DIAsource ImmunoAssays S.A.
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1400 Nivelles
Belgium

Instructions for use

3-CAT ELISA

REF KAPL10-1600

3x96

+8 +2 °C

IVD

CE

Adrenaline – Noradrenaline - Dopamine ELISA

1. Principle of the test

Enzyme Immunoassay for the quantitative determination of Adrenaline (Epinephrine), Noradrenaline Norepinephrine), and Dopamine in plasma and urine. For in-vitro diagnostic use only.

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-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 tetramethylbenzidine (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 ADR 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-6110	ADR-AS	**Adrenaline Antiserum**	1 x 6 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA E-6210	NAD-AS	**Noradrenaline Antiserum**	1 x 6 mL	from rabbit, ready for use, yellow coloured, yellow screw cap
BA E-6310	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**	2 x 4 mL	ready for use
BA R-6601	STANDARD A	**Standard A**	1 x 4 mL	ready for use
BA R-6602	STANDARD B	**Standard B**	1 x 4 mL	ready for use
BA R-6603	STANDARD C	**Standard C**	1 x 4 mL	ready for use
BA R-6604	STANDARD D	**Standard D**	1 x 4 mL	ready for use
BA R-6605	STANDARD E	**Standard E**	1 x 4 mL	ready for use
BA R-6606	STANDARD F	**Standard F**	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-6613	ASSAY-BUFF	**Assay Buffer**	1 x 6 mL	ready for use, contains 1 M HCl
BA R-6614	COENZYME	**Coenzyme**	1 x 2 mL	ready for use, S-adenosyl-L-methionine
BA R-6615	ENZYME	**Enzyme**	6 x 1 mL	lyophilized, contains the enzyme COMT
BA R-6617	EXTRACT-BUFF	**Extraction Buffer**	1 x 6 mL	ready for use
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
BA R-6651	CONTROL 1	**Control 1**	1 x 4 mL	ready for use
BA R-6652	CONTROL 2	**Control 2**	1 x 4 mL	ready for use
BA R-6609	STANDARD A/B	**Standard A/B**	1 x 4 mL	ready for use

⚠ **for the determination of dopamine in plasma the additional **Standard A/B** is mandatory!*

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

- Calibrated variable precision micropipettes (e.g. 10-100 µL / 100-1 000µL)
- Microtiter plate washing device, Absorbent material (paper towel)
- ELISA reader capable of reading absorbance at 450 nm and 620 or 650 nm
- Shaker (shaking amplitude 3mm; approx. 600 rpm)
- 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.

Urine

Spontaneous or 24-hours urine, collected in a bottle containing 10-15 mL of 6 M HCl, should be used.
Storage: for longer periods (up to 6 months) 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.

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

⚠ **for the determination of dopamine in plasma the additional **Standard A/B** is mandatory!*

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 **distilled water** 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 **175 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:

Dopamine (standards + urine)	**25 µL**	**Dopamine (plasma)**	**50 µL**
Noradrenaline	**20 µL**	**Adrenaline**	**100 µL**

6.3 Dopamine ELISA

1.	Pipette **25 µl** of the **Enzyme Solution** (refer to 6.1*)* into all wells of the **Dopamine Microtiter Strips**.
2.	Pipette **25 µL** of the extracted **standards, 25 µL** of the extracted **controls, 25 µL** of the extracted **urine samples** and **50 µL** of the extracted **plasma samples** into the respective tubes.
3.	Add **25 µL** of **Hydrochloric Acid** to the **standards, controls** and **urine samples.**
4.	Incubate for **30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
5.	Pipette **50 µL** of the **Dopamine Antiserum** into all wells and cover plate with **Adhesive Foil**.
6.	Incubate for **2 hours** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
7.	Remove the foil. Discard or aspirate the content of the wells and **wash** each well **3 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** (20-25°C) on a shaker (approx. 600 rpm).
10.	Discard or aspirate the content of the wells and **wash** each well **3 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 **25 ±5 min** at **RT** (20-25°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.

