Version 14-05/24

User's Manual

CatCombi Research ELISA Kit

REF **DEIA2078**

Σ **2 × 96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

General Description

Catecholamine is the name of a group of aromatic amines (noradrenaline, adrenaline, dopamine, and their derivatives) which act as hormones and neurotransmitter, 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 and noradrenaline in plasma and urine. Noradrenaline and adrenaline are extracted using a cis-diol-specific affinity gel and acylated to N-acylnoradrenaline and N-acyladrenaline and then converted enzymatically into N-acylnormetanephrine and N-acylmetanephrine.

Principles of Testing

The competitive CatCombi Research ELISA Kit uses the microtitre plate format. Adrenaline and noradrenaline, 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 anti-rabbit IgG / peroxidase. 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.

Reagents And Materials Provided

1. **Adhesive Foil**: Adhesive Foils in a resealable pouch. 10 pieces.
2. **Wash Buffer Concentrate**: Concentrated 50×. Buffer with a non-ionic detergent and physiological pH. 2 × 20 ml.
3. **Substrate:** Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide. 2 × 12 ml, ready for use.
4. **Stop Solution**: 0.3 M sulfuric acid. 2 × 12 ml, ready for use.
5. **Adrenaline-Metanephrine Microtiter Strips**: 1 × 96 well (12 × 8) antigen precoated microwell plate in a resealable blue pouch with desiccant.
6. **Noradrenaline- Normetanephrine Microtiter Strips**: 1 × 96 well (12 × 8) antigen precoated microwell plate in a resealable yellow pouch with desiccant.
7. **Adrenaline Antiserum**: Rabbit anti-adrenaline antibody. 1 × 6 ml, colour coded blue.
8. **Noradrenaline Antiserum**: Rabbit anti-noradrenaline antibody. 1 × 6 ml, colour coded yellow.
9. **Standard 1-7 and Control**: Ready to use.

Concentrations:

Standard	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

When determing urine samples only: Standard 2 can be omitted.

When determing plasma samples only: Standard 7 can be omitted.

10. **Control 1 & 2**: Ready for use. Each 4 ml.
11. **Hydrochloric Acid:** 0.025 M Hydrochloric Acid, yellow coloured. 1 × 21 ml, colour coded yellow orange.
12. **Acylation Reagent:** Acylation reagent in DMF and DMSO. 1 × 6 ml/vial (please note that solvent reacts with many plastic materials including plastic trays; it does not react with normal pipette tips and with glass devices). Danger, Warning!
13. **Acylation Buffer**: Buffer with light alkaline pH for the acylation. 1 × 20 ml/vial. Colour coded purple.
14. **POD Conjugate**: 2 × 12 ml, ready for use. Anti-rabbit IgG-POD conjugate/ peroxidase. Warning!
15. **Coenzyme:** S-adenosyl-L-methionine. 1 × 1 ml.
16. **Enzyme:** Catechol-O-methyltransferase. 3 × 2 ml. Lyophilized.
17. **Enzyme Buffer:** 1 × 2 ml, ready for use. Warning!
18. **Extraction Plate**: 2 × 48 well plates coated with boronate affinity gel in a resealable pouch.
19. **Extraction-Buffer (TE Buffer)**: TRIS-EDTA buffer. 1 × 6 ml/vial, colour coded purple.

Materials Required But Not Supplied

1. Pipettes for pipetting 20, 50, 300, 1000 µl
2. Repeating dispenser for 20, 50, 100, 150, 200, 250 µl und 1 ml
3. Horizontal shaker
4. Microplate washing device
5. Microplate photometer
6. Distilled water

Storage

Store the unopened reagents at 2–8°C until expiration date.

Specimen Collection And Preparation

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 and especially lipemic samples should not be used for the assay, because 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.

2. **Urine**

The total volume of urine excreted during a 24-hours period should be collected and mixed 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 tested, too. Urine samples can be stored at -20 °C for at least 6 months.

