Epinephrine ELISA Kit

Catalog Number KA1882

96 assays

Version: 04

Intended for research use only

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Introduction

Intended Use

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

Principle of the Assay

Adrenaline (epinephrine) is extracted by using a cis-diol-specific affinity gel, acylated and then derivatized 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 antiserum binding sites. After the system is in equilibrium, free antigen and free antigen-antiserum 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 for use	4 pack
Wash Buffer Concentrate	Concentrate. Dilute content with dist. water to a final volume of 1000 mL	20 mL
Enzyme Conjugate	ready for use, anti-rabbit IgG conjugated with peroxidase	12 mL
Substrate	ready for use, containing a solution of tetramethylbenzidine (TMB)	12 mL
Stop Solution	ready for use, containing 0.25 M H_2SO_4	12 mL
Adrenaline-Metanephrine Microtiter Strips	break apart, precoated, blue coloured	96(8x12) wells
Adrenaline Antiserum	from rabbit, ready for use, blue coloured, blue screw cap	6 mL
Adjustment Buffer	ready for use	4 mL
Standard A	ready for use	4 mL
Standard B	ready for use	4 mL
Standard C	ready for use	4 mL
Standard D	ready for use	4 mL
Standard E	ready for use	4 mL
Standard F	ready for use	4 mL
Acylation Buffer	ready for use	20 mL
Acylation Reagent	ready for use	3 mL
Assay Buffer	ready for use, contains 1 M HCl	6 mL
Coenzyme	ready for use, S-adenosyl-L-methionine	4 mL
Enzyme	lyophilized, contains the enzyme COMT	1 mL x 2
Extraction Buffer	ready for use	6 mL
Extraction Plate	coated with boronate affinity gel	48 wells x 2
Hydrochloric Acid	ready for use, yellow coloured, contains 0.025 M HCl	20 mL
Control 1	ready for use	4 mL
Control 2	ready for use	4 mL

Storage Instruction

Store the reagents at 2-8°C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Do not mix various lots of any kit component within an individual assay.

Materials Required but Not Supplied

- ✓ Calibrated variable precision micropipettes (e.g. 10-100 µL / 100-1000 µL)
- ✓ Microtiter plate washing device, Absorbent material (paper towel)
- ✓ ELISA reader capable of reading absorbance at 450 nm and 620 or 650 nm
- ✓ Shaker (shaking amplitude 3mm; approx. 600 rpm)
- ✓ Distilled water
- ✓ Vortex mixer

Precautions for Use

- Reliability of the test results

  In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, RILIBAK, etc.). Special attention must be paid to control checks for precision and correctness during the test; the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions. It is recommended that each laboratory establishes its own reference intervals. The values reported in this test instruction are only indicative.

- Complaints

  In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

- Warranty

  This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

- Disposal

Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.

- Interference

  Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

- Precautions

  Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation. All reagents of this test kit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.

Assay Protocol

Reagent Preparation

- Wash Buffer
  Dilute the 20 mL Wash Buffer Concentrate with distilled water to a final volume of 1000 mL. Storage: up to 6 months 4-8℃.

- Enzyme Solution
  Reconstitute the content of the vial labelled 'Enzyme' with 1 mL distilled water 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
  EDTA-Plasma should be used. Do not use haemolytic or lipemic samples. Storage: up to 6 hours at 2-8℃; for longer periods (up to 6 months) at -20℃. Repeated freezing and thawing should be avoided.

- Urine:
  Spontaneous or 24-hours urine, collected in a bottle containing 10-15 mL of 6 M HCl, should be used. Storage: for longer periods (up to 6 months) at -20℃. 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.

- 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 distilled water 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:

    Adrenaline, 100 µL

- Adrenaline ELISA

1. Pipette 25 µL of the Enzyme Solution into all wells of the Adrenaline Microtiter Strips.
2. Pipette 100 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 respective Adrenaline 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 and wash each well 3 times thoroughly with 300 µL Wash Buffer. Blot dry 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 and wash each well 3 times thoroughly with 300 µL Wash Buffer. Blot dry 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). *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 and a reference wavelength between 620 nm and 650 nm.

