Epinephrine/Norepinephrine ELISA Kit

Catalog Number KA3767

96 assays

Version: 03

Intended for research use only

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Introduction

Intended Use

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

Principle of the Assay

Adrenaline (epinephrine) and noradrenaline (norepinephrine) are extracted from a plasma sample* by using a cis-diol-specific affinity gel, acylated and then modified 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.
* Flexible sample volumes between 100 – 600 µL can be used with this assay.

General Information

Materials Supplied

List of component

Component	Description	Amount
Microtiter Plate	Empty in a resealable pouch.	96 wells
Extraction Plate	2 x 48 well plates coated with boronate affinity gel in a resealable pouch	48 wells x 2
Adhesive Foil	Ready to use	4 slides x 2
Wash Buffer Concentrate (50x)	Concentrated Buffer with a non-ionic detergent and physiological pH.	20 mL x 2
Enzyme Conjugate	Ready to use, goat anti-rabbit immunoglobulins, conjugated with peroxidase.	12 mL x 2
Substrate	Ready to use, chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide.	12 mL x 2
Stop Solution	Ready to use, containing 0.25 M Sulfuric acid (H_2SO_4).	12 mL x 2
Adrenaline Microtiter Strips	Ready to use, 96 wells (12 x 8), antigen precoated microwell plate in a resealable blue foil pouch with desiccant, blue coloured.	96 wells
Noradrenaline Microtiter Strips	Ready to use, 96 wells (12 x 8), antigen precoated microwell plate in a resealable yellow foil pouch with desiccant, yellow coloured.	96 wells
Adrenaline Antiserum	Rabbit anti-adrenaline antibody, blue coloured.	6 mL
Noradrenaline Antiserum	Rabbit anti-noradrenaline antibody, yellow coloured.	6 mL
Adjustment Buffer	Ready to use. TRIS buffer.	4 mL
Standard A	Ready to use. ADR (0 pg/mL), NAD (0 pg/mL); ADR (0 pmol/L), NAD (0 pmol/L). White.	4 mL
Standard B	Ready to use. ADR (20 pg/mL), NAD (80 pg/mL); ADR (109 pmol/L), NAD (473 pmol/L). Light yellow.	4 mL
Standard C	Ready to use. ADR (60 pg/mL), NAD (240 pg/mL); ADR (328 pmol/L), NAD (1418 pmol/L). Orange.	4 mL
Standard D	Ready to use. ADR (200 pg/mL), NAD (800 pg/mL); ADR (1092 pmol/L), NAD (4728 pmol/L). Dark blue.	4 mL
Standard E	Ready to use. ADR (800 pg/mL), NAD (3200 pg/mL); ADR (4368 pmol/L), NAD (18912 pmol/L). Light grey.	4 mL
Standard F	Ready to use. ADR (3200 pg/mL), NAD (12800 pg/mL); ADR (17472 pmol/L), NAD (75648 pmol/L). Black.	4 mL
TE Buffer	Ready to use, TRIS-EDTA buffer.	4 mL
Control 1	Ready to use, light green.	4 mL
Control 2	Ready to use, dark red.	4 mL

Acylation Buffer	Ready to use, buffer with light alkaline pH for the acylation, white cap.	20 mL
Acylation Reagent	Ready to use, acylation reagent in DMF and DMSO.	3 mL
Coenzyme	Ready to use, S-adenosyl-L-methionine.	4 mL
Enzyme	Lyophilized, Catechol-O-methyltransferase.	4 vials
Hydrochloric Acid	Ready to use, 0.025 M Hydrochloric Acid, yellow coloured.	20 mL

Conversation: Adrenaline (pg/mL) x 5.46 = Adrenaline (pmol/L)

Noradrenaline (pg/mL) x 5.91 = Noradrenaline (pmol/L)

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 25-700 µL; 1 mL
- ✓ Microtiter plate washing device (manual, semi-automated or automated)
- ✓ ELISA reader capable of reading absorbance at 450 nm an if possible 620 - 650 nm
- ✓ Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- ✓ Temperature controlled incubator (37°C) or similar heating device
- ✓ 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 a certain type of sample 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. Reagents of this kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
4. The principles of Good Laboratory Practice (GLP) have to be followed.
5. In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves

and protective glasses where necessary.

6. 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.
7. For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
8. 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.
9. Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
10. 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.
11. Incubation times do influence the results. All wells should be handled in the same order and time intervals.
12. To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
13. A calibrator curve must be established for each run.
14. The controls should be included in each run and fall within established confidence limits.
15. 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.
16. 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.
17. Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, rinse off immediately with water. NaN_3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.
18. 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.
19. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets (MSDS). The MSDS for this product is made available directly on the website of the manufacturer or upon request.
20. The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
21. The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.
22. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

✓ Limitations

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

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: up to 1 month at 2-8°C
- ✓ Enzyme Solution: Reconstitute the content of the vial labeled '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 by venipuncture into centrifuge tubes containing EDTA as anti-coagulant and centrifuged at room temperature immediatelyafter collection.
- Haemolytic and especially lipemic samples should not be used for the assay.
- ✓ Storage
- Up to 6 hours at 2-8°C, for longer period (up to 6 month) at -20°C.
- Repeated freezing and thawing should be avoided.

