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Instructions for use

Adrenaline ELISA Fast Track

REF IB89551R

2°C – 8°C

Σ 96

RUO

For research use only – Not for use in diagnostic procedures

Table of contents

Related Products:

- Noradrenaline ELISA Fast Track
- Dopamine ELISA Fast Track
- 2-CAT 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) in plasma and urine.
Adrenaline (epinephrine) is 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 ultra-pure 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 H_2SO_4. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.

(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 1,550 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 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 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 desiccant.

4. Materials

4.1 Contents of the kit

BA D-0090	**FOILS**	**Adhesive Foil** – ready to use
Content:	Adhesive foils in a resealable pouch	
Number:	1 x 4 foils	
BA E-0030	**WASH-CONC** **50x**	**Wash Buffer Concentrate** – concentrated 50x
Content:	Buffer with a non-ionic detergent and physiological pH	
Volume:	1 x 20 ml/vial, purple cap	
BA E-0040	**CONJUGATE**	**Enzyme Conjugate** – ready to use
Content:	Goat anti-rabbit immunoglobulins conjugated with peroxidase	
Volume:	1 x 12 ml/vial, red cap	
Description:	Species is goat	
BA E-0055	**SUBSTRATE**	**Substrate** – ready to use
Content:	Chromogenic substrate containing 3,3',5,5'-tetramethylbenzidine, substrate buffer and hydrogen peroxide	
Volume:	1 x 12 ml/vial, black cap	

BA E-0080	**STOP-SOLN**	**Stop Solution** – ready to use
Content:	0.25 M sulfuric acid	
Volume:	1 x 12 ml/vial, grey cap	
BA E-0131	**IU ADR MN**	**Adrenaline Microtiter Strips** – ready to use
Content:	1 x 96 wells (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 in buffer with proteins and non-mercury preservative, blue coloured	
Volume:	1 x 6 ml/vial, blue cap	
Description:	Species of antibody is rabbit, species of protein in buffer is bovine	
BA E-6612	**ACYL-REAG**	**Acylation Reagent** – ready to use
Content:	Acylation reagent in DMSO	
Volume:	1 x 3 ml/vial, white cap	
BA R-0050	**ADJUST-BUFF**	**Adjustment Buffer** – ready to use
Content:	TRIS buffer	
Volume:	1 x 4 ml/vial, green cap	
BA R-6611	**ACYL-BUFF**	**Acylation Buffer** – ready to use
Content:	Buffer with light alkaline pH for the acylation	
Volume:	1 x 20 ml/vial, white cap	
BA R-6613	**ASSAY-BUFF**	**Assay Buffer** – ready to use
Content:	1 M hydrochloric acid and a non-mercury preservative	
Volume:	1 x 6 ml/vial, grey cap	
Hazard pictograms:	 GHS05	
Signal word:	Danger	
Hazard statements:	H314 Causes severe skin burns and eye damage.	
Precautionary statements:	P280 Wear protective gloves, protective clothing, eye protection. 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. 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	
BA R-6615	**ENZYME**	**Enzyme** – lyophilized
Content:	Catechol-O-methyltransferase	
Volume:	2 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	

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
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	Concentration [ng/ml] ADR	Concentration [nmol/l] ADR	Volume/ Vial
BA E-6601	**STANDARD A**	white	0	0	4 ml
BA E-6602	**STANDARD B**	yellow	1	5.5	4 ml
BA E-6603	**STANDARD C**	orange	4	22	4 ml
BA E-6604	**STANDARD D**	blue	15	82	4 ml
BA E-6605	**STANDARD E**	grey	50	273	4 ml
BA E-6606	**STANDARD F**	black	200	1,092	4 ml
BA E-6651	**CONTROL 1**	green	Refer to QC-Report for expected value and acceptable range.		4 ml
BA E-6652	**CONTROL 2**	red			4 ml

Conversion: adrenaline [ng/ml] x 5.46 = adrenaline [nmol/l]

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

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

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.*

⚠ *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

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** (BA E-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.

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 µl** 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 µl** 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 µl** 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**

6.3 Adrenaline ELISA

1.	Pipette **25 µl** of the **Enzyme Solution** (refer to 6.1*)* into all wells of the **Adrenaline Microtiter Strips** **ш** **ADR** **MN**.
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 **ADR-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 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 µl** 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 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 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).

7. Calculation of results

Measuring range	Adrenaline	
	Urine	0.7 – 200 ng/ml
	Plasma	18 – 6,667 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.*

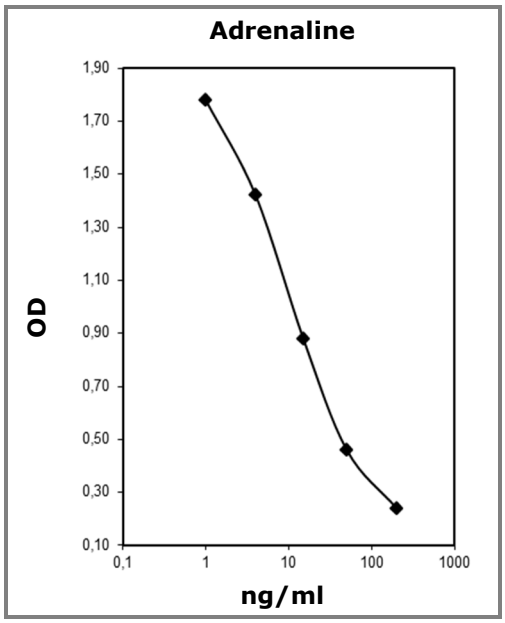
Urine samples and controls
The concentrations of the **urine samples** and the **Controls** can be read directly from the standard curve.
Calculate the 24 h excretion for each urine sample: **µg/24h = µg/l x l/24h**

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]

7.1 Typical standard curve

⚠*Example: Do not use for calculation!*



8. Control samples

The confidence limits of the kit controls are indicated on the QC-Report.

9. Assay characteristics

9.1 Performance data

Analytical Sensitivity		
	Adrenaline	
Limit of Blank (LOB)	Urine [ng/ml]	0.8
	Plasma [pg/ml]	9.3
Limit of Detection (LOD)	Urine [ng/ml]	0.9
	Plasma [pg/ml]	10
Limit of Quantification (LOQ)	Urine [ng/ml]	0.7
	Plasma [pg/ml]	18

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

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
Inter-Assay Urine (n = 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

Recovery				
		Range	Mean [%]	Range [%]
Adrenaline	Urine	4.5 – 53.5 ng/ml	106	94 – 120
	Plasma	9.1 – 4,268 pg/ml	105	88 – 117

Linearity				
		Serial dilution up to	Mean [%]	Range [%]
Adrenaline	Urine	1:512	108	92 – 123
	Plasma	1:512	105	94 – 115

10. 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-α, 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

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Use-by date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number		Distributor
	Date of manufacture			RUO	For research use only!