



Instructions for use

CAT ELISA

Enzyme Immunoassays for the
Quantitative Determination of
Adrenaline / Noradrenaline / Dopamine
in Plasma and Urine



Item No. EA603/288



3 x 96



2 – 8 °C

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Symbols

	In Vitro Diagnostic Medical Device		EC Declaration of conformity
	Content		Expiry Date
	Lot Number		Store at
	Manufactured by		Sufficient for ... determinations
	Catalogue Number of Manufacturer		Consult Instructions for Use

Hazard Pictograms



Warning



Danger

1 Introduction and Principle of the Test

Catecholamine is the name of a group of aromatic amines (noradrenaline, adrenaline, dopamine, and their derivatives) which act as hormones and neurotransmitters, respectively. Adrenaline and noradrenaline are formed from dopamine. They act on the cardiac musculature and the metabolism (adrenaline) as well as on the peripheral circulation (noradrenaline) and help the body to cope with acute and chronic stress.

An increased production of catecholamines can be found with tumours of the chromaffine system (pheochromocytoma, neuroblastoma, ganglioneuroma). An increased or decreased concentration of the catecholamines can also be found with hypertension, degenerative cardiac diseases, schizophrenia and manic-depressive psychosis. The measurement of dopamine and its derivatives is of special diagnostic value with children who are suspected to have a neuroblastoma.

The assay kit provides materials for the quantitative measurement of adrenaline, noradrenaline and dopamine in plasma and urine. Noradrenaline, adrenaline and dopamine are extracted using a cis-diol-specific affinity gel and acylated to N-acylnoradrenaline, N-acyladrenaline and N-acyl-dopamine and then converted enzymatically into N-acylnormetanephrine, N-acylmetanephrine and N-acyl-3-methoxytyramine.

The competitive CAT ELISA kit uses the microtitre plate format. Adrenaline, noradrenaline and dopamine, respectively, are bound to the solid phase of the microtiter plate. Acylated catecholamine from the sample and solid phase bound catecholamine compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase catecholamine is detected by peroxidase-conjugated anti-rabbit IgG. The substrate TMB / peroxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase catecholamine is inversely proportional to the catecholamine concentration of the sample.

2 Precautions

- For in vitro diagnostic use only. For professional use only!
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy; however these materials should be handled as potentially infectious.
- Individual components of different lots and test kits should not be interchanged. The expiry dates and storage conditions stated on the packaging and the labels of the individual components must be observed.
- Some components of this kit are containing hazardous reagents. These components are marked with the adequate hazard label. Further information is in section 4 and in the corresponding MSDS.
- Before carrying out the test, the instructions for use, as included in the kit, should be read completely and the content understood.
- When handling the reagents, controls and patient samples, the current laboratory safety guidelines and good laboratory practice should be observed.
- Wear protective clothing, disposable gloves, and eye protection while performing the test.
- Avoid any actions that could result in ingestion, inhalation or injection of the reagents. Never pipette by mouth.
- Avoid contact with individual reagents.
- Dispose of waste according to state and local environmental protection regulations.
- The quality control guidelines in the medical laboratory regarding the inclusion of control samples and/or pooled samples should be observed.

3 Storage and Stability

The kit is shipped at ambient temperature and is subsequently stable until the stated expiry date when stored between 2 – 8 °C. Once opened, the kit is stable until the expiry date.

The shelf life of the ready-to-use reagents is indicated on the respective bottle label. The shelf life and storage conditions of the prepared reagents is stated under 6.1.

Bring all reagents to room temperature before use and refrigerate immediately after use.

4 Contents of the Kit

4.1 Reagents for Sample Preparation:

Extraction Plate EX-PLATE 2 plates
48 wells, coated with boronate affinity gel

Extraction Buffer EX-BUFF 1 vial
6 ml, ready for use

HCl HCL 1 vial
21 ml, ready for use, 0.025 M HCl

Standards 1 – 7 CAL 1 - CAL 7 7 vials
4 ml each, ready for use, Concentrations:

Standards	1	2	3	4	5	6	7
Adrenaline (ng/ml)	0	0.5	1.5	5	15	50	150
Adrenaline (nmol/l)	0	2.7	8.2	27.3	81.9	273	819
Noradrenaline (ng/ml)	0	1.5	5	15	50	150	500
Noradrenaline (nmol/l)	0	8.9	29.6	88.9	296	887	2,955
Dopamine (ng/ml)	0	1.5	10	40	160	640	2,560
Dopamine (nmol/l)	0	9.8	65.3	261	1,045	4,179	16,717

When determining urine samples only: Standard 2 can be omitted.

