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Noradrenaline High Sensitive ELISA Assay Kit

High sensitivity and small sample volume

Catalog Number:
NOU39-K01

For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

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INTENDED USE

The Noradrenaline (Norepinephrine) High Sensitive ELISA Assay kit is an Enzyme-linked immunosorbent assay used for the quantitative and very sensitive determination of noradrenaline in biological samples including serum, plasma, tissue, and cell culture samples. The Noradrenaline (Norepinephrine) High Sensitive ELISA Assay kit is for research use only and should not be used in diagnostic procedures.

INTRODUCTION

The Noradrenaline High Sensitive ELISA provides materials for the quantitative measurement of noradrenaline in low concentrated samples and for small sample volumes. Noradrenaline is extracted using a cis-diol-specific affinity gel and acylated to N-acylnoradrenaline and then converted enzymatically into N-acylnormetanephrine.

PRINCIPLE OF THE ASSAY

The competitive Noradrenaline-Sensitive-ELISA kit uses the microtitre plate format. Noradrenaline is 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.

PRECAUTIONS

- For research use only
- Some reagents contain sodium azide as preservative. Avoid skin contact.
- 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.
- Some components of this kit are containing hazardous reagents. These components are marked with the adequate hazard label.

STORAGE AND STABILITY

On arrival, store the kit at 2-8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.

Do not use components beyond the expiration date shown on the labels.

Do not mix various lots of any kit component within an individual assay.

CONTENTS OF THE KIT

Reagents for Sample Preparation

4.1	Extraction Plate 48 wells Coated with bronate affinity gel	2 plates
4.2	Extraction Buffer 6 ml, ready for use Colour coded purple	2 vials



4.3 HCl 1 vial
21 ml, ready for use
0.025 M HCl
Colour coded yellow orange

4.4 Standards A-F 6 vials
Each 4 ml, ready for use
Concentrations:

Standard	A	A/B*	B	C	D	E	F
Noradrenaline (ng/ml)	0	0.05	0.15	0.5	1.5	5	25
Noradrenaline (nmol/l)	0	0.30	0.89	3.0	8.9	29.6	148

*prepare Standard A/B by mixing 400 µl Standard A and 200 µl Standard B in suitable polypropylene tubes or Eppendorf cups; prepare just prior to assay

4.5 Control 1&2 2 vials
Each 4 ml, ready for use
Concentrations: see q.c. certificate

4.6 Acylation Reagent 1 vial
6 ml, ready for use, contains DMSO and DMF
(please note that solvent reacts with many plastic materials including plastic trays; it does not react with normal pipette tips and with glass devices).

4.7 Acylation Buffer 1 vial
20 ml, ready for use
Colour coded purple

4.8 Enzyme 3 vials
Each 2 ml, lyophilized
Catechol-O-methyltransferase

4.9 Coenzyme 1 vial
1 ml, ready for use
S-adenosyl-L-methionine

4.10 Enzyme Buffer 1 vial
3.5 ml, ready for use

4.11 Enzyme Plate 1 piece
96 wells, ready for use

4.12 Sample Stabilizer 1 vial
96 wells, ready for use

Reagents for ELISA

4.13 Noradrenaline-Antiserum 1 vial
2.5 ml, ready for use, rabbit
colour coded yellow



- 4.14 MT-Strips 12 strips
8 wells each, break apart, precoated with:
noradrenaline (12 strips), colour coded yellow
- 4.15 POD Conjugate 1 vial
Each 12 ml, ready for use,
Anti-rabbit IgG-POD conjugate/peroxidase
- 4.16 Wash Buffer 2 vials
20 ml, concentrate
Dilute content with dist. water to 500 ml total volume
- 4.17 Substrate 1 vial
12 ml TMB solution, ready for use
- 4.10 Stop Solution 1 vial
12 ml, ready for use
Contains 0.3 M sulphuric acid
- 4.15 Adhesive Foil 10 pieces
Ready for use

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Pipettes 20, 50, 100, 150, 175, 280 μ l
- Repeating dispenser for 20, 25, 50, 100, 150 μ l and 1 ml
- Orbital shaker
- Multichannel pipette or Microplate washing device
- Microplate photometer
- Distilled water
- Heating cabinet with 37 °C (optional)

SAMPLE COLLECTION

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.

