Instructions for use T

2-CAT ELISA Reast Track

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BA E-6500R







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Related Products:

- Adrenaline ELISA Fast Track
- Noradrenaline ELISA Fast Track
- Dopamine ELISA Fast Track
- 3-CAT ELISA Fast Track

1. Introduction

1.1 Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of adrenaline (epinephrine) and noradrenaline (norepinephrine) in plasma and urine.

Adrenaline (epinephrine) and noradrenaline (norepinephrine) are extracted by using a cis-diol-specific affinity gel, acylated and then converted enzymatically.

The subsequent competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated standards, controls and samples compete with the solid phase bound analytes for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antigen-antibody 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 resulting in a colour reaction. The reaction is monitored at a wavelength of 450 nm.

Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations. Manual processing of the ELISA is recommended. The use of automatic laboratory equipment is the responsibility of the user.

This product is not intended to clinical diagnoses.

1.2 Background

In humans the catecholamines adrenaline (epinephrine), noradrenaline (norepinephrine) and dopamine are neurotransmitters of the sympathetic nervous system and are involved in many physiological processes. The sympathetic nervous system sets the body to a heightened state of alert, also called as the body's fight-or-flight response.

In the human body the catecholamines and their metabolites indicate the adaptation of the body to acute and chronic stress.

2. Procedural cautions, guidelines, warnings and limitations

2.1 Procedural cautions, guidelines and warnings

- (1) This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and must be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- (2) The principles of Good Laboratory Practice (GLP) must be followed.
- (3) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
- (4) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. For dilution or reconstitution purposes, use deionized, distilled, or ultrapure water. Avoid repeated freezing and thawing of reagents and specimens.
- (5) The microplate contains snap-off strips. Unused wells must be stored at 2 8 °C in the sealed foil pouch with desiccant and used in the frame provided. Microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up.
- (6) Duplicate determination of sample is highly recommended.
- (7) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials, and devices are prepared for use at the appropriate time.
- (8) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
- (9) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
- (10) A standard curve must be established for each run.
- (11) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report provided with the kit.
- (12) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
- (13) Avoid contact with Stop Solution containing 0.25 M H₂SO₄. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.

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- (14) TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Rinse contaminated items before reuse.
- (15) For information about hazardous substances included in the kit please refer to Safety Data Sheet (SDS). The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
- (16) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
- (17) In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components must not be used for a test run. They must be stored properly until the manufacturer decides what to do with them. If it is decided that they are no longer suitable for measurements, they must be disposed of in accordance with national regulations.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results

2.2.1 Interfering substances and proper handling of specimens

Plasma

Samples containing precipitates or fibrin strands or which are hemolytic or lipemic might cause inaccurate results. Hemolytic samples (up to 4 mg/ml hemoglobin), icteric samples (up to 50 mg/dl bilirubin) and lipemic samples (up to 800 mg/dl triglycerides) have no influence on the assay results.

If the concentrations cannot be estimated and there are doubts as to whether the above limit values for hemolytic, icteric or lipemic samples are complied with, the samples should not be used in the assay.

24-hour urine

Please note the sample collection! If the percentage of the final concentration of acid is too high, this will lead to incorrect results for the urine samples.

2.2.2 Drug and food interferences

There are no known substances (drugs) which ingestion catecholamine level in the sample interferes with the measurement of

2.2.3 High-Dose-Hook effect

No hook effect was observed in this test.

3. Storage and stability

3. Storage and stability

Store kit and reagents at 2 – 8 °C until expiration date. Do not use kit and components beyond the expiry date indicated on the kit labels. Once opened, the reagents are stable for 2 months when stored at 2 - 8 °C. Once the resealable pouch of the ELISA plate has been opened, care should be taken to close it tightly again including the desiceant.

