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LDN®

Instructions for use

3-CAT Plasma ELISA High Sensitive

REF BA E-4600

3x96

+2 +8 °C

IVD

CE

Adrenaline – Noradrenaline - Dopamine Plasma ELISA **High Sensitive**

1. Principle of the test

Enzyme Immunoassay for the quantitative determination of Adrenaline (Epinephrine), Noradrenaline (Norepinephrine), and Dopamine in plasma.

Adrenaline (epinephrine), noradrenaline (norepinephrine) and dopamine are extracted from a plasma sample***)** by using a cis-diol-specific affinity gel, acylated and then modified 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.

***)** Flexible sample volumes between 100 – 600 µl can be used with this assay.

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	MTP 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	MTP ARD MN	**Adrenaline-Metanephrine Microtiter Strips**	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, blue coloured
BA E-0231	MTP NAD NMN	**Noradrenaline-Normetanephrine Microtiter Strips**	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, yellow coloured
BA E-0331	MTP DOP	**Dopamine Microtiter Strips**	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, green coloured
BA E-4110	ADR-AS	**Adrenaline Antiserum**	1 x 6 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA E-4210	NAD-AS	**Noradrenaline Antiserum**	1 x 6 mL	from rabbit, ready for use, yellow coloured, yellow screw cap
BA E-4310	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-4601	STANDARD A	**Standard A**	1 x 4 mL	ready for use
BA R-4602	STANDARD B	**Standard B**	1 x 4 mL	ready for use
BA R-4603	STANDARD C	**Standard C**	1 x 4 mL	ready for use
BA R-4604	STANDARD D	**Standard D**	1 x 4 mL	ready for use
BA R-4605	STANDARD E	**Standard E**	1 x 4 mL	ready for use
BA R-4606	STANDARD F	**Standard F**	1 x 4 mL	ready for use
BA R-4617	TE-BUFF	**TE Buffer**	1 x 4 mL	ready for use
BA R-4651	CONTROL 1	**Control 1**	1 x 4 mL	ready for use
BA R-4652	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

Plasma

EDTA-Plasma should be used. Do not use haemolytic or lipemic samples.
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

A plasma volume between **100 µl-600 µl** is needed per single determination.

If a plasma volume **< 600 µl** is used, dist. water has to be added to a **final volume of 600 µl** and this **prediluted sample** has to be used for the extraction procedure (please refer to point 6.2 of this protocol).

This sample predilution has to be considered in the calculation of results (please refer to point 7 of this protocol).

Allow all reagents to reach room temperature and mix thoroughly by gentle inversion before use. 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 1000 mL.
Storage: up to 6 months 4–8°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, extraction and acylation

1.	Pipette **30 µL** of **standards**, **controls** and **600 µL** of **plasma samples** into the respective wells of the **Extraction Plate**.
2.	Add **500 µL** of **distilled water** to the wells with **standards** and **controls.**
3.	Pipette **25 µL** of **TE Buffer** into all wells.
4.	Cover the plate with adhesive foil. Shake **60 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm).
5.	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.
7.	Shake **5 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm).
8.	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 Reagent** into all wells.
12.	Shake **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.
15.	Shake **5 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
16.	Blot dry by tapping the inverted plate on absorbent material.
17.	**Wash one more time** as described (step 14, 15, 16).
18.	Pipette **200 µL** of **Hydrochloric Acid** into all wells.
19.	Cover plate with adhesive foil. Shake **10 min** at **RT** (20-25°C) on an shaker (approx. 600 rpm).
⚠	***Do not decant the supernatant thereafter!***
	190 µL of the supernatant is needed for the subsequent enzymatic conversion

6.3 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.
4.	Incubate for **2 hours** at **37°C**. The following volumes of the supernatants are needed for the subsequent ELISA: **Adrenaline 100 µL** | **Noradrenaline 10 µL** | **Dopamine 100 µL**

6.4 Adrenaline ELISA

1.	Pipette **100 µL** of **standards, controls** and **samples** from the **Enzyme Plate** (refer to 6.3) into the respective pre-coated **Adrenaline Mikrotiter Strips.**
2.	Pipette **50 µL** of the respective **Adrenaline 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.

6.5 Noradrenaline ELISA

1.	Pipette **10 µL** of **standards, controls** and **samples** from the **Enzyme Plate** (refer to 6.3) into the respective pre-coated **Noradrenaline Mikrotiter 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.

6.6 Dopamine ELISA

1.	Pipette **100 µL** of **standards, controls** and **samples** from the **Enzyme Plate** (refer to 6.3) into the respective pre-coated **Dopamine Mikrotiter Strips.**
2.	Pipette **50 µL** of the respective **Dopamine 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 standards refer to:

	Concentration of the standards					
Standard	**A**	**B**	**C**	**D**	**E**	**F**
Adrenaline (pg/mL)	0	20	60	200	800	3 200
Adrenaline (pmol/L)	0	109	328	1 092	4 368	17 472
Noradrenaline (pg/mL)	0	80	240	800	3 200	12 800
Noradrenaline (pmol/L)	0	473	1 418	4 728	18 912	75 648
Dopamine (pg/mL)	0	20	60	200	800	3 200
Dopamine (pmol/L)	0	131	392	1 306	5 224	20 896
Conversion:	Adrenaline (ng/mL) x 5.46 = Adrenaline (nmol/L) Noradrenaline (ng/mL) x 5.91 = Noradrenaline (nmol/L) Dopamine (ng/mL) x 6.53 = Dopamine (nmol/L)					

The calibration curves are 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 **undiluted plasma samples** and the **controls** can be read directly from the standard curve.