When determining plasma samples only: Standard 7 can be omitted.

Control 1 & 2 CON 1 & CON 2 2 vials
4 ml each, ready for use
Concentration: see QC Certificate

Acylation Reagent ACYL-REAG 1 vial
6 ml, ready for use, contains DMSO and DMF;
(Please note that DMSO/DMF reacts with many plastic materials including plastic trays. It does not react with normal pipette tips and with glass devices)



Warning



Danger

Acylation Buffer ACYL-BUFF 4 vials
20 ml, ready for use

Enzyme 2 ml, lyophilized, Catechol-O-methyltransferase	ENZYME	3 vials
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Coenzyme 1 ml, ready for use, S-adenosyl-L-methionine	COENZYME	2 vials
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Enzyme Buffer 2 ml, ready for use	ENZYME-BUFF	1 vial
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Warning

4.2 Reagents for ELISA

Adrenaline-Antiserum 6 ml, ready for use, rabbit, colour coded blue	AS-AD	1 vial
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Noradrenaline-Antiserum 6 ml, ready for use, rabbit, colour coded yellow	AS-NAD	1 vial
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Dopamine-Antiserum 6 ml, ready for use, rabbit, colour coded green	AS-DA	1 vial
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MT-Strips 8 wells each, break apart, precoated with: Derivatized adrenaline, colour coded blue	STRIPS-AD	12 strips
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MT-Strips 8 wells each, break apart, precoated with: Derivatized noradrenaline, colour coded yellow	STRIPS-NAD	12 strips
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MT-Strips 8 wells each, break apart, precoated with: Derivatized dopamine, colourless	STRIPS-DA	12 strips
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POD Conjugate 12 ml each , ready for use, anti-rabbit IgG-peroxidase conjugate	CONJ	3 vials
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Wash Buffer 20 ml each, concentrated Dilute each with dist. water to 1000 ml total volume	WASH	2 vials
Substrate 12 ml each, TMB solution, ready for use	SUB	3 vials
Stop Solution 12 ml each, ready for use, contain 0.3 M sulphuric acid	STOP	3 vials
Adhesive foil Ready for use	FOIL	10 pieces

Additional materials and equipment required but not provided:

- Pipettes for pipetting 20, 50, 300, 1000 μ l
- Repeating dispenser for 20, 50, 100, 150, 200, 250 μ l und 1 ml
- Horizontal shaker
- Microplate washing device or multichannel pipette
- Microplate photometer (450 nm)
- Distilled water

5 Sample Collection and Storage

5.1 Plasma

EDTA plasma samples are required for the assay. Physical and psychical stress usually causes a high increase of the catecholamine concentration. Therefore, it is recommended to let the patient rest for 20 to 30 minutes after the venipuncture and before collecting the blood sample.

Haemolytic, **icteric** and especially lipemic samples should not be used for the assay, as false low values will be obtained with such samples.

The plasma samples can be stored at 2 – 8 °C up to 6 hours. For a longer period (up to 1 week) the samples should be stored at -20 °C.

5.2 Urine

The total volume of urine excreted during a 24-hours period should be collected in a single bottle containing 10 - 15 ml of 6 M hydrochloric acid as preservative. Avoid exposure to direct sun light. Determine the total volume and take an aliquot for the measurement. For patients with suspected kidney disorders the creatinine concentration should be determined in addition. Urine samples can be stored at -20 °C for at least 6 months.

Mix and centrifuge before use.

6 Preparation of Reagents and Samples

6.1 Preparation of Reagents

6.1.1 Wash Buffer

Dilute the content (20 ml) of the bottle WASH with distilled water to a total volume of 1000 ml.