Materials

4.1 Contents of the kit

BA D-0090 FOILS Adhesive Foil - ready to use

Adhesive foils in a resealable pouch Content:

Number:

BA E-0030 WASH-CONC 50x Wash Buffer Concentrate - concentrated 50x

Content: Buffer with a non-ionic detergent and physiological pH

2 x 20 ml/vial, purple cap Volume:

CONJUGATE BA E-0040 **Enzyme Conjugate** – ready to use

Content: Goat anti-rabbit immunoglobulins conjugated with peroxidase

Volume: 2 x 12 ml/vial, red cap

Description: Species is goat

SUBSTRATE **BA E-0055** Substrate - ready to use

Content: Chromogenic substrate containing 3,3',5,5'-tetramethylbenzidine, substrate buffer

and hydrogen peroxide

2 x 12 ml/vial, black cap Volume:

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Noradrenaline Antiserum – ready to use preservative. Content: Volume: Description: **BA E-6210** Content: 1 x 6 ml/vial, yellow cap Volume: Species of antibody is rabbit, species of protein in buffer is bovine Description: **BA E-6612 ACYL-REAG** Acylation Reagent - ready to use Content: Acylation reagent in DMSO Volume: 1 x 3 ml/vial, white cap Adjustment Buffer -**BA R-0050** ADJUST-BUFF - ready to use Content: TRIS buffer Volume: 1 x 4 ml/vial, green cap Acylation Buffer - ready to use **BA R-6611 ACYL-BUFF** Buffer with light alkaline pH for the acylation Content: Volume: 1 x 20 ml/vial, white cap **ASSAY-BUFF BA R-6613** Assay Buffer - ready to use 1 M hydrochloric acid and a non-mercury preservative Content: Volume: 1 x 6 ml/vial grey cap Hazard pictograms: GH**S0**5 Danger Signal word: H314 Causes severe skin burns and eye damage. Hazard statements: Precautionary P280 Wear protective gloves, protective clothing, eye protection. statements: P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

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P310 Immediately call a doctor, a POISON CENTER.

P501 Dispose of contents/container to an authorised waste collection point.

BA R-6614 COENZYME Coenzyme – ready to use

Content: S-adenosyl-L-methionine Volume: 1 x 4 ml/vial, purple cap

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BA R-6615 ENZYME Enzyme – lyophilized Content: Catechol-O-methyltransferase Volume: 4 vials, pink cap Description: Catechol-O-methyltransferase from pig liver **BA R-6617 EXTRACT-BUFF** Extraction Buffer - ready to use Content: Buffer containing carbonate Volume: 1 x 6 ml/vial, brown cap **EXTRACT-PLATE** 48 Extraction Plate – ready to use **BA R-6618** Content: 2 x 48 well plates coated with boronate affinity gel in a resealable pouch ned with the kit **BA R-6619** HCL Hydrochloric Acid - ready to use Content: 0.025 M Hydrochloric Acid, yellow coloured Volume: 1 x 20 ml/vial, green cap

4.2 Calibration and Controls

Standards and Controls - ready to use

Cat. no.	Component	Colour/Cap	Concen [ng/	ml]	Solo	ol/I] 	Volume/ Vial
			ADR	NAD	ADR	NAD	
BA E-6601	STANDARD A	white	0	0	0	0	4 ml
BA E-6602	STANDARD B	yellow	1	55	5.5	30	4 ml
BA E-6603	STANDARD C	orange	4	20	22	118	4 ml
BA E-6604	STANDARD D	blue	15	75	82	443	4 ml
BA E-6605	STANDARD E	grey	50	250	273	1,478	4 ml
BA E-6606	STANDARD F	black	200	1,000	1,092	5,910	4 ml
BA E-6651	CONTROL 1	green	Refer to C			ted value	4 ml
BA E-6652	CONTROL 2	red	and accep	table ran	ge.		4 ml
Conversion:	adrenaline [ng/ml] x 5.46 = adrenaline [nmol/l] noradrenaline [ng/ml] x 5.91 = noradrenaline [nmol/l]						

Content: Acidic buffer with non-mercury stabilizer, spiked with defined quantity of adrenaline

and noradrenaline

4.3 Additional materials required but not provided in the kit

- Water (deionized, distilled, or ultra-pure)
- Absorbent material paper towel)

4.4 Additional equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 10 700 µl; 1 ml
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 650 nm
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)

Vortex mixer

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5. Sample collection, handling and storage

Plasma

Whole blood should be collected into centrifuge tubes containing EDTA as anti-coagulant and centrifuged according to manufacturer's instructions immediately after collection.

