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Instructions for use

3-CAT Urine ELISA Fast Track

REF **BA E-7600**

Σ 3 x 96

+2 +8 °C

IVD

CE

Adrenaline – Noradrenaline - Dopamine Urine ELISA

1. Intended use and principle of the test

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

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 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-0045	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	MTP ADR 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-7110	ADR-AS	**Adrenaline Antiserum**	1 x 12 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA E-7210	NAD-AS	**Noradrenaline Antiserum**	1 x 12 mL	from rabbit, ready for use, yellow coloured, yellow screw cap
BA E-7310	DOP-AS	**Dopamine Antiserum**	1 x 12 mL	from rabbit, ready for use, green coloured, green screw cap
BA R-0012	ACYL-CONC	**Acylation Concentrate**	1 x 0.5 ml	concentrated
BA R-0050	ADJUST-BUFF	**Adjustment Buffer**	1 x 4 mL	ready for use
BA R-0075	ACYL-DILUENT	**Acylation Diluent**	1 x 4 mL	ready for use
BA R-6611	ACYL-BUFF	**Acylation Buffer**	1 x 20 mL	ready for use
BA R-6614	COENZYME	**Coenzyme**	1 x 4 mL	ready for use, contains S-adenosyl-L-methionine
BA R-6615	ENZYME	**Enzyme**	4 x 1 mL	lyophilized, contains the enzyme catechol-O-methyltransferase
BA R-7601	STANDARD A	**Standard A**	1 x 4 mL	ready for use
BA R-7602	STANDARD B	**Standard B**	1 x 4 mL	ready for use
BA R-7603	STANDARD C	**Standard C**	1 x 4 mL	ready for use
BA R-7604	STANDARD D	**Standard D**	1 x 4 mL	ready for use
BA R-7605	STANDARD E	**Standard E**	1 x 4 mL	ready for use
BA R-7606	STANDARD F	**Standard F**	1 x 4 mL	ready for use
BA R-7617	TE-BUFF	**TE Buffer**	1 x 6 mL	ready for use
BA R-7618	EXTRACT-PLATE 96	**Extraction Plate**	1 x 96 wells	coated with boronate affinity gel
BA R-7626	RELEASE-BUFF	**Release Buffer**	1 x 20 mL	ready for use, yellow coloured, contains 0.025 M HCl
BA R-7651	CONTROL 1	**Control 1**	1 x 4 mL	ready for use
BA R-7652	CONTROL 2	**Control 2**	1 x 4 mL	ready for use

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
- ELISA reader capable of reading absorbance at 450 nm and 620 or 650 nm
- Shaker (shaking amplitude 3mm; approx. 600 rpm)
- Absorbent material (paper towel), distilled water, Vortex mixer

5. Sample collection and storage

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 2–8°C

Acylation Solution
The Acylation Concentrate is diluted 1 + 30 with Acylation-Diluent in a **glass or polypropylene-vial**.

Acylation Concentrate	10 µL	25 µL	50 µL	100 µL
Acylation-Diluent	300 µL	750 µL	1.5 mL	3 mL

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

6.2 Derivatization (extraction, acylation and O-methylation)

1.	Pipette **25 µL** of **standards**, **25 µL** of **controls,** and **25 µL** of **urine samples** into the respective wells of the **Extraction Plate**.
2.	Pipette **50 µL** of **TE Buffer** into all wells.
3.	Shake **15 min** at **RT** (20-25°C) on an orbital **shaker** (approx. 600 rpm).
4.	Discard or aspirate the contents 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.
5.	Pipette **100 µL** of **Acylation Buffer** into all wells.
6.	Pipette **25 µL** of **Acylation Solution** into all wells.
7.	Shake **10 min** at **RT** (20-25°C) on an orbital **shaker** (approx. 600 rpm).
8.	Discard or aspirate the contents 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.
9.	Pipette **150 µL** of **Release Buffer** into all wells.
10.	Shake **5 min** at **RT** (20-25°C) on an orbital **shaker** (approx. 600 rpm).
11.	Pipette **50 µL** of **Enzyme Solution** (*prepared freshly,* refer to 6.1*)* into all wells.
12.	Shake **30 min** at **RT** (20-25°C) on an orbital **shaker** (approx. 600 rpm). ⚠ ***Do not decant the supernatant thereafter!***

The following volumes of the eluate are needed for the ELISA:

Adrenaline	**50 µL**	**Noradrenaline**	**25 µL**	**Dopamine**	**25 µL**

6.3 Adrenaline ELISA

1.	Pipette **50 µL** of the **derivatized standards, controls** and **samples** into the appropriate wells.
2.	Pipette **100 µL** of the **Adrenaline Antiserum** into all wells.
3.	Incubate for **30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
4.	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.
5.	Pipette **100 µL** of the **Enzyme Conjugate** into all wells.
6.	Incubate for **15 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
7.	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 **Substrate** into all wells and incubate for **15 ± 2 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm). ***Avoid exposure to direct sun light!***
9.	Add **100 µL** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
10.	**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 **derivatized standards, controls and samples** into the appropriate wells.
2.	Pipette **100 µL** of the **Noradrenaline Antiserum** into all wells.
3.	Incubate for **30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
4.	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.
5.	Pipette **100 µL** of the **Enzyme Conjugate** into all wells.
6.	Incubate for **15 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
7.	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 **Substrate** into all wells and incubate for **15 ± 2 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm). ***Avoid exposure to direct sun light!***
9.	Add **100 µL** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
10.	**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 Dopamine ELISA

1.	Pipette **25 µL** of the **derivatized standards, controls and samples** into the appropriate wells.
2.	Pipette **100 µL** of the **Dopamine Antiserum** into all wells.
3.	Incubate for **30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
4.	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.
5.	Pipette **100 µL** of the **Enzyme Conjugate** into all wells.
6.	Incubate for **15 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
7.	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 **Substrate** into all wells and incubate for **15 ± 2 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm). ***Avoid exposure to direct sun light!***
9.	Add **100 µL** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
10.	**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**
Adrenaline (ng/mL)	0	1.5	4.5	15	60	240
Adrenaline (nmol/L)	0	8,19	24.6	81.9	328	1 310
Noradrenaline (ng/mL)	0	7.5	22.5	75	300	1 200
Noradrenaline (nmol/L)	0	44.3	133	443	1 773	7 092
Dopamine (ng/mL)	0	25	75	250	1 000	4 000
Dopamine (nmol/L)	0	163	490	1 633	6 530	26 120
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).

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

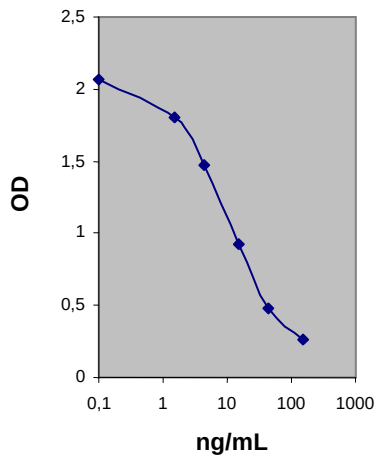
7.1 Quality control
It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are printed on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

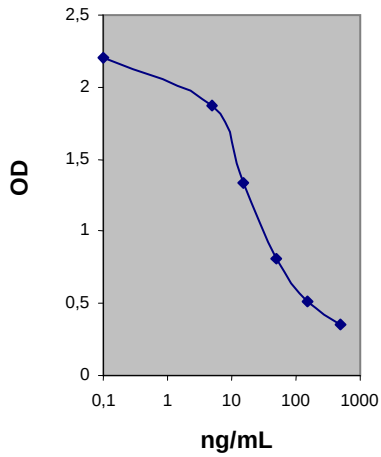
7.3 Typical calibration curves

ADRENALINE



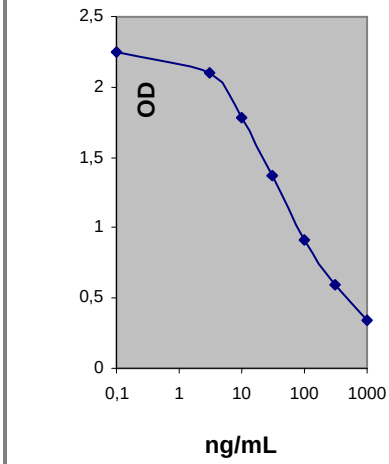
⚠ *Example. Do not use for calculation!*

NORADRENALINE



⚠ *Example. Do not use for calculation!*

DOPAMINE



⚠ *Example. Do not use for calculation!*

8. Assay characteristics

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

Analytical Sensitivity (Limit of Detection)	Mean signal (Zero-Standard) - 2SD			
		Adrenaline	**Noradrenaline**	**Dopamine**
	Urine	0.33 ng/mL	1.33 ng/mL	0.83 ng/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

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

Linearity			Range	Serial dilution up to	Range (%)
	Noradrenaline	Urine	20 - 339 ng/mL	1:16	85 - 123
	Adrenaline	Urine	4.6 – 81.4 ng/mL	1:16	86 – 124
	Dopamine	Urine	114 – 1 917 ng/mL	1:16	84 – 106

Recovery			Mean (%)	Range (%)	% Recovery after spiking
	Noradrenaline	Urine	109	83 – 115	
	Adrenaline	Urine	107	84 – 119	
	Dopamine	Urine	95	85 – 104	

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!