Store the diluted wash buffer at 2 – 8 °C for a maximum period of 4 weeks. Should the kit be used in several runs, then prepare only the required amount of wash buffer for each run.

6.1.2 Enzyme Mix

NOTE: The enzyme mix has to be prepared freshly prior to the assay (not longer than 10 - 15 minutes in advance). After use the reagent has to be discarded.

Reconstitute the content of one vial ENZYME with 2 ml distilled water.

Add 0.3 ml COENZYME and 0.3 ml ENZYME-BUFF (total volume: 2.6 ml) and mix thoroughly.

The three additional bottles of ENZYME allow another three runs of the test. If the whole kit is to be used in one run it is recommended to pool the contents of at least three vials of prepared enzyme mix.

All other reagents are ready for use.

6.2 Preparation of Samples

Preparation of standards, controls and samples is the same for the Adrenaline, Noradrenaline and Dopamine ELISAs and is performed in one extraction plate.

Allow reagents and samples to reach room temperature.

Determinations in duplicates are recommended.

Each 20 µl of Standards, Control 1 & 2 and urine samples are extracted.

Each 300 µl of plasma samples are extracted.

1. Pipette 20 µl Standard 1 – 7 **CAL 1 – 7** , 20 µl Control 1 & 2 **CON 1 & 2** and 20 µl Urine Sample into the respective wells of the extraction plate **EX-PLATE**. Add 250 µl of distilled water to these wells to correct for volume.
Pipette 300 µl Plasma Sample into the respective wells (no volume correction required).
2. Pipette 50 µl Extraction Buffer **EX-BUFF** into each well.
3. Incubate 60 minutes at room temperature on an orbital shaker (**medium speed**).
4. Decant the plate and remove residual liquid by **firmly** tapping the inverted plate on a paper towel.
5. Pipette 1 ml Wash Buffer **WASH** into each well and incubate for 5 minutes at room temperature on an orbital shaker (slow shaking).
6. Decant the plate and remove residual liquid by **firmly** tapping the inverted plate on a paper towel.
7. Pipette 150 µl Acylation Buffer **ACYL-BUFF** into each well.
8. Pipette 50 µl Acylation Reagent **ACYL-REAG** into each well and continue with step 9., immediately.
(please note that solvent reacts with many plastic materials including plastic trays; it does not react with normal pipette tips and with glass devices)
9. Incubate the plate for 20 minutes at room temperature on an orbital shaker (**medium speed**).
10. Decant the plate and remove residual liquid by **firmly** tapping the inverted plate on a paper towel.

11. Pipette 1 ml Wash Buffer **WASH** into each well and incubate for 5 minutes at room temperature on an orbital shaker (slow shaking).
12. Decant the plate and remove residual liquid by **firmly** tapping the inverted plate on a paper towel.
13. Repeat the wash steps 11. and 12.
14. Pipette 200 μ l HCl **HCL** into each well.
15. Incubate the plate covered with adhesive foil **FOIL** for 20 minutes at room temperature on an orbital shaker (**medium speed**).

Caution: Do not decant the supernatant thereafter.

Take each 50 μ l of the supernatant for the adrenaline assay, 50 μ l for the noradrenaline assay and 50 μ l for the dopamine assay.

7 Test Procedure ELISA

7.1 Adrenaline ELISA

1. Pipette 20 µl of freshly prepared Enzyme Mix (s. 6.1.2) into the required number of wells of STRIPS-AD (colour coded blue).
2. Add 50 µl each of prepared Standards, Controls and Patient Samples into the respective wells. A colour change to red occurs and indicates which wells have already been pipetted.
3. Incubate the plate covered with adhesive foil FOIL for 30 minutes at room temperature (20 – 25 °C) on an orbital shaker (medium speed).
4. Pipette 50 µl Adrenaline-Antiserum AS-AD (colour coded blue) into each well.
5. Cover the plate with adhesive foil FOIL, shake for 10 seconds and incubate for 12 - 20 hours (overnight) at 2 – 8 °C.
6. Discard or aspirate the contents of the wells and wash thoroughly with each 250 µl Wash Buffer WASH. Remove residual liquid by tapping the inverted plate on clean absorbent paper. Repeat the washing procedure 3 times.
7. Pipette 100 µl POD-Conjugate CONJ into each well.
8. Incubate for 30 minutes at room temperature on an orbital shaker (medium speed).
9. Washing: Repeat wash step 6.
10. Pipette 100 µl Substrate SUB into each well.
11. Shake for 10 seconds on an orbital shaker, cover with a box and incubate for 30 ± 5 minutes at room temperature (20 – 25 °C) without shaking.
12. Pipette 100 µl Stop Solution STOP into each well. Shake plate for 10 seconds.
13. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