In case of hemolytic, icteric or lipemic samples see 2.2.1.

Storage: up to 6 hours at 2 - 8 °C, for longer period (up to 6 months) at -20 °C.

Repeated freezing and thawing should be avoided.

Urine

Spontaneous urine or 24-hour urine, collected in a bottle containing 10 – 15 ml of 6 M HCl, can be used. If 24-hour urine is used please record the total volume of the collected urine.

Storage: up to 48 hours at 2 – 8 °C, up to 24 hours at room temperature, 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 and samples to reach room temperature and mix thoroughly by gentle inversion before use. Number the Extraction Plate and microwell plates (Microtiter Strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up). Duplicate determinations are recommended.

The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent. The higher the temperature, the higher the absorption values will be. Varying incubation times will have similar influences on the absorbance. The optimal temperature during the enzyme immunoassay is between 20 - 25 °C.

The use of a microtiter plate shaker with the following specifications is mandatory: shaking amplitude 3 mm; approx. 600 rpm. Shaking with differing settings might influence the results.

 \triangle In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm.

6.1 Preparation of reagents and further notes

Wash Buffer

Dilute the 20 ml Wash Buffer Concentrate **WASH-CONC 50X** with water to a final volume of 1000 ml.

Storage: 2 months at 2 - 8 °C

Enzyme Solution

Reconstitute the content of the vial **ENZYME** with 1 ml water (deionized, distilled, or ultra-pure) and mix thoroughly. Add 0.3 ml of **COENZYME** followed by 0.7 ml of **ADJUST-BUFF**. 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!

Adrenaline Microtiter Strips and Noradrenaline Microtiter Strips

In rare cases residues of the blocking and stabilizing reagent can be seen in the wells as small, white dots or lines. These residues do not influence the quality of the product.

Acylation Reagent

The **ACYL-REAG** (BAC-6612) has a freezing point of 18.5 °C. To ensure that it is liquid when being used, it must be ensured that the Acylation Reagent has reached room temperature and forms a homogeneous, crystal-free solution before being used.

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6.2 Sample preparation, extraction and acylation

- 1. Pipette 10 µl of standards, controls, urine samples and 300 µl of plasma samples into the respective wells of the EXTRACT-PLATE 48.
- 2. Add 250 µl of water (deionized, distilled, or ultra-pure) to the wells with standards, controls and urine samples.
- 3. Pipette 50 µl of ASSAY-BUFF into all wells.
- **4.** Pipette **50** μ I of **EXTRACT-BUFF** into all wells.
- **5.** Cover plate with **FOILS** 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 ACYL-BUFF into all wells.
- 10. Pipette 25 μ I of ACYL-REAG 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 **150** μ**I** of **HCL** into all wells.
- **15.** Cover plate with **FOILS**. 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:

Adrenaline 100 µl Noradrenaline 20 µl

6.3 Adrenaline ELISA

- 1. Pipette 25 μ I of the Enzyme Solution (refer to 6.1) into all wells of the Adrenaline Microtiter Strips \coprod ADR MN.
- 2. Pipette 100 μ I 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 ADR-AS into all wells and cover plate with FOILS.
- 5. Incubate for 2 hat RT (20 25 °C) on a shaker (approx. 600 rpm).
- 6. Remove the foil. Discard or aspirate the content of the wells. Wash the plate $3 \times 10^{\circ}$ x by adding $300 \mu l$ of Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
- 7. Pipette 100 μ I of the **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. Wash the plate 3 x by adding 300 µl of Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
- 10. Pipette 100 μ I of the SUBSTRATE into all wells and incubate for 25 \pm 5 min at RT (20 25 °C) on a shaker (approx. 600 rpm). \triangle Avoid exposure to direct sunlight!
- 11. Add 100 µl of the STOP-SOLN to all wells and shake the microtiter plate shortly.
- **12. Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** (if available a reference wavelength between 620 nm and 650 nm is recommended).