6.4 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 hours** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
6.	Remove the foil. Discard or aspirate the content of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry 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 and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry 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 sun light!***
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** and a reference wavelength between 620 nm and 650 nm.

6.5 Adrenaline ELISA

1.	Pipette **25 µl** of the **Enzyme Solution** (refer to 6.1*)* into all wells of the **Adrenaline Mikrotiter Strips**.
2.	Pipette **100 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 respective **Adrenaline Antiserum** into all wells and cover plate with **Adhesive Foil**.
5.	Incubate for **2 hours** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
6.	Remove the foil. Discard or aspirate the content of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry 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 and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry 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 sun light!***
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** and a reference wavelength between 620 nm and 650 nm.

7. Calculation of results

	Concentration of the standards						
Standard	**A**	**B**	**C**	**D**	**E**	**F**	**A/B***
Adrenaline (ng/mL)	0	1	4	15	50	200	
Adrenaline (nmol/L)	0	5.5	22	82	273	1 092	
Noradrenaline (ng/mL)	0	5	20	75	250	1 000	
Noradrenaline (nmol/L)	0	30	118	443	1 478	5 910	
Dopamine (ng/mL)	0	10	40	150	500	2 000	4.5
Dopamine (nmol/L)	0	65	261	980	3 265	13 060	29
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)						

⚠ ***for the determination of dopamine in plasma the additional ***Standard A/B*** is mandatory!***

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).

Urine samples and controls:
The concentrations of the **urine samples** and the **Controls 1 & 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:

Adrenaline and Noradrenaline:
The read concentrations of the **plasma samples** have to be **divided by 30.**

Dopamine:
The read concentrations of the **plasma samples** have to be **divided by 60.**

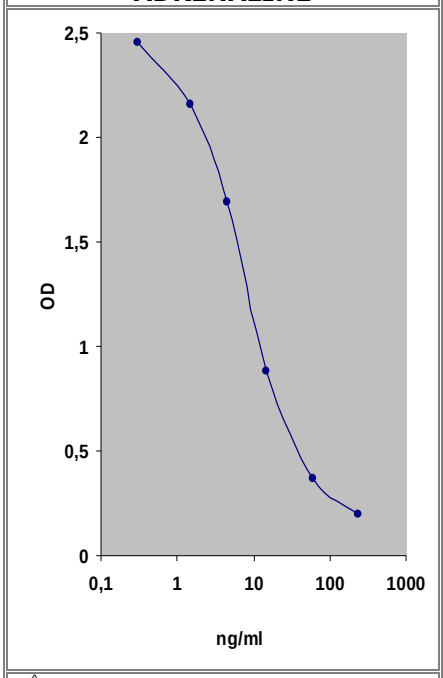
7.1 Quality control
It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are printed on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

