LABOR DIAGNOSTIKA NORD GmbH & Co.KG | Am Eichenhain 1 | 48531 Nordhorn | Germany | Tel. +49 5921 8197-0 | Fax +49 5921 8197-222 | info@ldn.de | www.ldn.de

DISTRIBUITO IN ITALIA DA:

Meridian Healthcare srl

Via G. Guglielmino, 68 - 95030 Tremestieri Etneo - CT

Tel. 095 725 68 69 - Fax: 095 725 44 54 e-mail: info@meridianhealthcare.it

Meridian Healthcare

Instructions for wise natine ELISA Fast Track

Adrenaline ELISA

Adrenaline ELISA

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1. Introduction

1.1 Intended use and principle of the test

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

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

1.2 Clinical application

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.

Next to the metanephrine/normetanephrine the catecholamines are important for the diagnosis and the follow-up of tumors of the sympathoadrenal system like the pheochromocytoma. The quantitative determination of catecholamines in urine is preferred for the diagnosis of these tumors, whereas the determination of catecholamines in plasma is medically sensible for the localization of the tumor and for function testing. Values above the cut-off can provide an indication for neuroendocrine tumors.

However, in literature various diseases like hypertension cardiovascular diseases, schizophrenia and manic depression are described with abnormal low or high levels of catecholamines.

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as under point "Procedural cautions, guidelines and warnings". Any laboratory result is only a part of the total clinical picture of the patient.

Only in cases where the laboratory results are in an acceptable agreement with the overall clinical picture of the patient it can be used for therapeutic consequences.

The test result itself should never be the sole determinant for deriving any therapeutic consequences.

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 has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- (2) This assay was validated for certain types of sample as indicated in *Intended Use* (please refer to Chapter 1). Any off-label use of this kit is in the responsibility of the user and the manufacturer cannot be held liable.
- (3) The principles of Good Laboratory Practice (GLP) have to be followed.
- (4) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
- (5) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- (6) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
- (7) The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant and used in the frame provided.
- (8) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
- (9) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- (10) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
- (11) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
- (12) A standard curve must be established for each run.

Version: 17.0 Effective: 2018-10-09 2/17

- (13) 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.
- (14) 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.
- (15) 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.
- (16) 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. Wash contaminated objects before reusing them.
- (17) For information on 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
- (18) The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
- (19) The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence (e.g. medication before a scheduled surgery) but have to be correlated to other diagnostic tests and clinical observations.
- (20) Kit reagents must be regarded as hazardous waste and disposed according to national regulations.

2.2 Limitations

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

2.2.1 Interfering substances

Plasma

Samples containing precipitates or fibrin strands or which are haemolytic or lipemic might cause inaccurate results.

24-hour urinePlease note the sample preparation! 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 interferences

2.2 Drug interferences

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

2.2.3 High-Dose-Hook effect

No hook effect was observed in this test.

3. Storage and stability

Store the unopened reagents at 200 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 - 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

4. Materials

4.1 Content of the kit

BA D-0090 Adhesive Foil - Ready to use **FOILS**

Content: Adhesive Foils in a resealable pouch

Volume: 1 x 4 foils

BA E-0030 Wash Buffer Concentrate - Concentrated 50x

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

Volume: 1 x 20 ml/vial, light purple cap

BA E-0040 Enzyme Conjugate - Ready to use CONJUGATE

Content: Goat anti-rabbit immunoglobulins, conjugated with peroxidase

Volume: 1 x 12 ml/vial, red cap

Version: 17.0 Effective: 2018-10-09 3/17

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

Content: Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen

peroxide

Volume: 1 x 12 ml/vial, black cap

BA E-0080 STOP-SOLN Stop Solution - Ready to use

Contents: 0.25 M sulfuric acid

Volume: 1 x 12 ml/vial, light grey cap

Hazards

identification:

H290 May be corrosive to metals.

H314 Causes severe skin burns and eye damage.

BA E-0131 MN ADR MN Adrenaline Microtiter Strips- Ready to use

Content: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable blue pouch with

desiccant

BA E-6110 ADR-AS Adrenaline Antiserum - Ready to use Content: Rabbit anti-adrenaline antibody, blue coloured Volume: 1 x 6 ml/vial, blue cap BA R-0050 ADJUST-BUFF Adjustment Buffer - Ready to use Content: TRIS buffer Volume: 1 x 4 ml/vial, green cap Standards and Controls - Ready to use							
Content: Volume:	TRIS buffer	aroon can		Olonia			
	1 x 4 ml/vial			Use .			
Standards an	id Controls - I	Ready to use	40				
Cat. no.	Component	Colour/ Cap	ng/ml	Concentration nmol/I ADR	Volume/ Vial		
BA E-6601	STANDARD A	white	1 1 4 15	0	4 ml		
BA E-6602	STANDARD B	light yellow	\oldots 1	5.5	4 ml		
BA E-6603	STANDARD C	orange	sion 4	22	4 ml		
BA E-6604	STANDARD D	dark blue	15	82	4 ml		
BA E-6605	STANDARD E	light grey	50	273	4 ml		
BA E-6606	STANDARD F	black	200	1 092	4 ml		
BA E-6651	CONTROL 1	light green	and the Cartain and the Cartai				
BA E-6652	CONTROL 2	dark red	acceptable range! 4 ml				