Assay Procedure

- ✓ A plasma volume between 100 µL-600 µL is needed per single determination.
- ✓ If a plasma volume < 600 µL is used, water (deionized, distilled or ultra-pure) has to be added to a final volume of 600 µL and this prediluted sample has to be used for the extraction procedure.
- ✓ This sample predilution has to be considered in the calculation of results
- ✓ Allow all reagents to reach room temperature and mix thoroughly by gentle inversion before use. Duplicate determinations are recommended.

- ✓ Sample preparation, extraction and acylation

1. Pipette 30 µL of standards, controls and 600 µL of plasma samples into the respective wells of the Extraction Plate.
2. Add 500 µL of water (deionized, distilled or ultra-pure) to the wells with standards and controls.
3. Pipette 25 µL of TE Buffer into all wells.
4. Cover the plate with adhesive foil. Incubate 60 min at RT (20-25°C) on a shaker (approx. 600 rpm).
5. Remove the foil and empty the plate. Blot dry by tapping the inverted plate on absorbent material.
6. Pipette 1 mL of Wash Buffer into all wells.

7. Incubate 5 min at RT (20-25°C) on a shaker (approx. 600 rpm).
8. Blot dry by tapping the inverted plate on absorbent material.
9. Wash one more time as described (step 6, 7 and 8)!
10. Pipette 150 µL of Acylation Buffer into all wells.
11. Pipette 25 µL of Acylation Reagent into all wells.
12. Incubate 20 min at RT (20-25°C) on a shaker (approx. 600 rpm).
13. Empty the plate and blot dry by tapping the inverted plate on absorbent material.
14. Pipette 1 mL of Wash Buffer into all wells.
15. Incubate 5 min at RT (20-25°C) on a shaker (approx. 600 rpm).
16. Blot dry by tapping the inverted plate on absorbent material.
17. Wash one more time as described (step 14, 15, 16).
18. Pipette 200 µL of Hydrochloric Acid into all wells.
19. Cover plate with adhesive foil. Incubate 10 min at RT (20-25°C) on an o shaker (approx. 600 rpm).
    Note: Do not decant the supernatant thereafter! 190 µL of the supernatant is needed for the subsequent enzymatic conversion

✓ Enzymatic conversion

1. Pipette 190 µL of the extracted standards, controls and samples into the respective wells of the Microtiter Plate.
2. Add 50 µL of Enzyme Solution to all wells.
3. Cover plate with Adhesive Foil. Incubate 1 min at RT (20-25°C) on a shaker (approx. 600 rpm).
4. Incubate for 2 hours at 37°C. The following volumes of the supernatants are needed for the subsequent ELISA:
   - Adrenaline 100 µL
   - Noradrenaline 10 µL

✓ Adrenaline ELISA

1. Pipette 100 µL of standards, controls and samples from the Enzyme Plate into the respective pre-coated Adrenaline Microtiter Strips.
2. Pipette 50 µL of the respective Adrenaline Antiserum into all wells.
3. Cover the plate with Adhesive Foil. Incubate 1 min at RT (20-25°C) on a shaker (approx. 600 rpm).
4. Incubate for 15 – 20 hours (overnight) at 2 – 8 °C.
5. Remove the foil and discard or aspirate the contents of the wells and wash each well 4 times thoroughly with 300 µL Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
6. Pipette 100 µL of Enzyme Conjugate into all wells.
7. Cover the plate with Adhesive Foil and incubate 30 min at RT (20-25°C) on a shaker (approx. 600 rpm).
8. Remove the foil. Discard or aspirate the contents of the wells. Wash the plate 4x by adding 300 µL of Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent

material.

9. Pipette 100 µL of Substrate into all wells.
10. Incubate 20-30 min at RT (20-25°C) on a shaker (approx. 600 rpm).
    Note: Avoid exposure to direct sun light!
11. Pipette 100 µL of Stop Solution into all wells.
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).

✓ Noradrenaline ELISA

1. Pipette 10 µL of standards, controls and samples from the Enzyme Plate into the respective pre-coated Noradrenaline Microtiter Strips.
2. Pipette 50 µL of the respective Noradrenaline Antiserum into all wells.
3. Cover the plate with Adhesive Foil. Incubate 1 min at RT (20-25°C) on a shaker (approx. 600 rpm).
4. Incubate for 15 – 20 hours (overnight) at 2 – 8 °C.
5. Remove the foil and discard or aspirate the contents of the wells and wash each well 4 times thoroughly with 300 µL Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
6. Pipette 100 µL of Enzyme Conjugate into all wells.
7. Cover the plate with Adhesive Foil and incubate 30 min at RT (20-25°C) on a shaker (approx. 600 rpm).
8. Remove the foil and discard or aspirate the contents of the wells and wash the plate 4x by adding 300 µL of Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
9. Pipette 100 µL of Substrate into all wells.
10. Incubate 20-30 min at RT (20-25°C) on a shaker (approx. 600 rpm).
    Note: Avoid exposure to direct sun light!
11. Pipette 100 µL of Stop Solution into all wells.
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: Adrenaline: 10-3200 pg/mL; Noradrenaline: 20-12800 pg/mL

The calibration curves are 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).