Immediately after collection the plasma samples should be centrifuged (preferable at 2 - 8 °C) and frozen. The samples are stable up to 1 week at -20 °C.

To improve the stability each sample should be enriched with the Sample Stabilizer STABILIZER before freezing (max. 20% of the sample volume), e.g.:

Sample volume	Stabilizer volume
20 μ l	4 μ l
50 μ l	10 μ l
100 μ l	20 μ l



200 µl	40 µl
300 µl	60 µl
500 µl	100 µl

Urine, Cell culture samples and various biological samples:

The stability of such samples depends on the sample type and the way of collection. Therefore, a general procedure for collection and storage is not possible. However, it is recommended to freeze the samples immediately after collection. The samples should be stable at -20 °C for up to 1 week.

To improve the stability each sample should be enriched with the Sample Stabilizer STABILIZER before freezing (max. 10% of the sample volume), e.g.:

Sample volume	Stabilizer volume
20 µl	2 µl
50 µl	5 µl
100 µl	10 µl
200 µl	20 µl
300 µl	30 µl
500 µl	50 µl

Acidified samples, which have a pH value of 5 or less must not be enriched with the Sample Stabilizer and have to be frozen immediately after collection.

Tissue samples

Tissue samples can be homogenized in 0.01 N HCl in the presence of 0.15 mM EDTA and 4 mM sodium metabisulfite.

The following basic principles should be followed:

- Avoid excess of acid. This might exceed the buffer capacity of the extraction buffer. After adding the extraction buffer a pH value of 7 or above is mandatory. If the pH value is below 7 it is necessary to repeatedly add 50 µl of Extraction Buffer until the pH value is at or above 7. Acidified samples, which have a pH value of 5 or less must not be enriched with the Sample Stabilizer.
- Avoid substances in the samples with a cis-diol-structure (boric acid, Sorbitol, mannitol, etc.). These substances reduce the recovery of extraction.

PREPARATION OF REAGENTS

Wash Buffer

Dilute the content of the bottle with distilled water to a total volume of 500 ml.

For further use the diluted wash buffer must be stored at 2 - 8 °C for a maximum period of 4 weeks.

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 labelled ENZYME with 2 ml distilled water.
Add 0.3 ml COENZYME and 0.7 ml ENZYME-BUFF , mix thoroughly (total volume: 3 ml).

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 sufficient to prepare one vial of enzyme mix.

All other reagents are ready for use.

PREPARATION OF SAMPLES

Allow reagents to reach room temperature.

Determinations in duplicates are recommended.

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

Each 1 µl - 300 µl of samples are extracted (alternatively: > 300 µl up to 500 µl).

1. Pipette each 20 µl Standard A, A/B, B - F, 20 µl Control 1 & 2 and each 1 µl - 300 µl Sample into the respective wells of the extraction plate. Correction for volume: Pipette 280 µl of distilled water into the wells of the standards and controls (final volume: 300 µl). Pipette as much distilled water into the wells of the samples to obtain a final volume of 300 µl, e.g. 100 µl sample + 200 µl distilled water. For sample volumes above 300 µl up to 500 µl: fill up all wells to a final volume of 500 µl. Within a run the final volume has to be the same in all wells (300 µl or 500 µl, respectively).
2. Pipette each 100 µl Extraction Buffer into all wells.
3. Cover the plate with adhesive foil and incubate for 60 minutes at room temperature on an orbital shaker (high shaking rate).
4. Decant the plate and remove residual liquid by tapping the inverted plate on a paper towel.
5. Pipette each 1 ml prepared Wash Buffer into all wells and incubate for 5 minutes at room temperature on an orbital shaker (slow shaking rate).
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 for 20 minutes at room temperature on an orbital shaker (medium shaking rate).
10. Decant the plate and remove residual liquid by tapping the inverted plate on a paper towel.
11. Pipette each 1 ml prepared Wash Buffer into all wells and incubate for 5 minutes at room temperature on an orbital shaker (slow shaking rate).
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 125 µl HCl (0.025 M) for elution into all wells.
15. Cover the plate with adhesive foil and incubate for 20 minutes at room temperature on an orbital shaker (medium shaking rate).
Caution: Do not decant the supernatant thereafter.
16. Transfer 100 µl from the extraction plate into the respective wells of the enzyme plate.
17. Pipette each 20 µl of freshly prepared Enzyme Mix (s. 6.1.2) into all wells of the enzyme plate. Colour changes to red.
18. Cover the plate with adhesive foil and incubate for 1 minute at room temperature on an orbital shaker (medium shaking rate).
19. Incubate the plate for 90 minutes at 37°C without shaking. (Alternatively: 120 minutes at room temperature (20 - 25°C) on an orbital shaker at medium shaking rate).



