Instructions for Use

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TriCat TM Urine ELISA

Enzyme immunoassay for the quantitative determination of adrenaline (epinephrine), noradrenaline (norepinephrine) and dopamine in human urine.

REF **30143814**

Σ **3x96**

2°C 8°C

EU: IVD CE

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REVISION HISTORY OF INSTRUCTIONS FOR USE

New Product

1. INTENDED USE

Enzyme immunoassay for the quantitative determination of adrenaline (epinephrine), noradrenaline (norepinephrine) and dopamine in human urine.

2. INTENDED PURPOSE

The TriCat TM Urine ELISA is intended for the quantitative determination of adrenaline (epinephrine), noradrenaline (norepinephrine) and dopamine in human urine.

This supplemental test is intended as an aid to diagnosis of pheochromocytoma in which the individual levels or a combination of the levels of adrenaline and/or noradrenaline and/or dopamine are elevated in comparison with a corresponding population of apparently healthy adult individuals due to the presence of a catecholamine secreting tumor.

This test is intended to monitor the response to therapy (surgical removal of pheochromocytoma) by recurrent testing of individuals to quantify the individual levels of adrenaline and/or noradrenaline and/or dopamine in comparison with a corresponding population of apparently healthy adult individuals to confirm absence of pheochromocytoma.

Additionally, other clinical observations such as blood pressure monitoring, quantification of plasma metanephrines, computer tomography or magnetic resonance imaging may be employed to assess the status of the pheochromocytoma.

The TriCat TM Urine ELISA is NOT appropriate as a first-line test for pheochromocytoma.

The TriCat TM Urine ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA), based on selective adsorption of catecholamines and the principle of immunoglobulin binding and measured on an absorbance reader. The assay is semi-automated requiring general purpose laboratory instruments and consumables such as absorbance microplate reader/washer, vortex mixer and pipettes to execute the test. The assay is adaptable by laboratory personnel to automate on open ELISA based liquid handler platforms; however, the programming of the steps and timing required by the manual kit assay test instructions must be strictly adhered to and verified by the laboratory. Test results may be calculated manually from a standard curve and compared to laboratory established reference ranges from healthy adults (i.e. normal ranges).

The test kit is intended for professional laboratory use by trained personnel. The test kit is not for home or layperson use.

3. SUMMARY AND EXPLANATION

Catecholamines (adrenaline, noradrenaline and dopamine) are physiologically active molecules, which can be found in circulation in the human body and are excreted in urine. They act as neurotransmitters and hormones. Biosynthesis and physiological functions of adrenaline, noradrenaline and dopamine have been reviewed in the scientific literature.[1]

Adrenaline, noradrenaline and dopamine are biomarkers in pheochromocytoma, because the cells of these tumors show an increased production of these compounds.[2] Their role in the diagnosis and treatment has been documented in numerous review articles[3-7] and text books [8-10]. Even though urinary catecholamines are not recommended as a stand-alone diagnostic device, they are used by clinicians as a supplemental assay to urinary or plasma metanephrines.[11,12] Additional information about patient population and testing algorithms for pheochromocytoma are given in a guidance document of the international Endocrine Society.[2]

4. TEST PRINCIPLE

The assay system is based on selective adsorption and followed by a solid phase enzyme-linked immunosorbent assay (ELISA) based on immunoglobulin binding.
During extraction step the catecholamines in standards, controls and samples are selectively adsorbed onto the wells of the extraction plate and afterwards biotinylated by use of the acylation reagent. The biotinylated catecholamines are released and transferred onto the microtiter plate, coated with a goat anti rabbit antibody. The extracted standards, controls and samples are incubated in the coated wells together with antiserum containing rabbit antibodies specific to the targeted catecholamine and enzyme conjugated streptavidin.
After the substrate reaction the intensity of the developed color is proportional to the amount of the targeted catecholamine. Results of samples can be determined directly using the standard curve.

5. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only by GLP trained professionals.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Broken glass may cause injury. Handle glass vessels with caution.
5. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
6. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
7. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
8. Chemicals and prepared, used, unused or expired reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
9. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
10. All serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
11. Avoid contact with Stop solution. It may cause skin irritations and burns.
12. Some reagents contain ProClin 300 as preservative. In case of contact with eyes or skin, flush immediately with water. When disposing reagents, flush with a large volume of water to avoid built-up.
13. The device contains material of animal origin, which is certified apparently free of infectious or contagious diseases and injurious parasites. Still the material should be handled with extreme caution.

6. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sunlight. The storage and stability of specimens and prepared reagents is stated in the corresponding chapters.

The microtiter strips and extraction plate are stable up to the indicated expiry date after the kit is opened. Make sure that the opened bag is tightly closed when stored at 2 to 8°C.

7. SPECIMEN COLLECTION AND STORAGE

Specimen
Urine

Specimen collection
It is possible to use spontaneous as well as 24 h urine. The total volume of urine excreted during a 24 hours period should be collected and mixed in a single bottle containing 10-15 mL of 6 M HCl as preservative. Determine total volume for calculation of results. **Mix and centrifuge samples before use in the assay.**

The in-vivo catecholamine and metanephrines release is influenced by several foods and drugs. Vitamin B, coffee and bananas, alpha-methyldopa, MAO and COMT inhibitors as well as medications related to hypertension or local anesthetics (tetracaine) should be discontinued for at least 72 hours prior to specimen collection.

Sample Collection Device
No special requirements.

Specimen storage
Samples can be stored at 2°C to 25°C for 24 hours.
It is recommended to freeze samples and store at -20°C for long time storage (< 6 months).
Avoid repeated freeze-thaw cycles. Keep away from heat or direct sunlight.

8. MATERIALS SUPPLIED

Quantity	Symbol	Component
3 x 12x8	MTP	**Microtiter Plate** Break apart strips. Coated with anti-rabbit IgG (goat, polyclonal).
1 x 6 x 2.5 mL	CAL A-F	**Standard A-F** Ready to use. Contains: [-] Adrenaline, [-] Noradrenaline, Dopamine, and 0.1 M HCl. Exact concentrations see vial labels or QC certificate.
1 x 2 x 2.5 mL	CONTROL 1+2	**Control 1+2** Ready to use. Contains: [-] Adrenaline, [-] Noradrenaline, Dopamine and 0.1 M HCl. Exact concentrations see vial labels or QC certificate.
3 x 400 µL	ENZCONJ CONC	**Enzyme Conjugate Concentrate** (51x) Contains: Streptavidin alkaline phosphatase, Tris buffer, stabilizers.
1 x	EXTRPLATE	**Extraction Plate** Plate with 96 wells. Coated with boronate affinity gel.
1 x 60 mL	EXTRBUF	**Extraction Buffer** Pink colored. Ready to use. Contains: 0.016 % NaN_3.
4 x 1.65 mL	COMT LYO	**COMT lyophilized** Contains: Catechol-O-methyltransferase (porcine liver), NaN_3.
4 x 1.25 mL	COENZ	**Coenzyme Solution** Ready to use. Contains: S-Adenosyl-L-Methionine, stabilizers.
2 x 3 mL	ENZBUF	**Enzyme Buffer** Ready to use. Contains: Tris buffer, HCl, stabilizers.
1 x 100 mL	RELEASEBUF	**Release Buffer** Yellow Colored. Ready to use. Contains: 0.1 M HCl, indicator.
1 x 4 mL	ACYLREAG	**Acylation Reagent** Ready to use. Contains: DMSO
2 x 100 mL	WASHBUF CONC	**Wash Buffer Concentrate** (10x) Contains: Tris buffer, HCl, Tween, 0.2 % NaN_3.
1 x 10 mL	ANTISERUM AD	**Adrenaline Antiserum** Green colored. Ready to use. Contains: antibodies against Adrenaline (rabbit), Buffer, stabilizers.
1 x 10 mL	ANTISERUM NAD	**Noradrenaline Antiserum** Blue colored. Ready to use. Contains: antibodies against Noradrenaline (rabbit), Buffer, stabilizers.
1 x 10 mL	ANTISERUM DO	**Dopamine Antiserum** Violet colored. Ready to use. Contains: antibodies against Dopamine (rabbit), Buffer, stabilizers.
3 x 13 mL	PNPP SUBS	**PNPP Substrate Solution** Ready to use. Contains: p-nitrophenyl phosphate (PNPP).
3 x 15 mL	PNPP STOP	**PNPP Stop Solution** Ready to use. Contains: 1 M NaOH, 0.25 M EDTA.
1 x	DILPLATE	**Dilution Plate** Plate with 96 wells (PP).
9 x	FOIL	**Adhesive Foil**

9. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 10; 10-100; 100-1000 µL
2. Orbital shaker (200-900 rpm) (e.g. EAS 2/4, SLT)
3. Vortex mixer
4. 8-Channel Micropipettor with reagent reservoirs
5. Wash bottle, automated or semi-automated microtiter plate washing system
6. Microtiter plate reader capable of reading absorbance at 405 nm (reference wavelength 600-650 nm)
7. Bidistilled or deionised water
8. Paper towels, pipette tips and timer
9. Disposable tubes for sample dilution
10. Hydrochloric acid (HCl, 0.1 M) for sample dilution

10. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pre-treatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25°C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine duplicates to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout. It is advised to use the same pipetting scheme for extraction and ELISA.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
7. Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
8. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

11. PRE-TEST SETUP INSTRUCTIONS

The contents of the kit for 3 x 96 determinations can be divided into 2 separate runs.

⚠	**Visible amounts of gel can be separated from surface of extraction plate during extraction. This has no influence on test results.**
⚠	**Air contamination by peroxygen containing disinfectants for cleaning of surfaces or equipment used as powder or as solutions, e.g. VIRKON® must be avoided in any case. They will strongly disturb assay performance.** VIRKON® is a trademark of DuPont.

11.1. Dilution of Samples

Samples suspected to contain concentrations above the highest standard must diluted as follows:

Sample	to be diluted	with	Remarks
Urine	> highest standard and / or > 500 ng/mL Dopamine	0.1 M HCl	prior to extraction step

12. TEST PROCEDURE

12.1. Extraction of Samples, Standards and Controls (Extraction Plate)

1.	Pipette **100 µL** of **Extraction Buffer** into each well.
2.	Pipette **25 µL** of each **Standard, Control and sample** into the respective wells of the extraction plate.
3.	**Extract 60 minutes** at **18-25°C** (room temperature) on an orbital shaker (500 rpm).
4.	Discard incubation solution. Wash plate **3 x** with **400 µL** of **bidist. water**. Remove excess solution by tapping the inverted plate on a paper towel.
5.	Pipette **100 µL** of **Extraction Buffer** into each well.
6.	Pipette **25 µL** of **Acylation Reagent** into each well.
7.	**Incubate 30 minutes** at **18-25°C** on an orbital shaker (500 rpm).
8.	Discard incubation solution. Wash plate **3 x** with **400 µL** of **bidist. water**. Remove excess solution by tapping the inverted plate on a paper towel.
9.	Pipette **200 µL** of **Release Buffer** into each well.
10.	**Incubate 15 minutes** at **18-25°C** on an orbital shaker (500 rpm).

Prepared samples should be assayed the same day. If this is not possible, the extraction plate can be stored over night at 2-8°C, covered with adhesive foil.

12.2. Enzymatic Derivatization of Samples, Standards and Controls (Microtiter Plate)

⚠	If pipetting with *positive displacement*, give the residual fluid from the tip of the pipette back to the corresponding wells of the extraction plate, otherwise the extracts may not be sufficient for the determination of the other analytes. When transferring liquid from the extraction plate to the ELISA plate, it is useful to hold the extraction plate in a sloping position. Before use of the microtiter plates, define and label the wells for Adrenaline, Noradrenaline and Dopamine.

12.2.1. Preparation of concentrated components

⚠	The volumes stated below are for one run with 3 ELISA plates (3 x 96 determinations)						
Dilute / dissolve	**Component**	**with**	**Diluent**	**Relation**	**Remarks**	**Storage**	**Stability**
100 mL	**WASHBUF CONC**	900 mL	bidist. water	1:10	Mix vigorously.	2-8°C	10 weeks
900 µL	**ENZCONJ CONC**	45 mL	**WASHBUF** (diluted)	1:51	Prepare freshly and use only once. Mix without foaming.	18-25°C	5 hours