The concentrations of the undiluted plasma samples and the controls can be read directly from the standard curve.

Concentration of diluted plasma samples:

If only a plasma volume < 600 µL was used for the extraction, the concentration read from the standard curve has to be multiplied with a volume-factor:

$$\text{Volume-factor} = \frac{600\ \mu L}{\text{used plasma volume } (\mu L)}$$

✓ Conversion

Adrenaline (pg/mL) x 5.46 = Adrenaline (pmol/L)

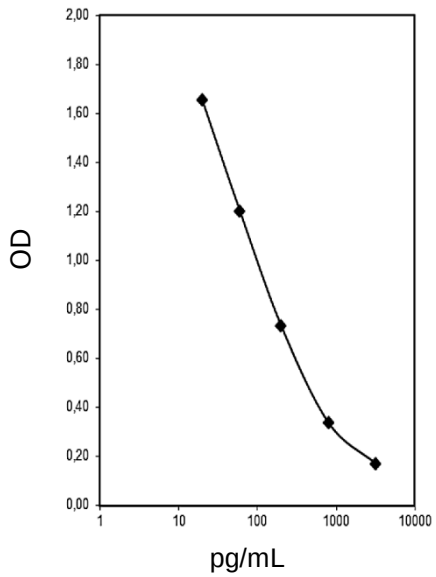
Noradrenaline (pg/mL) x 5.91 = Noradrenaline (pmol/L)

✓ Quality control

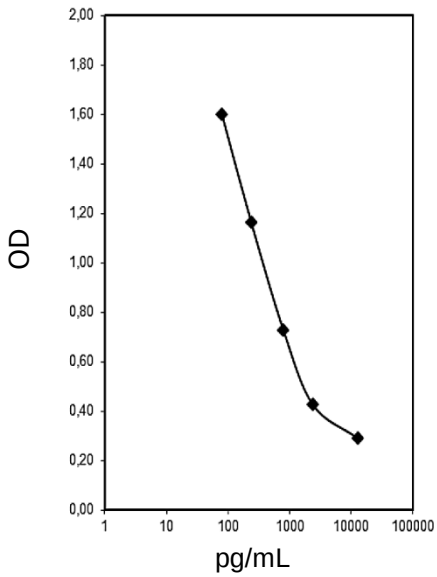
It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are printed on the QC Report.

✓ Typical calibration curves

Adrenaline



Noradrenaline



Note: Examples. Do not use for calculation!

Performance Characteristics

✓ Expected reference values

It is strongly recommended that each laboratory should determine its own reference values.

In a study conducted with apparently normal healthy adults, using this kit the following values are observed:

- Expected Reference Values

Adrenaline: < 100 pg/mL

Noradrenaline: < 600 pg/mL

✓ Analytical Specificity (Cross Reactivity)

Substance	**Cross Reactivity (%)**	
	Adrenaline	Noradrenaline
Derivatized Adrenaline	100	0.14
Derivatized Noradrenaline	0.20	100
Derivatized Dopamine	< 0.01	0.2
Metanephrine	0.64	< 0.01
Normetanephrine	< 0.01	0.48
3-Methoxytyramine	< 0.01	< 0.01
3-Methoxy-4-hydroxyphenylglycol	0.03	0.01
Tyramine	< 0.01	< 0.01
Phenylalanine, Caffeinic acid, L-Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid	< 0.01	< 0.01

✓ Analytical Sensitivity (600 µL undiluted sample)

Adrenaline	**Noradrenaline**
5.2 pg/mL	20 pg/mL

✓ Precision

Intra-Assay CV				
	Sample	Mean (pg/mL)	SD (pg/mL)	CV (%)
Adrenaline	low	54.0	9.1	16.8
	medium	113	13.2	11.7
	high	447	48.2	10.8
Noradrenaline	low	535	59.9	11.2
	medium	679	75.9	11.2
	high	2123	344	16.2

✓ Recovery

	Mean (%)	Range (%)	Measurement Range (pg/mL)
Adrenaline	110	93 – 121	38.9-2669
Noradrenaline	96	81 – 112	614-9606

✓ Linearity

	Serial dilution up to	Mean (%)	Range (%)
Adrenaline	1:512	90	73 – 105
Noradrenaline	1:512	96	76 – 107

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

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