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, 20 µl Control 1 & 2 and each 20 µl Urine Sample into the respective wells of the extraction plate. Add 250 µl of distilled water to these wells to correct for volume. Pipette each 300 µl Plasma Sample into the respective wells (no volume correction required).
2. Pipette each 50 µl Extraction Buffer into all wells.
3. Incubate 60 minutes at room temperature on an orbital shaker (400 - 600 r/min).
4. Decant the plate and remove residual liquid by tapping the inverted plate on a paper towel.
5. Pipette each 1 ml Wash Buffer into all wells and incubate for 5 minutes at room temperature on an orbital shaker (slow shaking).
6. Decant the plate and remove residual liquid by tapping the inverted plate on a paper towel.
7. Pipette each 150 µl Acylation Buffer into all wells.
8. Pipette each 50 µl Acylation Reagent into all wells 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 (400 - 600 r/min).
10. Decant the plate and remove residual liquid by tapping the inverted plate on a paper towel.
11. Pipette each 1 ml Wash Buffer into all wells and incubate for 5 minutes at room temperature on an orbital shaker (slow shaking).
12. Decant the plate and remove residual liquid by tapping the inverted plate on a paper towel.
13. Repeat the wash steps 11. and 12.
14. Pipette each 200 µl HCl (0.025 M) into all wells.
15. Incubate the plate with adhesive foil for 20 minutes at room temperature on an orbital shaker (400 - 600 r/min).

Caution: Do not decant the supernatant thereafter.

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

Reagent Preparation

1. **Wash Buffer**

Dilute the content of the bottle 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 or at -20°C until the indicated expiry date.

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 Buffer (total volume: 2.6 ml) and mix thoroughly.

The two additional bottles of Enzyme are allowing a second and a third run of the test. If the whole kit is to be used in one run it is recommended to pool the contents of at least two prepared enzyme mix.

Assay Procedure

Allow reagents to reach room temperature. Duplicates are recommended.

Adrenaline ELISA

1. Pipette each 20 µl of freshly prepared Enzyme Mix into all wells (colour coded blue).
2. Pipette each 50 µl prepared Standards, Controls and Patient Samples into the respective wells (colour coded blue).
3. Incubate the plate with adhesive foil for 30 minutes at room temperature (20 – 25°C) on an orbital shaker (400 - 600 r/min).
4. Pipette each 50 µl Adrenaline-Antiserum (colour coded blue) into all wells.
5. Cover the plate with adhesive 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. Remove residual liquid by tapping the inverted plate on clean absorbent paper. Repeat the washing procedure 4 times.
7. Pipette each 100 µl POD-Conjugate into all wells.
8. Incubate for 30 minutes at room temperature on an orbital shaker (400 - 600 r/min).
9. Washing: Repeat wash step 6.
10. Pipette each 100 µl Substrate into all wells.
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 into all wells.
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 each 20 µl of freshly prepared Enzyme Mix into all wells (colour coded yellow).
2. Pipette each 50 µl prepared Standards, Controls and Patient Samples into the respective wells (colour

coded yellow).

3. Incubate the plate with foil for 30 minutes at room temperature (20 – 25 °C) on an orbital shaker (400 - 600 r/min).
4. Pipette each 50 µl Noradrenaline-Antiserum (colour coded yellow) into all wells.
5. Cover the plate with adhesive 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. Remove residual liquid by tapping the inverted plate on clean absorbent paper. Repeat the washing procedure 4 times.
7. Pipette each 100 µl POD-Conjugate into all wells.
8. Incubate for 30 minutes at room temperature on an orbital shaker (400 - 600 r/min).
9. Washing: Repeat wash step 6.
10. Pipette each 100 µl Substrate into all wells.
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 into all wells.
13. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

Calculation

On a semilogarithmic graph paper 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).