12.2.2. Preparation of COMT Enzyme Solution

⚠	For one run with 3 ELISA plates (3 x 96 determinations) 3x COMT need to be prepared.					
Dilute / dissolve	**Component**	**with**	**Diluent**	**Remarks**	**Storage**	**Stability**
1x	**COMT LYO**	1.65 mL	bidist. water	Mix vigorously.	If not needed freeze immediately at -20°C	1 month
	COMT (reconstituted)	1.25 mL 1.25 mL	**COENZ** **ENZBUF**	Mix without foaming.	18-25°C	2 hours

12.2.3. Adrenaline ELISA

1.	Pipette **50 µL** of each extracted **Standard, Control and sample** into the respective wells.
2.	Pipette **50 µL** of freshly prepared **COMT Enzyme Solution** into each well of the **Microtiter Plate**. Shake plate briefly.
3.	Pipette **75 µL** of **Adrenaline Antiserum** (green colored) into each well.
4.	Cover plate with adhesive foil. **Incubate 120 minutes** at **18-25°C** on an orbital shaker (500 rpm).

12.2.4. Noradrenaline ELISA

1.	Pipette **15 µL** of each extracted **Standard, Control and sample** into the respective wells.
2.	Pipette **25 µL** of freshly prepared **COMT Enzyme Solution** into each well of the **Microtiter Plate**. Shake plate briefly.
3.	Pipette **75 µL** of **Noradrenaline Antiserum** (blue colored) into each well.
4.	Cover plate with adhesive foil. **Incubate 120 minutes** at **18-25°C** on an orbital shaker (500 rpm).

12.2.5. Dopamine ELISA

⚠	*Important for measurement of **Dopamine*** *Dilution of extracted **Standard, Controls and urine samples** must be performed prior to pipetting into wells of Microtiter Plate in extra tubes or the provided dilution plate.* Therefore, dilute all extracted Standards, Controls and urine samples 1:3 with Release Buffer (i.e. in the included Dilution Plate: 20 µL extracted samples + 40 µL Release Buffer).

1.	Pipette **15 µL** of each extracted and 1:3 prediluted **Standard, Control** and **sample** into the respective wells.
2.	Pipette **25 µL** of freshly prepared **COMT Enzyme Solution** into each well of the **Microtiter Plate**. Shake plate briefly.
3.	Pipette **75 µL** of **Dopamine Antiserum** (violet colored) into each well.
4.	Cover plate with adhesive foil. **Incubate 120 minutes** at **18-25°C** on an orbital shaker (500 rpm).

12.3. ELISA

The following procedure must be performed for Adrenaline, Noradrenaline and Dopamine.

1.	Remove adhesive foil. Discard incubation solution. Wash plate **4 x** with **300 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
2.	Pipette **100 µL** of freshly prepared **Enzyme Conjugate** into each well.
3.	Cover plate with new adhesive foil. **Incubate 60 minutes** at **18-25°C** on an orbital shaker (500 rpm).
4.	Remove adhesive foil. Discard incubation solution. Wash plate **4 x** with **300 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
5.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
6.	Pipette **100 µL** of **PNPP Substrate Solution** into each well.
7.	**Incubate 25 minutes** at **18-25°C** (without adhesive foil) on an orbital shaker (500 rpm).
8.	Stop the substrate reaction by adding **100 µL** of **PNPP Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
9.	**Measure** optical density with a photometer at **405 nm** (Reference-wavelength: 620-650 nm) within **60 minutes** after pipetting of the Stop Solution. No air bubbles should be visible.

13. AUTOMATION

Automated protocols can be provided for open ELISA systems, e.g.: Freedom EVOlyzer®.
For more information please contact: ProductSupport.IBL@tecan.com
For the use of products on automated instruments it is absolutely necessary to perform and maintain a validation according to legal requirements. A successful validation of the process is a prerequisite for diagnostic use. The responsibility for the implementation and documentation of validation in accordance with the country-specific requirements is assumed by the organization or institution.

14. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or comparable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

It is recommended to participate at appropriate quality assessment trials.

15. CALCULATION OF RESULTS

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logistics or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentrations for adrenaline, noradrenaline and dopamine of samples and kit Controls can be read in ng/mL, directly from the corresponding standard curve.