Adrenaline (ng/ml) x 5.46 = Adrenaline (nmol/l) Conversion:

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

BA R-6611 Acylation Buffer - Ready to use ACYL-BUFF

Content: Buffer with light alkaline pH for the acylation

Volume: 1 x 20 ml/vial, white cap

BA R-6612 Acylation Reagent - Ready to use ACYL-REAG

Content: Acylation reagent in DMF and DMSO

Volume: 1 x 3 ml/vial, light red cap

Hazards

identification:

H360D May damage the unborn child. H226 Flammable liquid and vapour.

H312 + H332 Harmful in contact with skin or if inhaled.

H319 Causes serious eye irritation.

Version: 17.0 Effective: 2018-10-09 4/17 **BA R-6613** Assay Buffer - Ready to use ASSAY-BUFF

Content: 1M hydrochloric acid and a non-mercury preservative

Volume: 1 x 6 ml/vial, light grey cap

BA R-6614 Coenzyme - Ready to use COENZYME

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

BA R-6615 ENZYME Enzyme - Lyophilized

Content: Catechol-O-methyltransferase

Volume: 2 vials, pink cap

BA R-6617 EXTRACT-BUFF Extraction Buffer - Ready to use

Content: Buffer containing carbonate 1 x 6 ml/vial, brown cap Volume:

BA R-6618 EXTRACT-PLATE 48 Extraction Plate - Ready to use

Content: 2 x 48 well plates coated with boronate affinity gel in a resealable pouch

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

Calibrated precision pipettes to dispense volumes between 10 700 µl· 1 miles

Microtiter plate washing device (manual, semi-automated and microtiter plate shaker (shall)

Microtiter plate shaker (shall)

Absorbert ELISA reader capable of reading absorbance at 450 nm and if possible 620 - 650 nm

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Water (deionized, distilled, or ultra-pure)

- Vortex mixer

5. Sample collection and storage

Plasma

Whole blood should be collected into centrifuge tubes containing EDTA as anti-coagulant (Monovette™ or Vacuette™ for plasma) and centrifuged according to manufacturer's instructions immediately after collection. Haemolytic and lipemic samples should not be used for the assay.

Storage: up to 6 hours at 2 - 8 °C, for longer period (up to 6 month) 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 month) 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. It is recommended to number the strips of the microwell plate before usage to avoid any mix-up.

The binding of the antiserum and the enzyme conjugate and the activity of the enzyme are temperature dependent, and the absorbance may vary if a thermostat is not used. The higher the temperature, the higher the absorbance will be. Varying incubation times will have a similar influence on the absorbance. The optimal temperature during the Enzyme Immunoassay is between 20 - 25 °C.

Version: 17.0 Effective: 2018-10-09 5/17

6.1 Preparation of reagents

Wash Buffer

Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 ml.

Storage: 1 month at 2 - 8 °C

Enzyme Solution

Reconstitute the content of the vial labelled '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 Adjustment Buffer. The total volume of the Enzyme Solution is 2.0 ml.



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

Adrenaline 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.

6.2 Sample preparation, extraction and acylation

- 1. Pipette 10 μ I of standards, controls, urine samples and 300 μ I of plasma samples into the respective wells of the Extraction Plate.
- 2. Add 250 μl of water (deionized, distilled, or ultra-pure) to the wells with standards, controls and urine samples.
- 3. Pipette 50 μ I of Assay Buffer into all wells.
- **4.** Pipette **50 μl** of **Extraction Buffer** into all wells.
- 5. Cover plate with **Adhesive Foil** and incubate **30 min** at **RT** (20 25 °C) on a **shaker** (approx. 600 rpm).
- **6.** Remove the foil. Empty plate and blot dry by tapping the inverted plate on absorbent material.
- 7. Pipette 1 ml of Wash Buffer into all wells. Incubate the plate for 5 min at RT (20 25 °C) on a shaker (approx. 600 rpm). Empty plate and blot dry by tapping the inverted plate on absorbent material.
- 8. Pipette another 1 ml of Wash Buffer into all wells. Incubate the plate for 5 min at RT (20 25 °C) on a shaker (approx. 600 rpm). Empty plate and blot dry by tapping the inverted plate on absorbent material.
- 9. Pipette 150 µl of Acylation Buffer into all wells.
- 10. Pipette 25 μl of Acylation Reagent into all wells.
- **11.** Incubate **15 min** at **RT** (20 25 °C) on a shaker (approx. 600 rpm).
- 12. Empty plate and blot dry by tapping the inverted plate on absorbent material.
- 13. Pipette 1 ml of Wash Buffer into all wells. Incubate the plate for 10 min at RT (20 25 °C) on a shaker (approx. 600 rpm). Empty plate and blot dry by tapping the inverted plate on absorbent material.
- 14. Pipette 150 μl of Hydrochloric Acid into all wells.
- **15.** Cover plate with **Adhesive Foil**. Incubate **10 min** at **RT** (20 25 °C) on a **shaker** (approx. 600 rpm). Remove the foil and discard.
- $\hat{\Lambda}$

Do not decant the supernatant thereafter!