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6.4 Noradrenaline ELISA

- 1. Pipette 25 μl of the Enzyme Solution (refer to 6.1) into all wells of the Noradrenaline Microtiter Strips W NAD NMN.
- **2.** Pipette **20** μ **I** 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 NAD-AS into all wells and cover plate with FOILS.
- **5.** Incubate for **2 h** at **RT** (20 25 °C) on a **shaker** (approx. 600 rpm).
- 6. Remove the foil. Discard or aspirate the content of the wells. Wash the plate $3 \times 10^{\circ}$ by adding $300 \ \mu l$ of Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
- 7. Pipette 100 μ I of the **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. Wash the plate 3 x by adding 300 µl of Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
- 11. Add 100 µl of the STOP-SOLN to all wells and shake the microtite plate shortly.
- **12. Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** (if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

		Adrenaline	Noradrenaline
Measuring range	Urine	0.7 200 ng/ml	2.5 - 1,000 ng/ml
	Plasma	18 - 6,667 pg/ml	93 – 33,333 pg/ml

The standard curve, which can be used to determine the concentration of the unknown samples, is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis) using a concentration of 0.001 ng/ml for Standard A (this alignment is mandatory because of the logarithmic presentation of the data). Use non-linear regression for curve fitting (e. g. 4-parameter, marquardt).

This assay is a competitive assay. This means: the OD-values are decreasing with increasing concentrations of the analyte OD-values found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.

Urine samples and controls

The concentrations of the **urine samples** and the **Controls** can be read directly from the standard curve.

Calculate the 24 h excretion for each urine sample: $\mu g/24h = \mu g/I \times I/24h$

Plasma samples

The read concentrations of the plasma samples have to be divided by 30.

Conversion:

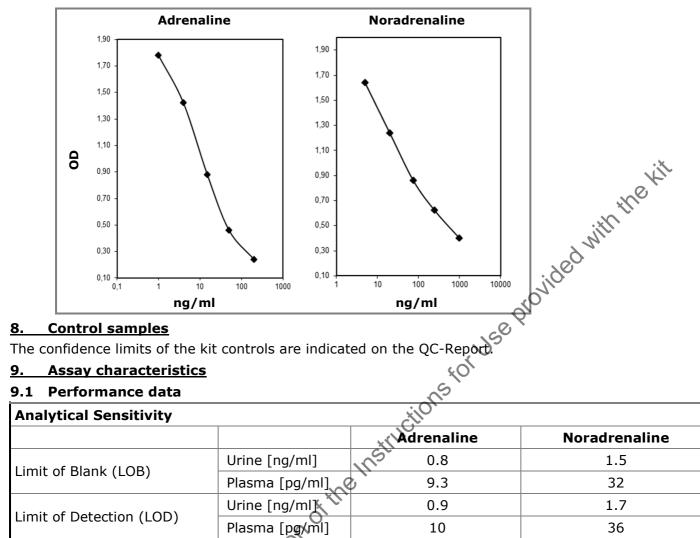
Adrenaline $[ng/ml] \times 5.46 = Adrenaline [nmol/l]$

Noradrenaline $[ng/ml] \times 5.91 = Noradrenaline [nmol/l]$

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7.1 Typical standard curve

AExamples: Do not use for calculation!