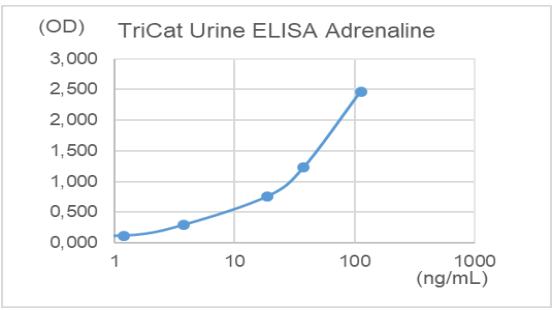
In case of diluted samples the values have to be multiplied with the corresponding dilution factor. Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and re-assayed.

Calculate the 24 hour excretion for each urine sample: µg/24h = µg/L x L/24h

Conversion: 1000 pg/mL = 1 ng/mL
Adrenaline (µg/L) x 5.458 = nmol/L
Noradrenaline (µg/L) x 5.911 = nmol/L
Dopamine (µg/L) x 6.528 = nmol/L

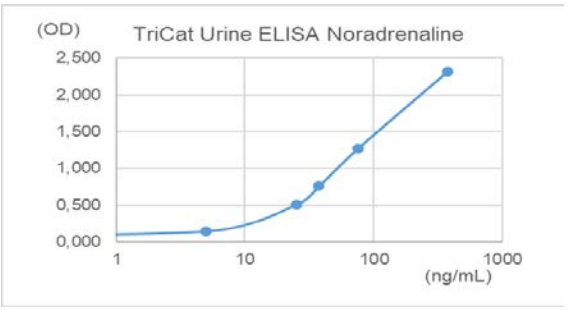
Typical Calibration Curve
(Example. Do not use for calculation!)

Standard	Adrenaline (ng/mL)	OD_{Mean}	OD/OD_{max} (%)
A	0	0.059	2.4
B	1	0.116	4.7
C	4	0.295	12.0
D	19	0.754	30.5
E	38	1.227	49.7
F	113	2.466	100



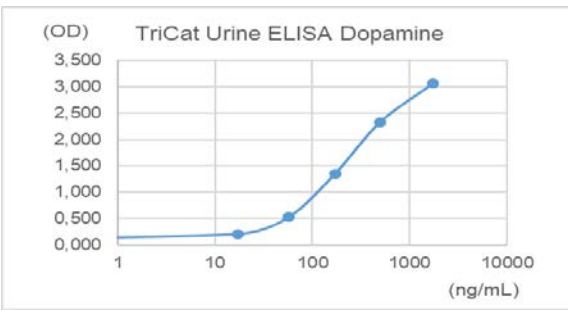
Measuring Range: from 3.5 ng/mL (LoQ) to 113 ng/mL (Standard F).

Standard	Noradrenaline (ng/mL)	OD_{Mean}	OD/OD_{max} (%)
A	0	0.049	2.1
B	5	0.143	6.1
C	25	0.510	22.0
D	38	0.760	32.8
E	75	1.266	54.6
F	375	2.319	100



Measuring Range: from 12.6 ng/mL (LoQ) to 375 ng/mL (Standard F).

Standard	Dopamine (ng/mL)	OD_{Mean}	OD/OD_{max} (%)
A	0	0.050	1.6
B	17	0.205	6.7
C	57	0.525	17.2
D	172	1.349	44.1
E	500	2.329	76.3
F	1725	3.055	100



Measuring Range: from 41.8 ng/mL (LoQ) to 500 ng/mL.

16. EXPECTED VALUES

Expected values for healthy and affected population are described in the literature [11, 12] and can be summarized as follows.

	Apparently healthy population Reference interval (µg/day)	Affected population (patients with pheochromocytoma) Median ± SD (µg/day)
Adrenaline	≤ 21	167 ± 231
Noradrenaline	15 - 80	481 ± 553
Dopamine	65 - 400	433 ± 892

The above stated reference interval for healthy population are comparable to reference ranges (2.5^{th} – 97.5^{th} percentile) established in an in-house study by testing urine samples derived from an apparently healthy European population.

	n	Range (ng/mL)	Median (ng/mL)	2.5^{th} - 97.5th Percentile (ng/mL)
Adrenaline	147	0.1 - 20.9	3.8	0.2 - 12.1
Noradrenaline	146	0.1 - 72.1	19.5	1.6 - 52.7
Dopamine	144	0.9 - 478	98.1	2.3 - 363

	n	Range (µg/day)	Median (µg/day)	2.5^{th} - 97.5th Percentile (µg/day)
Adrenaline	124	0.1 – 21.2	4.0	0.1 - 12.2
Noradrenaline	123	0.3 - 128	37.2	2.5 - 93.1
Dopamine	121	1.5 - 585	188	5.5 - 509

Conversion of data in µg/day was done for all donors where the total volume of urine per day was known.