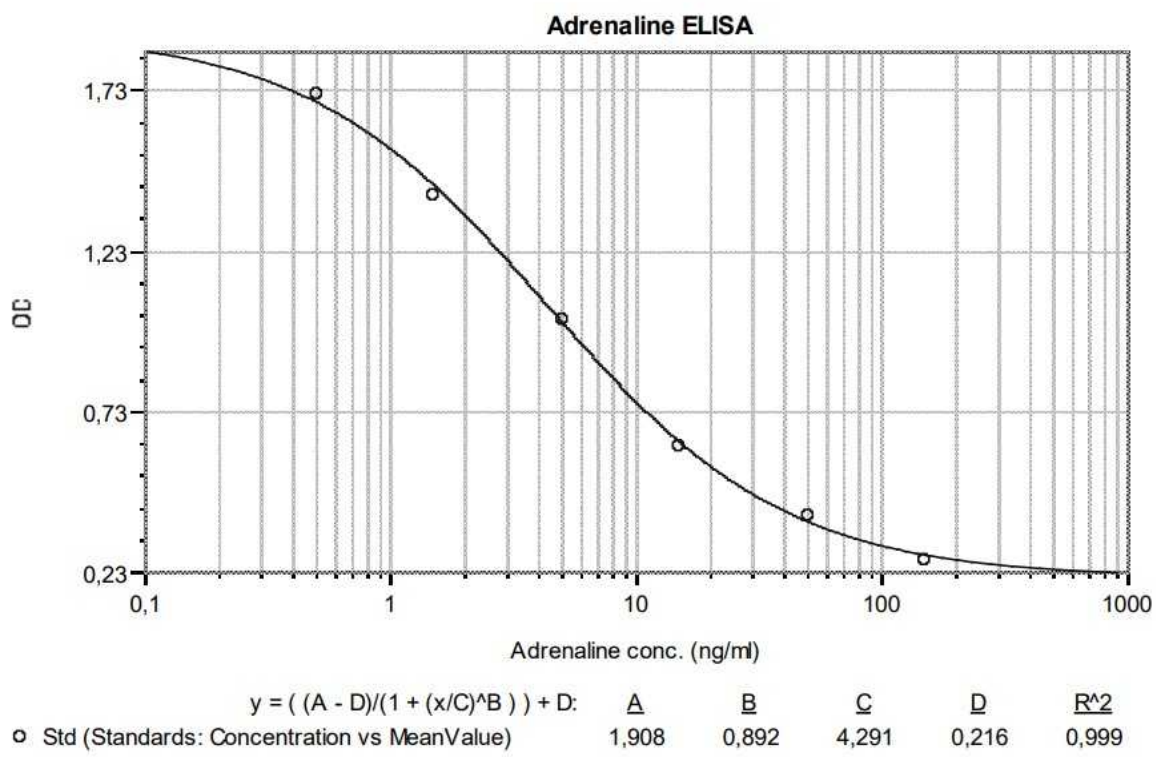
The concentration of the controls and urine samples can be read off the standard curve directly without any further conversion.