Concentration of diluted plasma samples:
If only a plasma volume **< 600 µl** was used for the extraction, the concentration read from the standard curve has to be **multiplied** with a **volume-factor**:

$$\textbf{Volume-factor} = \frac{\textbf{600 µl}}{\textbf{used plasma volume (µl)}}$$

7.1 Quality control

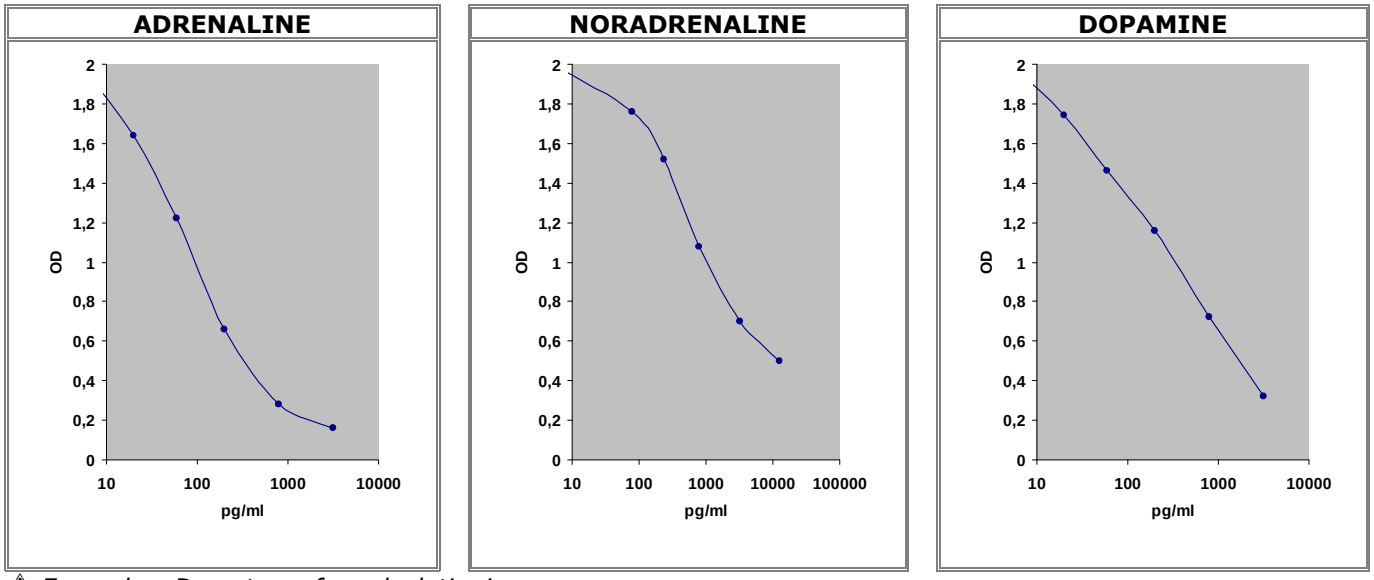
It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC Report.

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 curve



⚠ *Examples. Do not use for calculation!*

8. Assay characteristics

Expected Reference Values		**Adrenaline**	**Noradrenaline**	**Dopamine**
	Plasma	< 100 pg/mL	< 600 pg/mL	< 100 pg/mL

Analytical Specificity (Cross Reactivity)	**Substance**	**Cross Reactivity (%)**		
		Noradrenaline	Adrenaline	Dopamine
	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

Analytical Sensitivity (600 µl undiluted sample)	**Adrenaline**	**Noradrenaline**	**Dopamine**
	7 pg/mL	35 pg/mL	7 pg/mL

Functional Sensitivity (600 µl undiluted sample)	**Adrenaline**	**Noradrenaline**	**Dopamine**
	20 pg/mL	80 pg/mL	20 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

Recovery	Mean (%)	Range (%)	SD (%)	CV (%)
Adrenaline				
Human EDTA-Plasma	104.0	89.4 – 128.3	13.1	12.6
Noradrenaline				
Human EDTA-Plasma	116.5	104.8 – 125.6	8.0	6.9
Dopamine				
Human EDTA-Plasma	97.7	83.7 – 115.9	11.8	12.1

		Range	Serial dilution up to	Range (%)
Linearity	Noradrenaline	318 – 2 436 pg/mL	1:8	84 – 123
	Adrenaline	92 - 545 pg/mL	1:8	81 – 121
	Dopamine	98 - 798 pg/mL	1:8	74 – 131

⚠ ***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!