Warning: Very low values in affected population may result in concentration below limit of detection or limit of quantitation.

The results themselves should not be the only reason for any therapeutic consequences. They have to be correlated to other clinical observations and diagnostic tests. It is recommended that each laboratory establishes its own range of normal values.

17. LIMITATIONS OF THE PROCEDURE

Specimen collection and storage have a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details. For cross-reactivities, see PERFORMANCE.

The following substances do not have a significant effect on the test results up to the concentration stated below:	Biotin	3500 ng/mL
	Sodium azide	1 %
	ProClin	1 %

18. PERFORMANCE

18.1. Analytical Specificity (Cross Reactivity)

Cross reactivity study was conducted by spiking potential cross-reacting substances in analyte free matrix in different concentrations. Cross reactivity for the following structural similar molecules were no detectable or low enough that they had no significant influence on the determination of adrenaline, noradrenaline and dopamine.

Substance	**Adrenaline**	**Noradrenaline**	**Dopamine**
Adrenaline	N/A	0.38%	-
Noradrenaline	2.69%	N/A	-
Dopamine	-	-	N/A
Adenochrome	0.095%	-	-

No cross-reactivities were found with the following substances tested.

DL-Metanephrine; DL-Normetanephrine; DL-Synephrine; L-3,4-Dihydroxyphenylalanine; Homovanillyl alcohol; Homovanillic acid; Vanillic acid; N-Acetyl-L-Tryptophan; Ferulic Acid; DL- Octopamine; Mandelic acid; Caffeic acid; β-Phenylethylamine; 3,4 Dihydroxymandelic acid

18.2. Evaluation of Detection Capability

Limit of Blank (LoB)

The limit of blank study was conducted with five different blank samples, measuring four replicates per sample using two different kit lots for three days resulting in 60 observations per lot.

Adrenaline: Limit of Blank = 0.4 ng/mL
Noradrenaline: Limit of Blank = 3.3 ng/mL
Dopamine: Limit of Blank = 4.8 ng/mL

Limit of Detection (LoD)

The limit of detection study was conducted with five different blank samples and five different low concentrated samples, measuring four replicates per sample using two different kit lots for three days resulting in 120 observations per lot.

Adrenaline: Limit of Detection = 0.8 ng/mL
Noradrenaline: Limit of Detection = 6.6 ng/mL
Dopamine: Limit of Detection = 14.3 ng/mL

Limit of Quantitation (LoQ)

The limit of quantitation study was conducted with at least five low concentrated samples (different concentration levels), measuring four replicates per sample using two different kit lots for three days resulting in at least 60 observations per lot. The LoQ will was calculated for a coefficient of variation of 20 %.

Adrenaline: Limit of Quantitation = 3.5 ng/mL
Noradrenaline: Limit of Quantitation = 12.6 ng/mL
Dopamine: Limit of Quantitation = 41.8 ng/mL

18.3. Metrological Traceability

Trueness of measurement is demonstrated by metrological traceability of concentrations assigned to the kit standards (SI unit ng/mL) by use of gravimetrical manufacturing process and internal reference material which was calibrated against available reference standards from the EDQM (European Directorate for the Quality of Medicines & HealthCare) for each analyte. The overall uncertainty was calculated according to the GUM (Guide to the expression of uncertainty in measurement) Method.

Adrenaline: 22.7 %
Noradrenaline: 18.6 %
Dopamine: 21.8 %

18.4. Method Comparison

A method comparison to HPLC reference method (Labor Lademannbogen - HPLC with electrochemical detection and sample preparation by solid phase extraction) was performed. Measurement of >100 urine samples, analyzed by Passing-Bablok regression analysis, showed the following results.