7.2 Noradrenaline ELISA

1. Pipette 20 µl each of freshly prepared Enzyme Mix (s. 6.1.2) into the required number of wells of STRIPS-NAD (colour coded yellow).
2. Add 50 µl each of prepared Standards, Controls and Patient Samples into the respective wells. A colour change to red occurs and indicates which wells have already been pipetted.
3. Incubate the plate with adhesive foil FOIL for 30 minutes at room temperature (20 – 25 °C) on an orbital shaker (medium speed).
4. Pipette 50 µl Noradrenaline-Antiserum AS-NAD (colour coded yellow) into each well.
5. Cover the plate with adhesive foil FOIL, shake for 10 seconds and incubate for 12 - 20 hours (overnight) at 2 – 8 °C.
6. Discard or aspirate the contents of the wells and wash thoroughly with each 250 µl Wash Buffer WASH. Remove residual liquid by tapping the inverted plate on clean absorbent paper. Repeat the washing procedure 3 times.
7. Pipette 100 µl POD-Conjugate CONJ into each well.
8. Incubate for 30 minutes at room temperature on an orbital shaker (medium speed).
9. Washing: Repeat wash step 6.
10. Pipette 100 µl Substrate SUB into each well.
11. Shake for 10 seconds, cover with a box and incubate for 30 ± 5 minutes at room temperature (20 – 25 °C) without shaking.
12. Pipette 100 µl Stop Solution STOP into each well. Shake plate for 10 seconds.
13. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

7.3 Dopamine ELISA

1. Pipette 20 µl each of freshly prepared Enzyme Mix (s. 6.1.2) into the required number of wells of STRIPS-DA (colourless).
2. Add 50 µl each of prepared Standards, Controls and Patient Samples into the respective wells. A colour change to red occurs and indicates which wells have already been pipetted.
3. Incubate the plate with adhesive foil FOIL for 30 minutes at room temperature (20 – 25 °C) on an orbital shaker (medium speed).
4. Pipette 50 µl Dopamine-Antiserum AS-DA (colour coded green) into each well.
5. Cover the plate with adhesive foil FOIL, shake for 10 seconds and incubate for 12 - 20 hours (overnight) at 2 – 8 °C.
6. Discard or aspirate the contents of the wells and wash thoroughly with each 250 µl Wash Buffer WASH. Remove residual liquid by tapping the inverted plate on clean absorbent paper. Repeat the washing procedure 3 times.
7. Pipette 100 µl POD-Conjugate CONJ into each well.
8. Incubate for 30 minutes at room temperature on an orbital shaker (medium speed).
9. Washing: Repeat wash step 6.
10. Pipette 100 µl Substrate SUB into each well.
11. Shake for 10 seconds, cover with a box and incubate for 30 ± 5 minutes at room temperature (20 – 25 °C) without shaking.
12. Pipette 100 µl Stop Solution STOP into each well. Shake plate for 10 seconds.
13. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

8 Calculation of Results

The concentration of the standards (x-axis, logarithmic) are plotted against their corresponding optical density (y-axis, linear). Alternatively, the optical density of each standard and sample can be related to the optical density of the zero standard, expressed as the ratio OD/OD_{max} , and then plotted on the y-axis.

A good fit is provided with 4 Parameter Logistic (alternatively Log-Logit or Cubic Spline).

The concentration of the controls and urine samples **in ng/ml** can be read off the standard curve directly without any further conversion.

