Norepinephrine ELISA Kit

Catalog Number KA1891

96 assays

Version: 09

Intended for research use only

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Introduction

Intended Use

Enzyme Immunoassay for the quantitative determination of Noradrenaline (Norepinephrine) in plasma and urine.

Principle of the Assay

Noradrenaline (norepinephrine) is extracted by using a cis-diol-specific affinity gel, acylated and then converted enzymatically.
The competitive ELISA kit uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The derivatized standards, controls and samples and the solid phase bound analytes compete 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. The reaction is monitored at 450 nm. Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations.

General Information

Materials Supplied

List of component

Component	Description	Amount
Adhesive Foil	Ready to use. Adhesive foils in a resealable pouch.	4 slices
Wash Buffer Concentrate (50X)	Concentrated buffer with a non-ionic detergent and physiological pH.	20 mL
Enzyme Conjugate	Ready to use, goat anti-rabbit immunoglobulins, conjugated with peroxidase.	12 mL
Substrate	Ready to use, chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide.	12 mL
Stop Solution	Ready to use, 0.25 M H_2SO_4.	12 mL
Noradrenaline Microtiter Strips	Ready to use, antigen precoated microwell plate in a resealable yellow pouch with desiccant.	96 (12x8) wells
Noradrenaline Antiserum	Ready to use, rabbit anti-noradrenaline antibody, yellow coloured.	6 mL
Adjustment Buffer	Ready to use. TRIS buffer.	4 mL
Acylation Buffer	Ready to use, buffer with light alkaline pH for the acylation.	20 mL
Acylation Reagent	Ready to use, Acylation reagent in DMF and DMSO. Highly flammable liquid and vapour. May damage fertility or the unborn child. Causes serious eye irritation.	3 mL
Assay Buffer	Ready to use, contains 1 M hydrochloric acid and a non-mercury preservative.	6 mL
Coenzyme	Ready to use, S-adenosyl-L-methionine.	4 mL
Enzyme	Lyophilized, Catechol-O-methyltransferase.	2 vials
Extraction Buffer	Ready to use, buffer containing carbonate.	6 mL
Extraction Plate	Ready to use, coated with boronate affinity gel in a resealable pouch.	48 wells x 2
Hydrochloric Acid	Ready to use, 0.025 M HCl, yellow coloured.	20 mL

Standards and Controls - Ready to use

Component	Concentration (ng/mL)	Concentration (nmol/mL)	Amount
Standard A	0	0	4 mL
Standard B	5	30	4 mL
Standard C	20	118	4 mL
Standard D	75	443	4 mL
Standard E	250	1478	4 mL
Standard F	1000	5910	4 mL
Control 1	Refer to QC report for expected value and acceptable range.		4 mL
Control 2	Refer to QC report for expected value and acceptable range.		4 mL

Conversion: Noradrenaline (ng/mL) x 5.91 = Noradrenaline (nmol/L)

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

Storage Instruction

Store the unopened reagents at 2-8°C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2-8°C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

Materials Required but Not Supplied

- ✓ 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 3mm; approx. 600 rpm)
- ✓ Absorbent material (paper towel)
- ✓ Water (deionized, distilled, or ultra-pure)
- ✓ Vortex mixer

Precautions for Use

- 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 has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. This assay was validated for certain types of samples as indicated in Intended Use. Any off-label use of this kit is in the responsibility of the user and the manufacturer cannot be held liable.
3. The principles of Good Laboratory Practice (GLP) have to be followed.
4. In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
7. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant and used in the frame provided.
8. Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
9. Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
10. Incubation times do influence the results. All wells should be handled in the same order and time intervals.
11. To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
12. A standard curve must be established for each run.
13. The controls should be included in each run and fall within established confidence limits.
14. 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.
15. 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.
16. 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. Wash contaminated objects before reusing them.
17. For information on hazardous substances included in the kit please refer to Material Safety Data Sheet (MSDS). The Material Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
18. The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.

19. Kit reagents must be regarded as hazardous waste and disposed according to national regulations.

- Limitations

  Any inappropriate handling of samples or modification of this test might influence the results.

- Interfering substance

✓ Plasma: Samples containing precipitates or fibrin stands or which are haemolytic or lipemic might cause inaccurate results.

✓ 24-hour urine: please note the sample preparation and storage! If the percentage of the final concentration of acid is too high, the buffer capacity of the Extraction Buffer is insufficient. As a consequence noradrenaline will not be extracted adequately.

- High-Dose-Hook effect

  Ni hook effect was observed in this test.

Assay Protocol

Reagent Preparation

- ✓ Wash Buffer: Dilute the 20 mL Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 mL.
  Storage: 1 month at 2-8°C.

- ✓ Enzyme Solution: Reconstitute the content of the vial labelled '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 Adjustment Buffer. The total volume of the Enzyme Solution is 2.0 mL.
  Note: The Enzyme Solution has to be prepared freshly prior to the assay (not longer than 10-15 minutes in advance). Discard after use!

Sample Preparation

- Plasma
  Whole blood should be collected into centrifuge tubes containing EDTA as anti-coagulant and centrifuged according to manufacturer's instructions immediately after collection. Haemolytic and lipemic samples should not be used for the assay.
  Storage: up to 6 hours at 2 - 8°C; for longer periods (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.