Adrenaline: IBL ELISA = 0.979 HPLC – 1.070; r = 0.919, n = 104
Noradrenaline: IBL ELISA = 0.941 HPLC – 5.108; r = 0.936, n = 102
Dopamine: IBL ELISA = 0.869 HPLC –26.910; r = 0.857, n = 102

18.5. Linearity

For the linearity study high and low concentrated samples were mixed in different proportions (at least 8 levels) with the high sample exceeding the concentration of the standard range. Three sample panels were diluted for each analyte and every level was tested in duplicate. The concentration relationship between the samples is known.

Predicted values were calculated by linear fit for the measurement results depending on the dilution proportion and compared to the measured results. Linearity was given when the measured valued did not differ more than 35 % from the predicted value.

Linear Range	Sample panel I	Sample panel II	Sample panel III
Adrenaline	3.1 - 109 ng/mL	3.9 – 101 ng/mL	6.0 - 107 ng/mL
Noradrenaline	20.8 - 389 ng/mL	32.0 - 124 ng/mL	20.2 - 369 ng/mL
Dopamine	18.9 - 1130 ng/mL	37.1 - 519 ng/mL	20.0 - 449 ng/mL

18.6. Recovery

The recovery study was performed with three different urine samples spiked with different amounts of the respective analyte. Each sample (spiked/ not spiked) was measured in duplicate. Measured concentrations of adrenaline, noradrenaline and dopamine were then compared to the expected values.

	Recovery (%)	Average Recovery (%)	Range (ng/mL)
Adrenaline	71 - 100	82	5.5 - 36.6
Noradrenaline	85 - 111	98	19.6 – 258
Dopamine	93 -132	103	23.9 - 497

18.7. Precision

The precision study for the intra- and inter- assay variation was conducted determining the concentration of 5 different urine samples in duplicate on two runs per day for 20 days.

Sample	Mean conc. Adrenaline (ng/mL)	Intra-Assay (within run)		Inter-Assay (between run)	
		SD (ng/mL)	CV	SD (ng/mL)	CV
1	2.5	0.6	23.1%	0.6	25.3%
2	3.3	0.5	14.5%	0.8	24.3%
3	9.2	1.0	10.6%	1.1	12.2%
4	15.0	1.1	7.6%	2.6	17.3%
5	18.3	1.3	7.1%	2.7	14.7%
		Mean CV	12.6%	Mean CV	18.8%

The precision study for adrenaline resulted in a mean intra-assay variation (within run CV) of 12.6 % (7.1 - 23.1%) and a mean inter-assay variation (total CV) of 18.8 % (12.2% - 25.3%) for five different samples with a concentration range from 2.5 ng/mL – 18.3 ng/mL.

Sample	Mean conc. Noradrenaline (ng/mL)	Intra-Assay (within run)		Inter-Assay (between run)	
		SD (ng/mL)	CV	SD (ng/mL)	CV
1	14.2	1.3	9.2%	3.2	22.5%
2	25. 8	1.6	6.4%	6.0	23.1%
3	41.5	2.1	5.1%	6.8	16.3%
4	74.3	3.9	5.2%	11.6	15.7%
5	66.5	3.5	5.3%	9.6	14.4%
		Mean CV	6.2%	Mean CV	18.4%

The precision study for noradrenaline resulted in a mean intra-assay variation (within run CV) of 6.2% (5.1 - 9.2%) and mean inter-assay variation (total CV) of 18.4 % (14.4 - 23.1%) for five different samples with a concentration range from 14.2 ng/mL – 74.3 ng/mL.

Sample	Mean conc. Dopamine (ng/mL)	Intra-Assay (within run)		Inter-Assay (between run)	
		SD (ng/mL)	CV	SD (ng/mL)	CV
1	109	8.4	7.8%	21.0	19.3%
2	25.6	2.7	10.4%	5.2	20.1%
3	204	22.4	11.0%	33.0	16.2%
4	367	35.6	9.7%	60.8	16.5%
5	369	39.0	10.6%	74.8	20.3%
		Mean CV	9.9%	Mean CV	18.5%

The precision study for dopamine resulted in a mean intra-assay variation (within run CV) of 9.9% (7.8 - 11.0%) and mean inter-assay variation (total CV) of 18.5 % (16.2 - 20.3%) for five different samples with a concentration range from 25.6 ng/mL – 369 ng/mL.