The read concentrations of adrenaline, noradrenaline and dopamine in **plasma samples** have to be **divided by 15** due to the use of 300 μ l plasma sample in relation to 20 μ l standard.

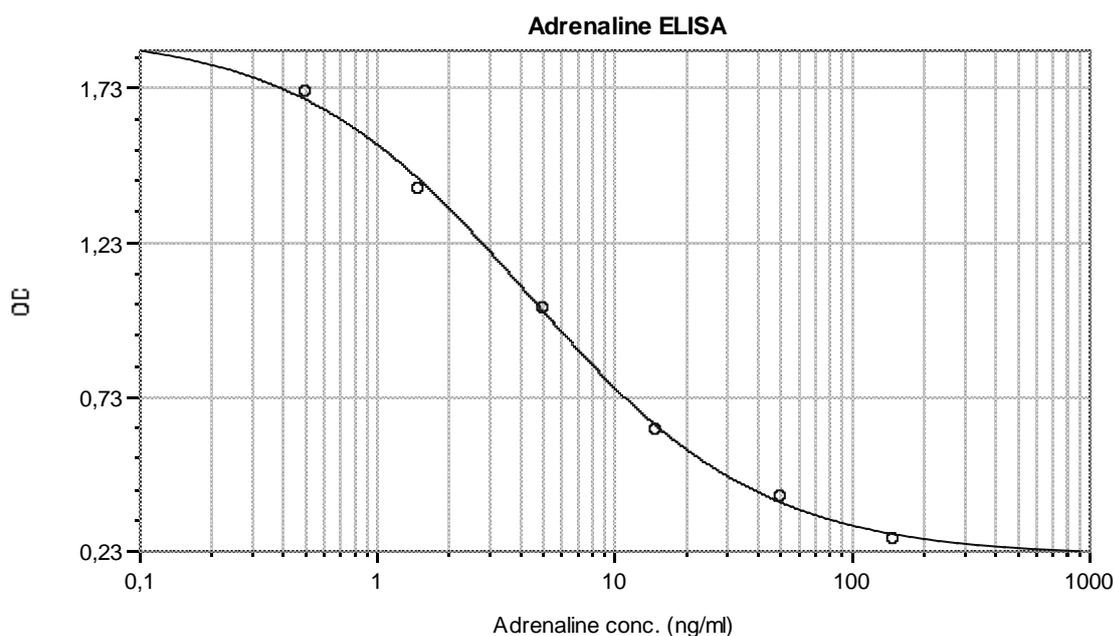
Adrenaline: 1 ng/ml = 5.46 nmol/l

Noradrenaline: 1 ng/ml = 5.91 nmol/l

Dopamine: 1 ng/ml = 6.53 nmol/l

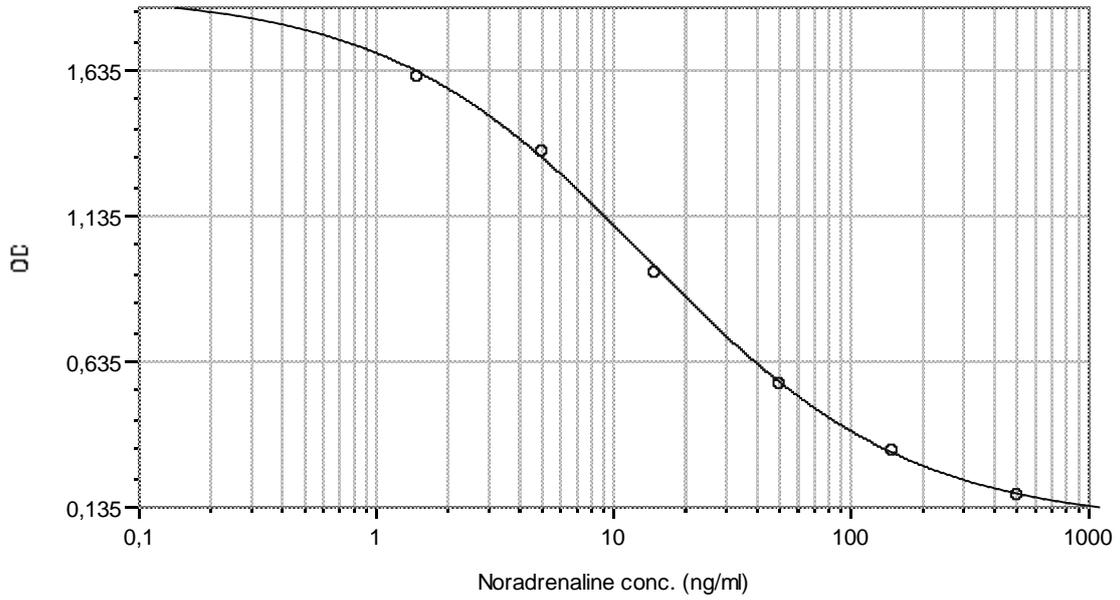
Quality Control: Test results are valid only if the kit controls are within the ranges specified on the QC Certificate. Otherwise, the test should be repeated.