The following volumes of the supernatant are needed for the subsequent ELISA:

Adrenaline 100 µl

Version: 17.0 Effective: 2018-10-09 6/17

6.3 Adrenaline ELISA

- 1. Pipette 25 μl of the Enzyme Solution (refer to 6.1) into all wells of the Adrenaline Microtiter Strips.
- 2. Pipette 100 µl of the extracted standards, controls and samples into the appropriate wells.
- 3. Incubate for 30 min at RT (20 25 °C) on a shaker (approx. 600 rpm).
- 4. Pipette 50 μl of the respective Adrenaline Antiserum into all wells and cover plate with Adhesive Foil.
- 5. Incubate for 2 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 x by adding 300 μl of Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
- 7. Pipette 100 μI of the Enzyme Conjugate into all wells.
- **8.** Incubate for **30 min** at **RT** (20 25 °C) on a **shaker** (approx. 600 rpm).
- **9.** Discard or aspirate the content of the wells. 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** µl of the **Substrate** into all wells and incubate for **25** ± **5** min at **RT** (20 − 25 °C) on a **shaker** (approx. 600 rpm). △ **Avoid exposure to direct sunlight!**
- 11. Add 100 μ I of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
- **12. Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** (if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

		Adrenaline
Measuring range	Urine	0.7 - 200 ng/ml
	Plasma	18 - 6667 pg/ml

The standard curve 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). Use a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

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) x 5.46 = Adrenaline (nmol/l)

Expected reference values

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

	Adrenaline
24-hour urine	< 20 μg/day
	(110 nmol/day)
Plasma	< 100 pg/ml

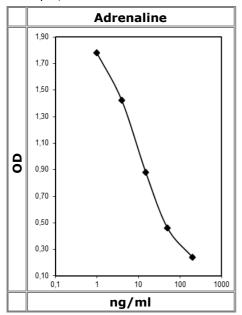
7.1 Quality control

It is recommended to use control samples according to national 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.

Version: 17.0 Effective: 2018-10-09 7/17

7.2 Typical standard curve

Example, do not use for calculation!



8. Assay characteristics

be provided with the kit Adrenaline Urine (ng/ml)
Plasma (pg/ml) 0.9 LOD 10 **Analytical Sensitivity**

	LOQ	Urine (ng/ml)	0.7		
		Plasma (pg/ml)	18		
	Substance	se instru	Cross Reactivity (%)		
	Substance		Adrenaline		
	Derivatized Adrenaline		100		
Analytical Specificity	Derivatize	ed Noradrenaline	0.13		
(Cross Reactivity)	Derivatize	ed Dopamine	< 0.01		
	Metanephrine		0.18		
	Normetar	iephrine	< 0.01		
	3-Methox	ytyramine	< 0.01		
	3-Methox	y-4-hydroxyphenylglycol	< 0.01		
	Tyramine		< 0.01		
Phenylalanine, Caffeinic acid, I		•	< 0.01		
OVE TEE	Dopa, Ho	movanillic acid, Tyrosine,			
Pic	3-Methox	y-4-hydroxymandelic acid			

Precision									
Intra-Assay Urine (n = 60)				Intra-Assay Plasma (n = 60)					
Sample Range (ng/ml) CV (%)				Sample	Range (pg/ml)	CV (%)			
	1	6.2 ± 1.1	17.4	Adrenaline	1	64.7 ± 15.9	24.7		
Adrenaline	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		
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		1	76.4 ± 11.1	14.5		
	2	17.8 ± 2.1	11.7	Adrenaline	2	247 ± 27.5	11.1		
	3	54.2 ± 6.6	12.1		3	771 ± 101	13.1		

			Serial dilution up to	Range (%)	Mean (%)
Linearity	Adrenaline	Urine	1:512	92 - 123	108
		Plasma	1:512	94 - 115	105

Version: 17.0 Effective: 2018-10-09 8/17

			Mean (%)	Range (%)	Range
Recovery	Advanalina	Urine	106	94 - 120	4.5 - 53.5 ng/ml
	Adrenaline	Plasma	105	88 - 117	9.1 - 4268 pg/ml

9. References/Literature

- (1) Kim et al. Vitamin C 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)



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

Symbols:

+2 +8 °C	Storage temperature	***	Manufacturer	Σ	Contains sufficient for <n> tests</n>
	Expiry date	LOT	Batch code	IVD	For in-vitro diagnostic use only!
i	Consult instructions for use	CONT	Content	CE	CE labelled
\triangle	Caution	REF	Catalogue number		

Version: 17.0 Effective: 2018-10-09 9/17