Caution: Do not decant the supernatant thereafter.

Take each 100 µl of the supernatant for the Noradrenaline ELISA.

TEST PROCEDURE ELISA

1. Pipette each 100 µl prepared Standards, Controls and Samples into the respective wells (colour coded yellow).
2. Pipette each 20 µl Noradrenaline-Antiserum (colour coded yellow) into all wells.
3. Cover the plate with adhesive foil, shake briefly and incubate for 15 – 20 hours (overnight) at 2 - 6 °C.
4. Discard or aspirate the contents of the wells and wash thoroughly with each 250 µl prepared Wash Buffer. Remove residual liquid by tapping the inverted plate on clean absorbent paper. Repeat the washing procedure 3 times.
5. Pipette each 100 µl POD-Conjugate into all wells.
6. Incubate for 60 minutes at room temperature on an orbital shaker (medium shaking rate).
7. Washing: Repeat wash step 4.
8. Pipette each 100 µl Substrate into all wells.
9. Incubate for 35 to 45 minutes at room temperature (20 – 25 °C) on an orbital shaker (medium shaking rate). Avoid exposure to direct sun light.
10. Pipette 100 µl Stop Solution into all wells.
11. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

CALCULATION OF THE RESULTS

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 can be read off the standard curve directly without any further conversion.

The read concentrations of the samples have to be divided by a correction factor due to the use of 1 µl - 300 µl sample volume in relation to 20 µl standard.

$$\text{Correction factor} = \frac{\text{Sample volume for extraction } (\mu\text{l})}{20 \mu\text{l (Standard volume)}}$$

Example:

300 µl sample was extracted and the concentration read off from the standard curve is 0.6 ng/ml.

$$\text{Correction factor} = 300 \mu\text{l} / 20 \mu\text{l} = 15$$

$$\text{Concentration of the sample} = 0.6 \text{ ng/ml} / 15 = 0.040 \text{ ng/ml} = 40 \text{ pg/ml}$$

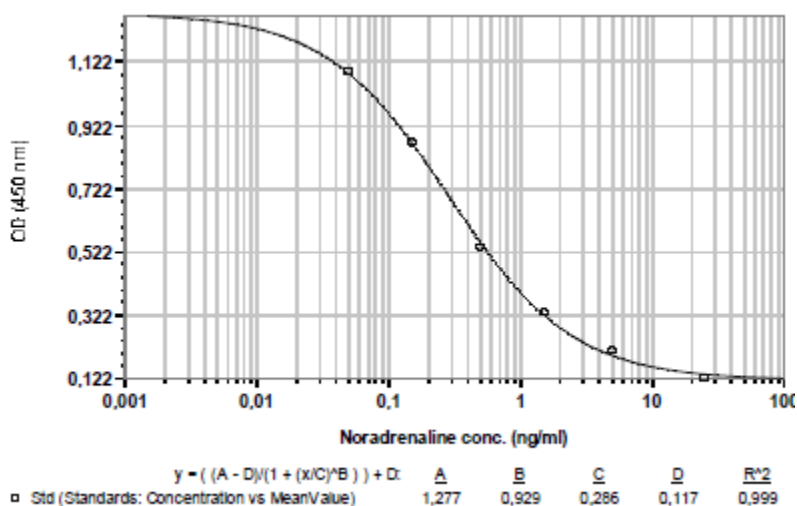
Conversion into pmol/l:

$$\text{Noradrenaline: } 1 \text{ pg} / \text{ml} = 5,91 \text{ pmol} / \text{l}$$



Typical Standard Curve:

Below a typical example of a standard curve with the Noradrenaline-Sensitive ELISA is shown:



ASSAY CHARACTERISTICS

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. The sensitivity depends on the sample volume and can be calculated with the corresponding correction factor (see 8. Calculation of Results)