Typical Example (do not use for calculation of results):



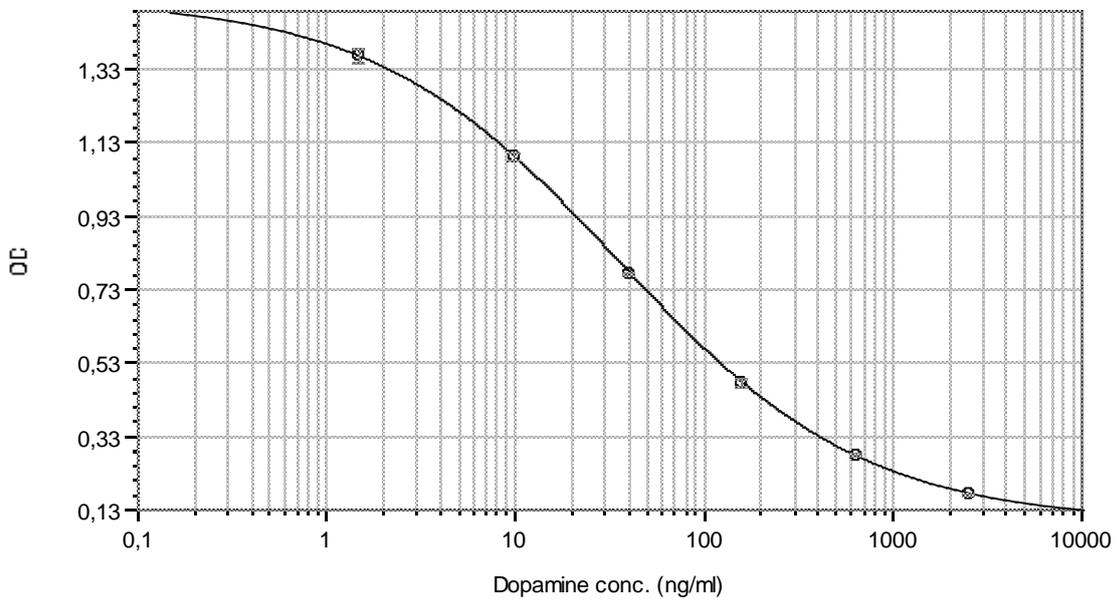
$y = ((A - D) / (1 + (x/C)^B)) + D$: A B C D R²
 ○ Std (Standards: Concentration vs MeanValue) 1,908 0,892 4,291 0,216 0,999

Noradrenaline ELISA



$y = ((A - D)/(1 + (x/C)^B)) + D$: A B C D R²
 ○ Std (Standards: Concentration vs MeanValue) 1,898 0,788 13,744 0,079 1

Dopamine ELISA



$y = ((A - D)/(1 + (x/C)^B)) + D$: A B C D R²
 ○ Std (Standards: Concentration vs MeanValue) 1,521 0,668 34,936 0,099 1

9 Assay Characteristics

9.1 Reference Ranges

The reference ranges given below should only be taken as a guideline. It is recommended that each laboratory should establish its own normal values.