Assay Procedure

Allow all reagents to reach room temperature and mix thoroughly by gentle inversion before use. Duplicate determinations are recommended.
The binding of the antiserum and the enzyme conjugate and the activity of the enzyme are temperature dependent, and the absorbance may vary if a thermostat is not used. The higher the temperature, the higher the absorbance will be. Varying incubation times will have a similar influence on the absorbance. The optimal temperature during the Enzyme Immunoassay is between 20-25°C.

- 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 Extraction Plate.
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 Buffer into all wells.
4. Pipette 50 µL of Extraction Buffer into all wells.
5. Cover plate with adhesive foil 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 Acylation Buffer into all wells.
10. Pipette 25 µL of Acylation Reagent 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 Hydrochloric Acid into all wells.
15. Cover plate with Adhesive Foil. Incubate 10 min at RT (20-25°C) on a shaker (approx. 600 rpm). Remove the foil and discard.

    Note: Do not decant the supernatant thereafter!

    The following volumes of the supernatant are needed for the subsequent ELISA: Noradrenaline: 20 µL

- Noradrenaline ELISA

1. Pipette 25 µL of the Enzyme Solution (refer to Preparation of reagents) into all wells of the Noradrenaline Microtiter Strips.
2. Pipette 20 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 Noradrenaline Antiserum into all wells and cover plate with Adhesive Foil.
5. Incubate for 2 hours 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 3x 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 Enzyme 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 3x 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).
    Note: Avoid exposure to direct sun light!
11. Add 100 µL of the Stop Solution to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
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).

Data Analysis

Calculation of Results

- Measuring range of Noradrenaline
  Urine: 2.5 to 1000 ng/mL
  Plasma: 93-33,333 pg/mL
  The standard curve 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).
  Use a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

Note: 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 1 and 2 can be read directly from the standard curve.
  Calculate the 24 h excretion for each urine sample: $\mu g/24h = \mu g/L \times L/24h$

- Plasma samples
  The read concentrations of the plasma samples have to be divided by 30.

- Conversion
  Noradrenaline (ng/mL) x 5.91 = Noradrenaline (nmol/L)

- Expected reference values
  It is strongly recommended that each laboratory should determine its own normal and abnormal values.
  24-hour Urine: < 90 µg/day (535 nmol/day)
  Plasma: < 600 pg/mL

- Quality Control
  It is recommended to use control samples according to national regulations. Use controls at both normal and abnormal levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are printed on the QC-Report.

- Typical standard curve

  Example, do not use for calculation!

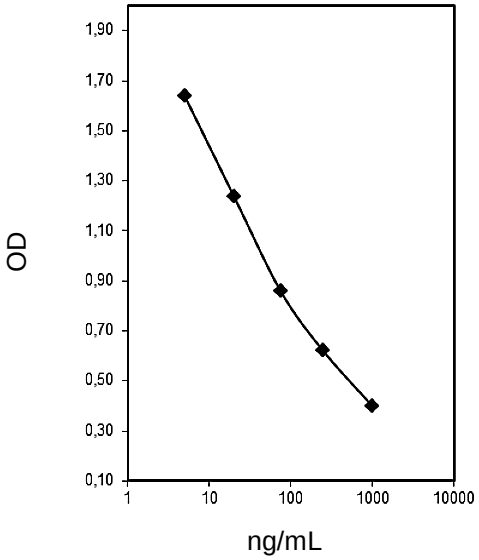

Figure 1: Typical Standard Curve for Norepinephrine ELISA Kit.

Performance Characteristics

- Analytical Sensitivity

	Sample	Noradrenaline
LOD	Urine (ng/mL)	1.7
	Plasma (pg/mL)	0.04
LOQ	Urine (ng/mL)	2.5
	Plasma (pg/mL)	93

- Analytical Specificity (Cross Reactivity)

Substance	Cross Reactivity (%)
	Noradrenaline
Derivatized Adrenaline	0.08
Derivatized Noradrenaline	100
Derivatized Dopamine	0.03
Metanephrine	< 0.01
Normetanephrine	0.16
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)			
	Sample	Range (ng/mL)	CV (%)
Noradrenaline	1	26.1 ± 3.6	13.8
	2	97 ± 12.8	13.4
	3	267 ± 35	13.1

Intra-Assay Plasma (n=60)			
	Sample	Range (pg/mL)	CV (%)
Noradrenaline	1	510 ± 65	12.8
	2	1358 ± 194	14.3
	3	3363 ± 374	11.1

Inter-Assay Urine (n=33)			
	Sample	Range (ng/mL)	CV (%)
Noradrenaline	1	19.5 ± 3.9	20.0
	2	80.6 ± 10.6	13.2
	3	226 ± 39.5	17.4

Inter-Assay Plasma (n=18)			
	Sample	Range (pg/mL)	CV (%)
Noradrenaline	1	445 ± 40.9	9.2
	2	1232 ± 134	10.9
	3	3283 ± 302	9.2

- Linearity

	Sample	Serial dilution up to	Range (%)	Mean (%)
Noradrenaline	Urine	1:512	100-127	112
	Plasma	1:512	102-125	112

- Recovery

		Mean (%)	Range (%)	Range
Noradrenaline	Urine	103	91 – 113	58.6-230 ng/mL
	Plasma	87	75 – 107	51-16,735 pg/mL

Resources

References

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).

Plate Layout

12								
11								
10								
9								
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7								
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1								
	A	B	C	D	E	F	G	H