Wash one more time** as described (step 13, 14, 15).		
17.	Pipette **200 µL** of **Hydrochloric Acid** into all wells.		
18.	Cover plate with adhesive foil. Shake **10 min** at **RT** (20-25°C) on an o shaker (approx. 600 rpm). ⚠ ***Do not decant the supernatant thereafter!*** **190 µL of the supernatant is needed for the subsequent enzymatic conversion**		

6.4 Enzymatic Conversion

1.	Pipette **190 µL** of the **extracted standards, controls** and **samples** into the respective wells of the **Microtiter Plate.**
2.	Add **50 µL** of **Enzyme Solution** (refer to 6.1*)* to all wells.
3.	Cover plate with **Adhesive Foil**. Shake **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: **Adrenaline 75 µL** **Noradrenaline 75 µL** **Dopamine 75 µL**

6.5 Adrenaline, Noradrenaline and Dopamine ELISA

1.	Pipette **75 µL** of **standards, controls** and **samples** from the **Enzyme Plate** (refer to 6.4) into the respective pre-coated **Mikrotiter Strips (*1)**.
2.	Pipette **50 µL** of the respective **Antiserum (*2)** 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.

⚠ (*1): **Adrenaline Microtiter Strips, Noradrenaline Microtiter Strips, Dopamine Microtiter Strips**
(*2): **Adrenaline Antiserum, Noradrenaline Antiserum, Dopamine Antiserum**

7. Calculation of results

The calibration curve from which the concentrations of 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**
Adrenaline	0	0.5	1.5	5	20	80
Noradrenaline	0	0.2	0.6	2	8	32
Dopamine	0	0.5	1.5	5	20	80

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

$$\textbf{Correction factor} = \frac{\textbf{30 µL (volume of standards extracted)}}{\textbf{sample volume (µL) extracted}}$$

Example: 750µL of the sample is extracted and the concentration taken from the standard curve is 0.15 ng/mL Noradrenaline.
Correction factor = 30/750 = 0.04
Concentration of the sample = 0.15 ng/mL x 0.04 = 0.006 ng/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	Adrenaline	Dopamine
Analytical Specificity (Cross Reactivity)	Derivatized Adrenaline	0.14	100	0.03
	Derivatized Noradrenaline	100	0.20	0.87
	Derivatized Dopamine	0.2	< 0.0007	100
	Metanephrine	< 0.003	0.64	< 0.007
	Normetanephrine	0.48	0.0009	0.008
	3-Methoxytyramine	< 0.003	< 0.0007	0.55
	3-Methoxy-4-hydroxyphenylglycol	0.01	0.03	< 0.007
	Tyramine	< 0.003	< 0.0007	0.13
	Phenylalanine, Caffeinic acid, L-Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid	< 0.003	< 0.0007	< 0.007

Sensitivity (Limit of Detection)	**Adrenaline**	**Noradrenaline**	**Dopamine**
	0.25 ng/mL x C*	0.1 ng/mL x C*	0.25 ng/mL x C*

C* = Correction factor (refer to 7.)

Analytical Sensitivity (750 µl undiluted sample)	**Adrenaline**	**Noradrenaline**	**Dopamine**
	10 pg/mL	4 pg/mL	10 pg/mL

Functional Sensitivity (750 µl undiluted sample)	**Adrenaline**	**Noradrenaline**	**Dopamine**
	15 pg/mL	6 pg/mL	15 pg/mL

Precision				
Intra-Assay Human EDTA-Plasma				
	Sample	Mean ± 3 SD (pg/mL)	SD (pg/mL)	CV (%)
Adrenaline	high	1329.3 ± 372.6	124.2	9.3
	medium	412.1 ± 129.6	43.2	10.5
	low	37.9 ± 19.5	6.5	17.1
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
Dopamine	high	1438.6 ± 465.6	155.2	10.8
	medium	565.9 ± 246.3	82.1	14.5
	low	56.4 ± 36.3	12.1	21.5
Intra-Assay Cell Culture Medium (RPMI)				
	Sample	Mean ± 3 SD (pg/mL)	SD (pg/mL)	CV (%)
Adrenaline	high	1649.6 ± 555.0	185	11.2
	medium	526.2 ± 186.6	62.2	11.8
	low	38.7 ± 18.9	6.3	16.3
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
Dopamine	high	2784.5 ± 1238.7	412.9	14.8
	medium	1003.7 ± 526.2	175.4	17.5
	low	74.7 ± 51.6	17.2	23.0

Recovery	Mean (%)	Range (%)	SD (%)	CV (%)
Adrenaline				
Human EDTA-Plasma	104.0	89.4 – 128.3	13.1	12.6
Cell Culture Medium	95.5	81.6 – 109.6	8.3	8.7
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
Dopamine				
Human EDTA-Plasma	97.7	83.7 – 115.9	11.8	12.1
Cell Culture Medium	98.6	77.7 – 113.4	12.1	12.2

⚠ ***For current 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	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!