Matrix	Adrenaline	Noradrenaline	Dopamine
Urine	< 20 µg/day	< 90 µg/day	< 600 µg/day
EDTA-Plasma	< 100 pg/ml	< 600 pg/ml	< 100 pg/ml

9.2 Sensitivity

Matrix	Adrenaline	Noradrenaline	Dopamine	Calculation
Urine	0.08 ng/ml	0.67 ng/ml	0.43 ng/ml	$OD_{Cal1} - 2xSD$
EDTA-Plasma	5 pg/ml	45 pg/ml	29 pg/ml	$OD_{Cal1} - 2xSD$

9.3 Specificity (Cross Reactivity)

Components	Cross Reactivity (%)	Cross Reactivity (%)	Cross Reactivity (%)
	Adrenaline-Ab	Noradrenaline-Ab	Dopamine-Ab
Adrenaline	100	< 0.01	< 0.02
Noradrenaline	0.053	100	0.45
Dopamine	< 0.01	0.37	100
Metanephrine	< 0.01	< 0.01	< 0.01
Normetanephrine	< 0.001	< 0.01	< 0.01
3-Methoxytyramine	< 0.001	< 0.01	< 0.01
L-Dopa	< 0.001	< 0.01	< 0.01
Tyramine	< 0.001	< 0.01	< 0.01
Tyrosine	< 0.001	< 0.001	< 0.002
Homovanillic acid	< 0.0001	< 0.001	< 0.001
Vanillic mandelic acid	< 0.0001	< 0.001	< 0.001

9.4 Recovery after spiking

Adrenaline

Matrix	Range (ng/ml)	Mean (%)	Recovery (%)
Urine	2.1 – 30.3	102	100 - 105
EDTA-Plasma	0.02 – 1.39	101	94 – 103

Noradrenaline

Matrix	Range (ng/ml)	Mean (%)	Recovery (%)
Urine	32.4 – 113.2	93	89 - 98
EDTA-Plasma	0.20 – 4.91	104	91 – 109

Dopamine

Matrix	Range (ng/ml)	Mean (%)	Recovery (%)
Urine	136 – 479	92	90 - 96
EDTA-Plasma	0.01 – 17.1	101	91 – 110

9.5 Linearity

Adrenaline

Matrix	Range (ng/ml)	Max. dilution	Mean (%)	Recovery (%)
Urine	4.4 – 59.7	1:15 (dist. water)	108	100 - 112
EDTA-Plasma	0.11 – 1.52	1:15 (dist. water)	107	104 - 111

Noradrenaline

Matrix	Range (ng/ml)	Max. dilution	Mean (%)	Recovery (%)
Urine	9.9 – 132.3	1:15 (dist. water)	105	98 - 112
EDTA-Plasma	0.33 – 4.87	1:15 (dist. water)	103	100 - 108

Dopamine

Matrix	Range (ng/ml)	Max. dilution	Mean (%)	Recovery (%)
Urine	48 – 750	1:15 (dist. water)	98	95 - 101
EDTA-Plasma	0.45 – 5.86	1:15 (dist. water)	107	97 - 115

9.6 Reproducibility

Adrenaline

Matrix	Range (ng/ml)	Intra-Assay-CV	Range (ng/ml)	Inter-Assay-CV
Urine	3.1 – 15.2	7.6 – 7.3 %	2.6 – 16.6	6.7 – 9.6 %
EDTA-Plasma	0.12 – 1.19	9.6 – 9.5 %		

Noradrenaline

Matrix	Range (ng/ml)	Intra-Assay-CV	Range (ng/ml)	Inter-Assay-CV
Urine	21.8 – 76.4	8.7 – 9.2 %	23.1 – 83.9	11.1 – 8.7 %
EDTA-Plasma	0.76 – 4.85	8.4 – 9.7 %		

Dopamine

Matrix	Range (ng/ml)	Intra-Assay-CV	Range (ng/ml)	Inter-Assay-CV
Urine	111 – 426	9.3 – 10.1 %	108 – 400	10.1 – 10.7 %
EDTA-Plasma	0.55 – 5.10	13.0 – 8.2 %		

9.7 Method Comparison

Adrenaline

Matrix	Method	Correlation
Urine	HPLC	$Y = 0.94 \times \text{HPLC} - 0.21$; $R = 0.987$; $N = 32$

Noradrenaline

Matrix	Method	Correlation
Urine	HPLC	$Y = 0.90 \times \text{HPLC} + 6.3$; $R = 0.983$; $N = 32$

Dopamine

Matrix	Method	Correlation
Urine	HPLC	$Y = 0.90 \times \text{HPLC} + 24$; $R = 0.982$; $N = 32$

9.8 Calibration

The calibration is carried out by weighing the pure substance. The correctness of the method was determined by comparing with the reference range and comparison of method (9.7).

