DIAGNOSTIKA GMBH

Instructions for use

CAT ELISA

Enzyme Immunoassays for the
Quantitative Determination of
Adrenaline / Noradrenaline / Dopamine
in Plasma and Urine

CE IVD

Item No. EA603/288

 3 x 96

 2 – 8 °C

Table of contents

Symbols

IVD	In Vitro Diagnostic Medical Device	CE	EC Declaration of conformity
CONT	Content		Expiry Date
LOT	Lot Number		Store at
	Manufactured by	Σ	Sufficient for ... determinations
REF	Catalogue Number of Manufacturer		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 measurement of dopamine and its derivatives is of special diagnostic value with children who are suspected to have a neuroblastoma.

The assay kit provides materials for the quantitative measurement of adrenaline, noradrenaline and dopamine in plasma and urine. Noradrenaline, adrenaline and dopamine are extracted using a cis-diol-specific affinity gel and acylated to N-acylnoradrenaline, N-acyladrenaline and N-acyl-dopamine and then converted enzymatically into N-acylnormetanephrine, N-acylmetanephrine and N-acyl-3-methoxytyramine.

The competitive CAT ELISA kit uses the microtitre plate format. Adrenaline, noradrenaline and dopamine, respectively, are bound to the solid phase of the microtiter plate. Acylated catecholamine from the sample and solid phase bound catecholamine 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 catecholamine 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 catecholamine is inversely proportional to the catecholamine 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
Noradrenaline (ng/ml)	0	1.5	5	15	50	150	500
Noradrenaline (nmol/l)	0	8.9	29.6	88.9	296	887	2,955
Dopamine (ng/ml)	0	1.5	10	40	160	640	2,560
Dopamine (nmol/l)	0	9.8	65.3	261	1,045	4,179	16,717

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


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Danger

Acylation Buffer **ACYL-BUFF** 4 vials

20 ml, ready for use

Enzyme 2 ml, lyophilized, Catechol-O-methyltransferase	**ENZYME**	3 vials
Coenzyme 1 ml, ready for use, S-adenosyl-L-methionine	**COENZYME**	2 vials
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, colour coded blue	**AS-AD**	1 vial
Noradrenaline-Antiserum 6 ml, ready for use, rabbit, colour coded yellow	**AS-NAD**	1 vial
Dopamine-Antiserum 6 ml, ready for use, rabbit, colour coded green	**AS-DA**	1 vial
MT-Strips 8 wells each, break apart, precoated with: Derivatized adrenaline, colour coded blue	**STRIPS-AD**	12 strips
MT-Strips 8 wells each, break apart, precoated with: Derivatized noradrenaline, colour coded yellow	**STRIPS-NAD**	12 strips
MT-Strips 8 wells each, break apart, precoated with: Derivatized dopamine, colourless	**STRIPS-DA**	12 strips
POD Conjugate 12 ml each , ready for use, anti-rabbit IgG-peroxidase conjugate	**CONJ**	3 vials

Wash Buffer 20 ml each, concentrated Dilute each with dist. water to 1000 ml total volume	**WASH**	2 vials
Substrate 12 ml each, TMB solution, ready for use	**SUB**	3 vials
Stop Solution 12 ml each, ready for use, contain 0.3 M sulphuric acid	**STOP**	3 vials
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 und 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 venipuncture 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 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 has to 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 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 three additional bottles of ENZYME allow another three runs of the test. If the whole kit is to be used in one run it is recommended to pool the contents of at least three vials of prepared enzyme mix.

All other reagents are ready for use.

6.2 Preparation of Samples

Preparation of standards, controls and samples is the same for the Adrenaline, Noradrenaline and Dopamine ELISAs and is performed in one extraction plate.

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 20 µl Standard 1 – 7 [CAL 1 – 7] , 20 µl Control 1 & 2 [CON 1 & 2] and 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 300 µl Plasma Sample into the respective wells (no volume correction 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 (slow shaking).
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 (slow shaking).
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.
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, 50 µl for the noradrenaline assay and 50 µl for the dopamine assay.

7 Test Procedure ELISA

7.1 Adrenaline ELISA

1. Pipette 20 µl of freshly prepared Enzyme Mix (s. 6.1.2) into the required number of wells of STRIPS-AD (colour coded blue).
2. Add 50 µl each of 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 AS-AD (colour coded blue) 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 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.

7.2 Noradrenaline ELISA

1. Pipette 20 µl each of freshly prepared Enzyme Mix (s. 6.1.2) into the required number of wells of STRIPS-NAD (colour coded yellow).
2. Add 50 µl each of 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 with adhesive foil FOIL for 30 minutes at room temperature (20 – 25 °C) on an orbital shaker (medium speed).
4. Pipette 50 µl Noradrenaline-Antiserum AS-NAD (colour coded yellow) 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 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, 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.

7.3 Dopamine ELISA

1. Pipette 20 µl each of freshly prepared Enzyme Mix (s. 6.1.2) into the required number of wells of STRIPS-DA (colourless).
2. Add 50 µl each of 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 with adhesive foil FOIL for 30 minutes at room temperature (20 – 25 °C) on an orbital shaker (medium speed).
4. Pipette 50 µl Dopamine-Antiserum AS-DA (colour coded green) 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 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, 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.