The inter lot precision study was conducted by determining the concentration of 5 different urine samples (5 replicates per sample) using three different kit lots on 5 days

Sample	Mean conc. Adrenaline (ng/mL)	Inter-Lot	
		SD (ng/mL)	CV
1	3.1	0.6	17.7%
2	6.1	1.1	18.6%
3	9.5	1.8	19.2%
4	13.5	2.3	17.1%
5	27.4	2.4	8.8%
		Mean CV	16.3%

The between lot precision study for adrenaline resulted in a mean CV of 16.3% (8.8% - 19.2%) for five different urine samples with a concentration range from 3.1 ng/mL – 27.4 ng/mL.

Sample	Mean conc. Noradrenaline (ng/mL)	Inter-Lot	
		SD (ng/mL)	CV
1	27.3	5.7	20.7%
2	31.9	6.4	20.2%
3	42.9	10.0	23.3%
4	69.6	17.7	25.4%
5	98.8	20.6	20.9%
		Mean CV	22.1%

The between lot precision study for noradrenaline resulted in a mean CV of 22.1% (20.2% - 25.4%) for five different urine samples with a concentration range from 27.3 ng/mL -98.8 ng/mL.

Sample	Mean conc. Dopamine (ng/mL)	Inter-Lot	
		SD (ng/mL)	CV
1	145	7.0	4.8%
2	135	13.7	10.2%
3	240	26.8	11.2%
4	384	50.0	13.0%
5	509	61.7	12.1%
		Mean CV	10.3%

The between lot precision study for dopamine resulted in a mean CV of 10.3% (4.8% - 13.0%) for five different urine samples with a concentration range from 145 ng/mL – 509 ng/mL.

19. PRODUCT LITERATURE REFERENCES

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20. SHORT OVERVIEW ASSAY PROCEDURE

Extraction:

100 µL of Extraction Buffer into each well
25 µL of each Standard, Control and urine sample
Extract 60 minutes at 18-25°C (room temperature) on an orbital shaker (500 rpm)

⇩

Wash plate 3 x with 400 µL of bidist. water, remove excess solution

⇩

100 µL of Extraction Buffer
25 µL of Acylation Reagent into each well
Incubate 30 minutes at 18-25°C on an orbital shaker (500 rpm)

⇩

Wash plate 3 x with 400 µL of bidist. water, remove excess solution

⇩

Pipette 200 µL of Release Buffer into each well
Incubate 15 minutes at 18-25°C on an orbital shaker (500 rpm)

⇩

ELISA:

Adrenaline	**Noradrenaline**	**Dopamine**
-	-	Dilute extracted Standard, Control and sample 1:3
50 µL extracted Standard, Control and sample 50 µL prepared COMT into each well 75µl Adrenaline Antiserum into each well	15 µL extracted Standard, Control and sample 25 µL prepared COMT into each well 75 µL Noradrenaline Antiserum into each well	15 µL of 1:3 diluted extracted Standard, Control and sample 25 µL prepared COMT into each well 75 µL Dopamine Antiserum into each well

Cover plate and incubate 120 minutes at 18-25°C on orbital shaker (500 rpm)

⇩

Wash plate 4 x with 300 µL of Wash Buffer, remove excess solution

⇩

100 µL Enzyme Conjugate into each well
Incubate 60 minutes at 18-25°C on orbital shaker (500 rpm)

⇩

Wash plate 4 x with 300 µL of Wash Buffer, remove excess solution

⇩

100 µL PNPP Substrate Solution into each well
Incubate 25 minutes at 18-25°C on orbital shaker (500 rpm)

⇩

100 µL PNPP Stop Solution into each well
Measure with photometer at 405 nm (reference-wavelength: 620-650 nm)

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Store at: 2-8°C / Lagern bei: 2-8°C / Stocker à: 2-8°C / Almacene a: 2-8°C / Armazenar a: 2-8°C / Conservare a: 2-8°C / Αποθήκευση στους: 2-8°C
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Distributor: / Distributor: / Distributeur: / Distributor: / Distribuidor: / Distributore: / Διανομέας:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
	Symbols of the kit components see MATERIALS SUPPLIED. Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. Voir MATERIEL FOURNI pour les symbôles des composants du kit. Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

Generic table, not all symbols are present in the product

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

The labelling of hazardous substances is according to European directive.
For further country-specific classifications, please refer to the corresponding safety data sheet.

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