9.9 Limitations of Method

The result of the CAT ELISA is to be seen in connection with other diagnostic procedures and the anamnesis and the resulting questions. Samples measured above the highest standard must be diluted with the appropriate medium as stated in 9.5 and reassayed. The values of diluted samples must be multiplied by the appropriate dilution factor.

9.10 Interferences

Hemolytic, lipemic and icteric specimens should not be used.

Do not use non-acidified urine collection.

10 Changes to declare

Version _10 (valid as of lot AND122): Hazard symbol was removed from POD Conjugate. Further changes are highlighted in gray.

Pipetting Scheme - Sample Preparation

(Adrenaline, Noradrenaline, Dopamine)

		Standards	Controls	Urine	Plasma
EX-PLATE:					
CAL 1 - 7	µl	20			
CON 1 & 2	µl		20		
Urine	µl			20	
EDTA-Plasma	µl				300
Dist. Water	µl	250	250	250	
EX-BUFF	µl	50	50	50	50

Shake 60 minutes at RT

Decant plate and remove residual liquid

WASH	ml	1	1	1	1
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Incubate 5 minutes at RT (slow shaking)

Decant plate and remove residual liquid

ACYL-BUFF	µl	150	150	150	150
ACYL-REAG	µl	50	50	50	50

Immediately shake 20 minutes at RT

Decant plate and remove residual liquid

WASH	ml	1	1	1	1
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Incubate 5 minutes at RT (slow shaking)

Decant plate and remove residual liquid

WASH	ml	1	1	1	1
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Incubate 5 minutes at RT (slow shaking)

Decant plate and remove residual liquid

HCl	µl	200	200	200	200
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Cover with **FOIL**; shake 20 minutes at RT**Caution: Do not decant the supernatant thereafter**

For the ELISAs take each

50 µl for Adrenaline ELISA

50 µl for Noradrenaline ELISA

50 µl for Dopamine ELISA

Pipetting Scheme - ELISA

	Prepared Standards	Prepared Controls	Prepared Samples
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Adrenaline STRIPS-AD (blue):				
Enzyme mix (fresh)	μl	20	20	20
Transfer from EX-PLATE into STRIPS-AD (blue)	μl	50	50	50
Cover with FOIL; shake 30 min at room temperature				
AS-AD (blue)	μl	50	50	50

Noradrenaline STRIPS-NAD (yellow):				
Enzyme mix (fresh)	μl	20	20	20
Transfer from EX-PLATE into STRIPS-NAD (yellow)	μl	50	50	50
Cover with FOIL; shake 30 min at room temperature				
AS-NAD (yellow)	μl	50	50	50

Dopamine STRIPS-DA (colourless):				
Enzyme mix (fresh)	μl	20	20	20
Transfer from EX-PLATE into STRIPS-DA (colourless)	μl	50	50	50
Cover with FOIL; shake 30 min at room temperature				
AS-DA (green)	μl	50	50	50

Cover with **FOIL** and shake for 10 seconds
 Incubate 12 – 20 hours (overnight) at 2-8 °C
 Wash 4 x with 250 μl **WASH** per well

CONJ	μl	100	100	100
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Shake 30 minutes at RT
 Wash 4 x with 250 μl **WASH** per well

SUB	μl	100	100	100
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Shake for 10 seconds
 Incubate 30 ± 5 minutes at RT, covered with a box, without shaking

STOP	μl	100	100	100
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Shake for 10 seconds
 Read absorbance at 450 nm (ref. 570 - 650 nm)