Sensitivity	15 pg/ml (89 pmol/l) Correction factor
Example for 300 µl Sample (Correction factor 15):	15 pg/ml 1.0 pg/ml = 15 5.9 pmol/l

Specificity (Cross Reactivity)

Structural related components were tested for possible interference:

Components	Cross Reactivity (%)
Adrenaline	<0.012
Noradrenaline	100
Dopamine	0.092
Metanephrine	<0.012
Normetanephrine	0.16
3-Methoxytyramine	<0.012
L-Dopa	<0.005
Tyramine	<0.005
Tyrosine	<0.005
Homovanillic acid	<0.005
Vanillic mandelic acid	<0.005



Recovery

Increasing amounts of noradrenaline were added to an EDTA plasma sample and to a cell culture medium (RPMI 1640). 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 pg/ml

EDTA-Plasma				cell culture medium			
added	measured	expected	% recovery	added	measured	expected	% recovery
0.0	4.5			0.0	1.1		
6.7	10.4	11.2	92	6.7	7.1	7.8	91
10.9	14.5	15.4	94	10.9	13.2	12.0	110
15.2	20.4	19.7	104	15.2	15.4	16.3	95
22.4	27.5	26.9	102	22.4	22.7	23.5	97
45.5	45.5	50.0	91	45.5	48.9	46.6	105
67.2	58.9	71.7	82	67.2	59.1	68.3	87
88.2	80.3	92.8	87	88.2	82.9	89.4	93
151.5	131.7	156.0	84	151.5	108.7	152.6	71
294.1	246.0	298.7	82	294.1	246.6	295.3	84
		mean:	91			mean:	92

Reproducibility

Intra-Assay

The reproducibility of the ELISA method was investigated by determining the intra-assay-coefficients of variation (cv) by repeated measurements for EDTA-Plasma and cell culture medium (RPMI 1640) with different concentrations.

Concentrations in pg/ml

Sample	N	Mean value	Sd	CV (%)
EDTA-Plasma	16	113.1	7.57	6.7
Cell culture medium	16	21.9	1.07	4.9



Pipetting Scheme Sample Preparation

	Standards	Controls	Samples
Standard A, A/B, B - F μ l	20		
Control 1 & 2 μ l		20	
Sample μ l			1 - 300
Dist. Water μ l	280	280	fill up to 300
Extraction Buffer μ l	100	100	100

Cover the plate with adhesive foil; shake for 60 minutes at room temperature

Decant plate and remove residual liquid

Wash Buffer	ml	1	1	1
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Shake for 5 minutes at room temperature (slow shaking rate)

Decant plate and remove residual liquid

Acylation Buffer	μ l	150	150	150
Acylation Reagent	μ l	50	50	50

Immediately: Shake for 20 minutes at room temperature

Decant plate and remove residual liquid

Wash Buffer	ml	1	1	1
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Shake for 5 minutes at room temperature (slow shaking rate)

Decant plate and remove residual liquid

Wash Buffer	ml	1	1	1
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Shake for 5 minutes at room temperature (slow shaking rate)

Decant plate and remove residual liquid

HCl	μ l	125	125	125
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Cover the plate with adhesive foil; Shake for 20 min at room temperature

Caution: Do not decant the supernatant thereafter

Transfer to Enzyme Plate	μ l	100	100	100
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Enzyme Mix (fresh)	μ l	20	20	20
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Cover the plate with adhesive foil; Shake for 1 minute at room temperature

Incubate for 90 minutes at 37°C

Caution: Do not decant the supernatant thereafter

For the ELISA transfer each 100 μ l



Pipetting Scheme ELISA

		Standards	Controls	Samples
Noradrenaline (yellow)				
Standard A, A/B, B - F	µl	100		
Controls 1 & 2	µl		100	
Samples	µl			100
Noradrenaline Antiserum	µl	20	20	20

Cover the plates with adhesive foil and shake briefly
Incubate for 15 – 20 hours (overnight) at 2 - 8 °C

4 x washing

POD-Conjugate	µl	100	100	100
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Shake for 60 minutes at room temperature

4 x washing

Substrate	µl	100	100	100
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Shake for 35 – 45 minutes at room temperature

Stop Solution	µl	100	100	100
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Reading of absorbance at 450 nm

Warranty Information

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.