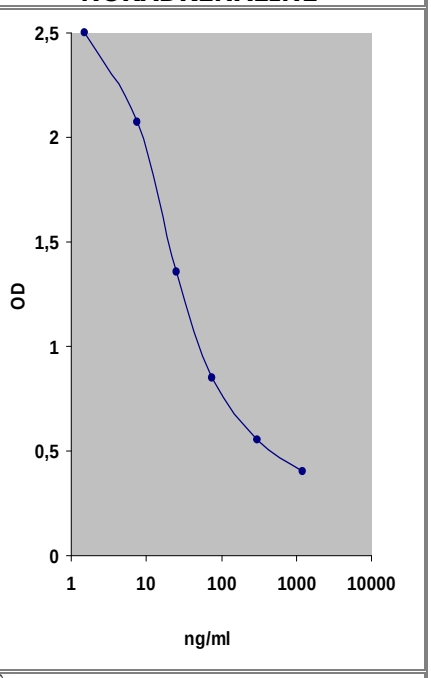
7.3 Typical calibration curves

ADRENALINE



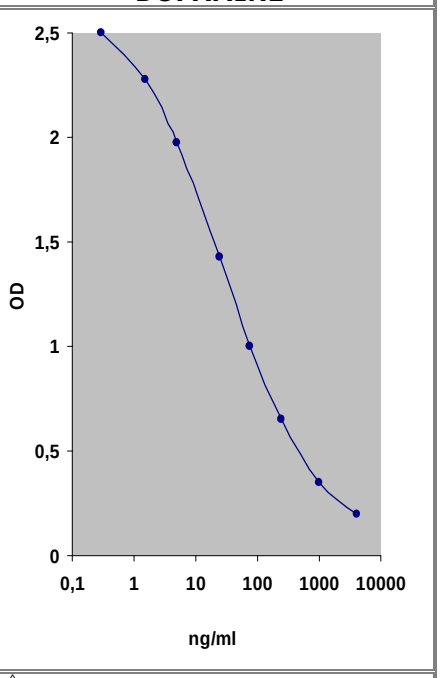
⚠ *Example. Do not use for calculation!*

NORADRENALINE



⚠ *Example. Do not use for calculation!*

DOPAMINE



⚠ *Example. Do not use for calculation!*

8. Assay characteristics

		Adrenaline	Noradrenaline	Dopamine
Expected Reference Values	Urine	< 20 µg/day (110 nmol/day)	< 90 µg/day (535 nmol/day)	< 600 µg/day (3 900 nmol/day)
	Plasma	< 100 pg/mL	< 600 pg/mL	< 100 pg/mL

		Adrenaline	Noradrenaline	Dopamine
Analytical Sensitivity (Limit of Detection)	Urine	0.3 ng/mL	1.5 ng/mL	4.5 ng/mL
	Plasma	10 pg/mL	50 pg/mL	25 pg/mL

	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

Precision

Intra-Assay				**Inter-Assay**			
	Sample	Range (ng/mL)	CV (%)		Sample	Range (ng/mL)	CV (%)
Noradrenaline	1	24.4 ± 3.9	16.1	Noradrenaline	1	39.8 ± 3.4	8.5
	2	92.7 ± 9.0	9.8		2	135 ± 20	15.0
Adrenaline	1	2.5 ± 0.4	15.0	Adrenaline	1	8.8 ± 1.1	13.2
	2	11.7 ± 0.8	6.9		2	34.2 ± 5.2	15.4
Dopamine	1	120 ± 11.5	9.5	Dopamine	1	148 ± 23.6	15.9
	2	1 074 ± 169	15.8		2	589 ± 107	18.2

		Range	Serial dilution up to	Range (%)
Linearity	Noradrenaline	4.9 – 339 ng/mL	1:64	87 - 120
	Adrenaline	1.3 – 81.4 ng/mL	1:64	88 – 122
	Dopamine	14 – 917 ng/mL	1:64	86 – 104

			Mean (%)	Range (%)	% Recovery after spiking
Recovery	Noradrenaline	Urine	109	83 – 115	
		Plasma	97	85 – 108	
	Adrenaline	Urine	107	84 – 119	
		Plasma	92	80 – 113	
	Dopamine	Urine	95	85 – 104	
		Plasma	90	74 – 110	

Method Comparison versus HPLC*	Noradrenaline	HPLC = 1.27 ELISA – 0.04	r = 0.96; n = 30
	Adrenaline	HPLC = 1.17 ELISA – 0.06	r = 0.99; n = 30
	Dopamine	HPLC = 0.98 ELISA + 0.08	r = 0.95; n = 30
* The concentrations were assessed using both the ELISA and the HPLC method (external QC samples from UK NEQAS). The correlation between ELISA and HPLC is excellent. This means, that the ELISA measure equally good when compared to the UK NEQAS HPLC data. Please take in mind, that the UK control values are the mean of about 40 different HPLC users, and contain always one pathological sample per sending.			

⚠ ***For updated 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!