Data Analysis

Calculation of Results

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).

	Concentration of the standards					
Standard	A	B	C	D	E	F
Adrenaline (ng/mL)	0	1	4	15	50	200
Adrenaline (nmol/L)	0	5.5	22	82	273	1092
Conversion:	Adrenaline (ng/mL) x 5.46 = Adrenaline (nmol/L)					

- Urine samples and controls

  The concentrations of the urine samples and the Controls 1 & 2 can be read directly from the standard curve. Calculate the 24 h excretion for each urine sample: µg/24h = µg/L x L/24h

- Plasma samples

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

- Quality control

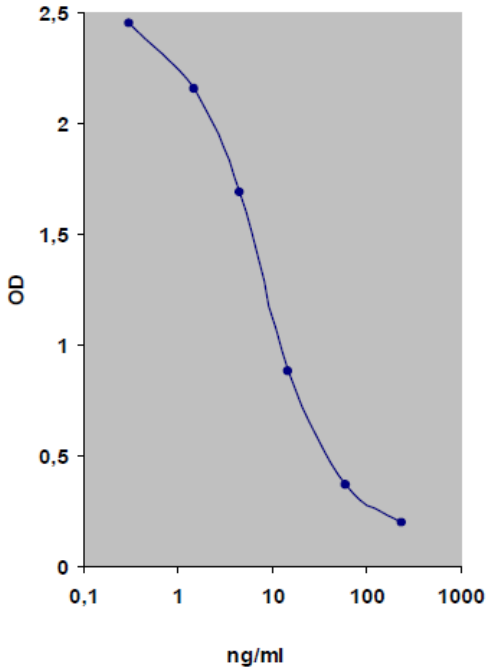
  It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are printed on the QC Report.

- Calibration

  The binding of the antisera and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the extinction values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. The extinction values also depend on the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20-25°C.

  Note: In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm.

Figure 1: Typical Standard Curve for Epinephrine ELISA Kit. (example – do not use for calculation)



Performance Characteristics

- Expected Reference Values

	Adrenaline
Urine	< 20 µg/day (110 nmol/day)
Plasma	< 100 pg/mL

- Analytical Sensitivity (Limit of Detection)

	Adrenaline
Urine	0.3 ng/mL
Plasma	10 pg/mL

- Analytical Specificity (Cross Reactivity)

Substance	Cross Reactivity (%)
	Adrenaline
Derivatized Adrenaline	100
Derivatized Noradrenaline	0.20
Derivatized Dopamine	< 0.0007
Metanephrine	0.64
Normetanephrine	0.0009
3-Methoxytyramine	< 0.0007
3-Methoxy-4-hydroxyphenylglycol	0.03
Tyramine	< 0.0007
Phenylalanine, Caffeinic acid, L-Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid	< 0.0007

- Precision

Intra-Assay			Inter-Assay		
Sample	Range (ng/mL)	CV (%)	Sample	Range (ng/mL)	CV (%)
1	2.5 ± 0.4	15.0	1	8.8 ± 1.1	13.2
2	11.7 ± 0.8	6.9	2	34.2 ± 5.2	15.4

- Linearity (Adrenaline)

	Range	Serial dilution up to	Range (%)
Urine	4.6 – 81.4 ng/mL	1:16	86 – 124
Plasma	92 - 545 pg/mL	1:8	81 – 121

- Recovery (Adrenaline)

	Mean (%)	Range (%)	% Recovery after spiking
Urine	107	84 – 119	
Plasma	92	80 – 113	

- Method Comparison versus HPLC*

Adrenaline	HPLC = 1.17 ELISA – 0.06	r = 0.99; n = 30

* The concentrations were assessed using both the ELISA and the HPLC method (external QC samples from UK NEQAS). The correlation between ELISA and HPLC is excellent. This means, that the ELISA measure equally good when compared to the UK NEQAS HPLC data. Please take in mind, that the UK control values are the mean of about 40 different HPLC users, and contain always one pathological sample per sending.

Resources

Plate Layout

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								