DIAGNOSTIKA GMBH

Instructions for Use

Adrenaline ELISA

Enzyme Immunoassay for the
Quantitative Determination of
Adrenaline in Plasma and Urine

CE IVD

Item No. EA604/96

12 x 8

2 – 8 °C

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Symbols

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

Hazard Pictograms


Warning


Danger

1 Introduction and Principle of the Test

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

An increased production of catecholamines can be found with tumours of the chromaffine system (pheochromocytoma, neuroblastoma, ganglioneuroma). An increased or decreased concentration of the catecholamines can also be found with hypertension, degenerative cardiac diseases, schizophrenia and manic-depressive psychosis.

The assay kit provides materials for the quantitative measurement of adrenaline in plasma and urine. Adrenaline is extracted using a cis-diol-specific affinity gel and acylated to N-acyladrenaline and then converted enzymatically into N-acylmetanephrine.

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

2 Precautions

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

3 Storage and Stability

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

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

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

4 Contents of the Kit

4.1 Reagents for Sample Preparation:

Extraction Plate **EX-PLATE** 2 plates

48 wells, coated with boronate affinity gel

Extraction Buffer **EX-BUFF** 1 vial

6 ml, ready for use

HCl **HCL** 1 vial

21 ml, ready for use, 0.025 M HCl

Standards 1 – 7 **CAL 1** - **CAL 7** 7 vials

4 ml each, ready for use, Concentrations:

Standards	1	2	3	4	5	6	7
Adrenaline (ng/ml)	0	0.5	1.5	5	15	50	150
Adrenaline (nmol/l)	0	2.7	8.2	27.3	81.9	273	819

When determining urine samples only: Standard 2 can be omitted.
When determining plasma samples only: Standard 7 can be omitted.

Control 1 & 2 **CON 1** & **CON 2** 2 vials

4 ml each, ready for use
Concentration: see QC Certificate

Acylation Reagent **ACYL-REAG** 1 vial

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


Warning


Danger

Acylation Buffer **ACYL-BUFF** 1 vial

20 ml, ready for use

Enzyme **ENZYME** 3 vials

2 ml, lyophilized, Catechol-O-methyltransferase

Coenzyme 1 ml, ready for use, S-adenosyl-L-methionine	**COENZYME**	1 vial
Enzyme Buffer 2 ml, ready for use	**ENZYME-BUFF**  Warning	1 vial

4.2 Reagents for ELISA:

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

Additional materials and equipment required but not provided:

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

5 Sample Collection and Storage

5.1 Plasma

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

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

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

5.2 Urine

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

Mix and centrifuge urine before use.

6 Preparation of Reagents and Samples

6.1 Preparation of Reagents

6.1.1 Wash Buffer

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

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

6.1.2 Enzyme Mix

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

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

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

The two additional bottles of ENZYME allow a second and a third run of the test. If the whole kit is to be used in one run one bottle of enzyme mix is sufficient.

All other reagents are ready for use.

6.2 Preparation of Samples

Allow reagents and samples to reach room temperature.

Determinations in duplicates are recommended.

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

Each 300 µl of plasma samples are extracted.

1. Pipette each 20 µl Standard 1 - 7 CAL 1 - 7, 20 µl Control 1 & 2 CON 1 & 2 and each 20 µl Urine Sample into the respective wells of the extraction plate EX-PLATE. Add 250 µl of distilled water to these wells to correct for volume.
   Pipette each 300 µl Plasma Sample into the respective wells (volume correction not required).
2. Pipette 50 µl Extraction Buffer EX-BUFF into each well.
3. Incubate 60 minutes at room temperature on an orbital shaker (medium speed).
4. Decant the plate and remove residual liquid by firmly tapping the inverted plate on a paper towel.
5. Pipette 1 ml Wash Buffer WASH into each well and incubate for 5 minutes at room temperature on an orbital shaker (low speed).
6. Decant the plate and remove residual liquid by firmly tapping the inverted plate on a paper towel.
7. Pipette 150 µl Acylation Buffer ACYL-BUFF into each well.
8. Pipette 50 µl Acylation Reagent ACYL-REAG into each well and continue with step 9, immediately.
   (please note, that solvent reacts with many plastic materials including plastic trays; it does not react with normal pipette tips and with glass devices)
9. Incubate the plate for 20 minutes at room temperature on an orbital shaker (medium speed).
10. Decant the plate and remove residual liquid by firmly tapping the inverted plate on a paper towel.

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

Caution: Do <u>not</u> decant the supernatant thereafter.

Take each 50 µl of the supernatant for the adrenaline assay.