Analytical Sensitivity						
		Adrenaline	Noradrenaline			
Limit of Blank (LOB)	Urine [ng/ml]	0.8	1.5			
Limit of Blank (LOB)	Plasma [pg/ml]	9.3	32			
Limit of Detection (LOD)	Urine [ng/ml]	0.9	1.7			
Limit of Detection (LOD)	Plasma [pg/ml]	10	36			
Limit of Quantification (LOO)	Urine [ng/ml]	0.7	2.5			
Limit of Quantification (LOQ)	Plasma [pg/ml]	18	93			

Analytical Specificity (Cross Reactivity)							
Substance	Cross Reactivity [%]						
Substance	Adrenaline	Noradrenaline					
Derivatized Adrenaline	100	0.08					
Derivatized Noradrenaline	0.13	100					
Derivatized Dopamine	< 0.01	0.03					
Metanephrine	0.18	< 0.01					
Normetanephrine	< 0.01	0.16					
3-Methoxytyramine	< 0.01	< 0.01					
3-Methoxy-4-hydroxyphenylglycol	< 0.01	< 0.01					
Tyramine	< 0.01	< 0.01					
Phenylalanine, Caffeinic acid, L-Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid	< 0.01	< 0.01					

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Precision								
Intra-Assay Urine (n = 60)				Intra-Assay Plasma (n = 60)				
	Sample	Range [ng/ml]	CV [%]		Sample	Range [pg/ml]	CV [%]	
Adrenaline	1	6.2 ± 1.1	17.4	Adrenaline	1	64.7 ± 15.9	24.7	
	2	21.4 ± 2.7	12.4		2	258 ± 32.5	12.7	
	3	59.4 ± 7.8	13.1		3	948 ± 105	11.0	
Noradrenaline	1	26.1 ± 3.6	13.8	Noradrenaline	1	510 ± 65	12.8	
	2	97 ± 12.8	13.4		2	1,358 ± 194	14.3	
	3	267 ± 35	13.1		3	3,363 ± 374	11.1	
Inter-Assay Ur	ine (n = 3	33)		Inter-Assay Plasma (n = 18)				
	Sample	Range [ng/ml]	CV [%]		Sample	Range [pg/ml] 💥	CV [%]	
Adrenaline	1	5.2 ± 0.9	17.9	Adrenaline	1	76.4 ± 11.1	14.5	
	2	17.8 ± 2.1	11.7		2	247 ± 27.5	11.1	
	3	54.2 ± 6.6	12.1		3	771 ± 101	13.1	
Noradrenaline	1	19.5 ± 3.9	20.0	Noradrenaline	1	445 ± 40.9	9.2	
	2	80.6 ± 10.6	13.2		2010	1,232 ± 134	10.9	
	3	226 ± 39.5	17.4		3	3,283 ± 302	9.2	

Recovery							
		Range	Mean [%]	Range [%]			
A 1 1:	Urine	4.5 - 53.5 ng/ml	106	94 - 120			
Adrenaline	Plasma	9.1 - 4,268 pg/ml	105	88 - 117			
Noradrenaline	Urine	58.6 – 260 ng/ml	103	91 - 113			
	Plasma	51 – 14,251 pg/m	87	75 – 107			

Linearity								
		Serial dilution up to	Mean [%]	Range [%]				
A 1 1.	Urine	. 1.512	108	92 – 123				
Adrenaline	Plasma	1:512	105	94 – 115				
Noradrenaline	Urine	1:512	112	100 - 127				
	Plasma	1:512	112	102 – 125				

10. References/Literature

- (1) Kim et al. Vitamin of prevents stress-induced damage on the heart caused by the death of cardiomyocytes, through the down-regulation of the excessive production of catecholamine, TNF-a, and ROS production in GULO(-I-) Vit C-Insufficient mice. Free Radical Biology and Medicine, 65:573-583 (2013)
- (2) Bada et al. Peripheral vasodilatation determines cardiac output in exercising humans: insight from atrial pacing. The Journal of Physiology, 590(8):2051-2060 (2012)
- (3) Parks et al. Employment and work schedule are related to telomere length in women. Occupational & Environmental Medicine 68(8):582-589 (2011)

For updated literature or any other information please contact your local supplier.

11. Changes

Version	Release Date	Chapter	Change
19.0-r	2023-11-28	4.1	- Hazard labelling updated according to SDS

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Symbols:

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	$\dot{\mathbb{N}}$	Caution	REF	Catalogue number		Distributor
	سا	Date of manufacture			RUO	For research use only!

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