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

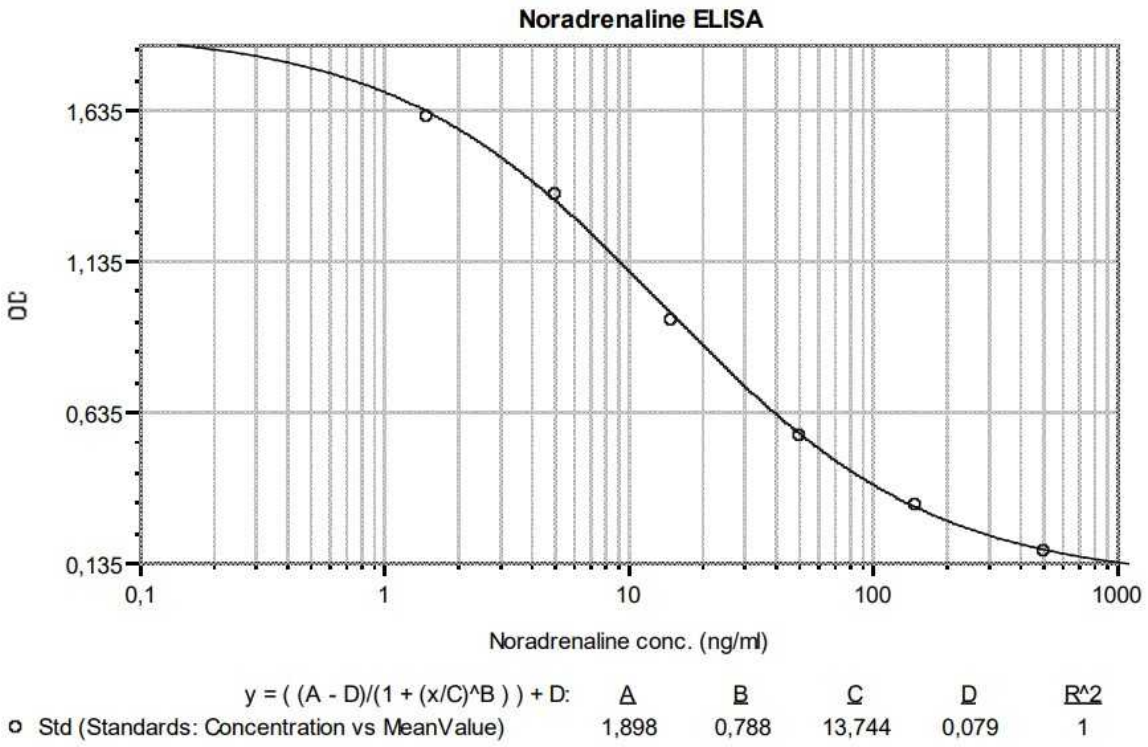
Typical Standard Curve

Below are listed typical examples of standard curves with the Adrenaline ELISA and Noradrenaline ELISA:

Adrenaline ELISA



Noradrenaline ELISA



Reference Values

The reference ranges given below should only be taken as a guideline. It is recommended that each

laboratory should establish its own normal values.

	Adrenaline	Noradrenaline
Urine	< 20 µg/day	< 90 µg/day
Plasma	< 100 pg/ml	< 600 pg/ml

Performance Characteristics

Method Comparision

Adrenaline

Matrix	Method	Correlation
Urine	HPLC	Y = 0.94 x HPLC - 0.21; R = 0.987; N = 32

Noradrenaline

Matrix	Method	Correlation
Urine	HPLC	Y = 0.90 x HPLC + 6.3; R = 0.983; N = 32

Sensitivity

The lower limit of detection was determined by taking the 2fold standard deviation of the absorbance of the Zero Reference and reading the corresponding value from the standard curve.

	Adrenaline	Noradenaline
Sensitivity (Urine):	0.08 ng/ml	0.67 ng/ml
Sensitivity (Plasma):	5 pg/ml	45 pg/ml

Specificity

Structural related components were tested for possible interference with the antisera against adrenaline and noradrenaline used in the ELISA method.

Components	Cross Reactivity (%) Adrenaline-Ab	Cross Reactivity (%) Noradrenaline-Ab
Adrenaline	100	< 0,01
Noradrenaline	0,053	100
Dopamine	< 0,01	0,037
Metanephrine	< 0,01	< 0,01
Normetanephrine	< 0,001	< 0,01
3-Methoxytyramine	< 0,001	< 0,01
L-Dopa	< 0,001	< 0,01
Tyramine	< 0,001	< 0,01
Tyrosine	< 0,001	< 0,001
Homovanillic acid	< 0,0001	< 0,001
Vanillic mandelic acid	< 0,0001	< 0,001

Linearity

The linearity of the ELISA method was investigated using different dilutions of an urine and a plasma sample.

Concentrations in ng/ml

Adrenaline

Matrix	Range (ng/ml)	Max. dilution	Mean (%)	Recovery (%)
Urine	9.9 – 132.3	1:15 (dist. water)	105	98 - 112
EDTA-Plasma	0.33 – 4.87	1:15 (dist. water)	103	100 - 108

Noradrenaline

Matrix	Range (ng/ml)	Max. dilution	Mean (%)	Recovery (%)
Urine	9.9 – 132.3	1:15 (dist. water)	105	98 - 112
EDTA-Plasma	0.33 – 4.87	1:15 (dist. water)	103	100 - 108

Recovery

Increasing amounts of adrenaline and noradrenaline were added to an urine and to a plasma sample. Each spiked sample was assayed. The analytical recovery was estimated at different concentrations by using the theoretically expected and the actually measured values.

Concentrations in ng/ml

Adrenaline

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

Noradrenaline

Matrix	Range (ng/ml)	Mean (%)	Recovery (%)
Urine	32.4 – 113.2	93	89 - 98
EDTA-Plasma	0.20 – 4.91	104	91 – 109

Reproducibility

Adrenaline

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 %		

Noradrenaline

Matrix	Range (ng/ml)	Intra-Assay-CV	Range (ng/ml)	Inter-Assay-CV
Urine	21.8 – 76.4	8.7 – 9.2 %	23.1 – 83.9	11.1 – 8.7 %
EDTA-Plasma	0.76 – 4.85	8.4 – 9.7 %		

Precautions

1. Some reagents contain sodium azide as preservative. Avoid skin contact.
2. Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.
3. Some components of this kit are containing hazardous reagents.