7 Test Procedure ELISA

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

8 Calculation of Results

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

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

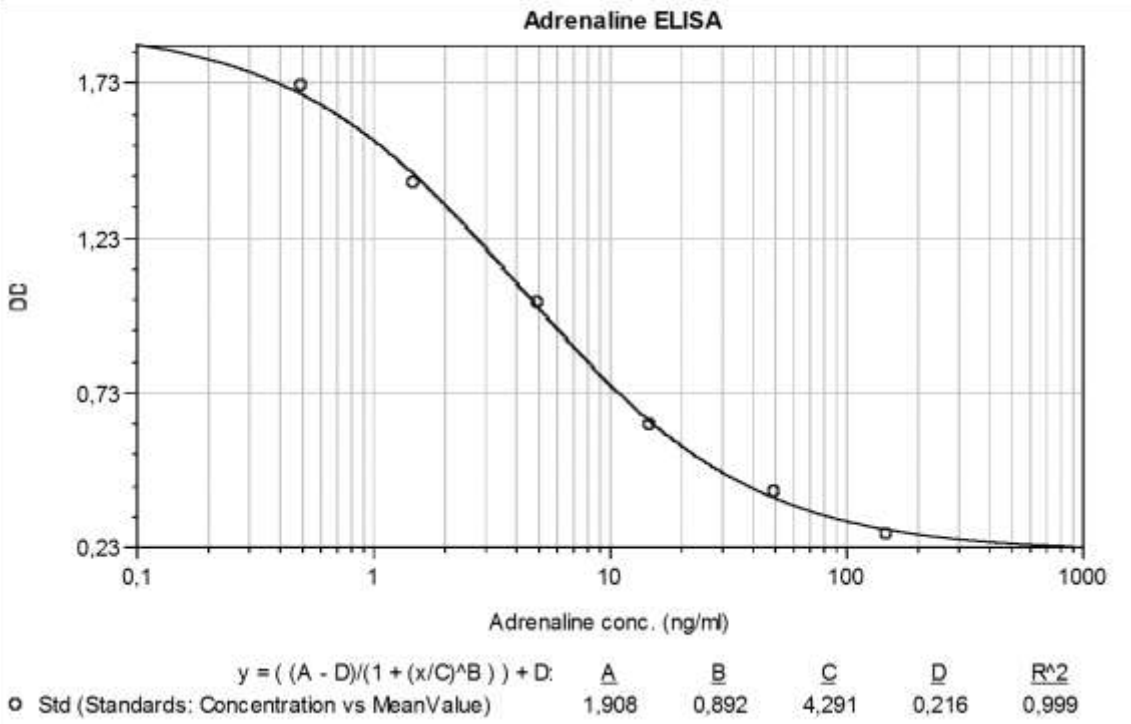
The concentration of the controls and urine samples in ng/ml can be read off the standard curve directly without any further conversion.

The read concentrations of adrenaline in plasma samples have to be divided by 15 due to the use of 300 µl plasma sample in relation to 20 µl standard.

Adrenaline: 1 ng/ml = 5.46 nmol/l

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

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



9 Assay Characteristics

9.1 Reference Ranges

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

Matrix	Reference Range Adrenaline
Urine	< 20 µg/day
EDTA-Plasma	< 100 pg/ml

9.2 Sensitivity

Matrix	LLOD Adrenaline	Calculation
Urine:	0.08 ng/ml	OD_{Cal1} – 2xSD
EDTA-Plasma:	5 pg/ml	OD_{Cal1} – 2xSD

9.3 Specificity (Cross Reactivity)

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

9.4 Recovery after spiking

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

9.5 Linearity

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

9.6 Reproducibility

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

9.7 Method Comparison

Matrix	Method	Correlation
Urine	HPLC	Y = 0.94 x HPLC - 0,21; R = 0.987; N = 32

9.8 Calibration

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

9.9 Limitations of Method

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

9.10 Interferences

Hemolytic, lipemic and icteric specimens should not be used. Do not use non-acidified urine collection.

10 Changes to declare

Version _10 (valid as of lot A122): Hazard symbol was removed from POD Conjugate. Further changes are highlighted in gray.

Pipetting Scheme - Sample Preparation

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

Shake 60 minutes at RT
Decant plate and remove residual liquid

WASH	ml	1	1	1	1

Incubate 5 minutes at RT (slow shaking)
Decant plate and remove residual liquid

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

Immediately, shake 20 minutes at RT
Decant plate and remove residual liquid

WASH	ml	1	1	1	1

Incubate 5 minutes at RT (slow shaking)
Decant plate and remove residual liquid

WASH	ml	1	1	1	1

Incubate 5 minutes at RT (slow shaking)
Decant plate and remove residual liquid

HCL	µl	200	200	200	200

Cover with FOIL; shake 20 minutes at RT

Caution: Do not decant the supernatant thereafter

Pipetting Scheme - ELISA

		Acyl. Standards	Acyl. Controls	Acyl. Samples
STRIPS-AD (blue):				
Enzyme mix (fresh)	µl	20	20	20
Transfer from EX-PLATE into STRIPS-AD	µl	50	50	50

Cover with FOIL; shake 30 min at RT

AS-AD	µl	50	50	50

Cover with FOIL
Shake for 10 seconds
Incubate 12 – 20 hours (overnight) at 2-8 °C

Wash 4 x with 250 µl WASH per well

CONJ	µl	100	100	100

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

SUB	µl	100	100	100

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

STOP	µl	100	100	100

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