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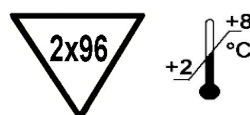
Instructions for use

2-CAT ELISA
(Adrenaline - Noradrenaline)

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

For Research use only-
Not for use in diagnostic procedures

REF **IB89519**



RUO

1. Principle of the test

Adrenaline (Epinephrine) and Noradrenaline (Norepinephrine) are 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 solid phase bound analytes 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 is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm.

The determination of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations.

2. Storage and stability

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date shown on the kit labels.

3. Contents of the kit

BA 1611	ACYL-BUFF	Acylation Buffer	1 x 20 mL	ready for use
BA 1612	ACYL-REAG	Acylation Reagent	2 x 1.5mL	ready for use
BA 1613	ASSAY-BUFF	Assay Buffer	2 x 4 mL	ready for use, contains 1 M HCl
BA 1614	COENZYME	Coenzyme	2 x 0.75 mL	ready for use, S-adenosyl-L-methionine
BA 1615	ENZYME	Enzyme	4 x 1 mL	lyophilized, contains the enzyme catechol-O-methyltransferase
BA 1617	EXTRACT-BUFF	Extraction Buffer	2 x 4 mL	ready for use
BA 1618	EXTRACT-PLATE 48	Extraction Plate	2 x 48 wells	coated with boronate affinity gel
BA 1619	HCL	Hydrochloric Acid	1 x 20 mL	ready for use, yellow coloured, contains 0.025 M HCl
BA 3050	ADJUST-BUFF	Adjustment Buffer	1 x 4 mL	ready for use
BA 10-0025	WASH-CONC 25x	Wash Buffer Concentrate	3 x 20 mL	Concentrate. Dilute content with dist. water to a final volume of 500 mL
BA 10-0040	CONJUGATE	Enzyme Conjugate	2 x 11 mL	ready for use, anti-rabbit IgG conjugated with peroxidase
BA 10-0055	SUBSTRATE	Substrate	2 x 11 mL	ready for use, containing a solution of tetramethylbenzidine (TMB)
BA 10-0080	STOP-SOLN	Stop Solution	2 x 11 mL	ready for use, containing 0.25 M H ₂ SO ₄
BA 10-0090	FOILS	Adhesive Foil	2 x 4	ready for use
BA 10-0110	ADR-AS	Adrenaline Antiserum	1 x 6 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA 10-0131	ADR MN	Adrenaline-Metanephrine Microtiter Strips	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, blue coloured
BA 10-0210	NAD-AS	Noradrenaline Antiserum	1 x 6 mL	from rabbit, ready for use, yellow coloured, yellow screw cap
BA 10-0231	NAD NMN	Noradrenaline-Normetanephrine Microtiter Strips	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, yellow coloured
BA 10-1601	STANDARD A	Standard A	1 x 1 mL	ready for use
BA 10-1602	STANDARD B	Standard B	1 x 1 mL	ready for use
BA 10-1603	STANDARD C	Standard C	1 x 1 mL	ready for use
BA 10-1604	STANDARD D	Standard D	1 x 1 mL	ready for use
BA 10-1605	STANDARD E	Standard E	1 x 1 mL	ready for use
BA 10-1606	STANDARD F	Standard F	1 x 1 mL	ready for use
BA 10-1651	CONTROL 1	Control 1	1 x 1 mL	ready for use
BA 10-1652	CONTROL 2	Control 2	1 x 1 mL	ready for use

4. **Additional materials and equipment required but not provided in the kit**

- Calibrated variable precision micropipettes (e.g. 10-100 μL / 100-1.000 μL)
- Microtiter plate washing device
- ELISA reader capable of reading absorbance at 450 nm and 620 or 650 nm
- Shaker (shaking amplitude 3mm; approx. 600 rpm)
- Absorbent material (paper towel), distilled water, Vortex mixer

5. **Sample collection and storage**

Plasma

EDTA-Plasma should be used. Do not use haemolytic or lipemic samples.

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 or 24-hour 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°C.

Repeated freezing and thawing should be avoided. Avoid exposure to direct sunlight.

6. **Test procedure**

Allow all reagents to reach room temperature and mix thoroughly by gentle inversion before use. Duplicate determinations are recommended.

6.1 **Preparation of reagents**

Wash Buffer

Dilute 20 mL of the Wash Buffer Concentrate with distilled water to a final volume of 500 mL. Store the diluted Wash Buffer Concentrate (Wash Buffer) at 2 – 8 °C. Shelf life: please refer to the expiry date indicated on the kit.

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.

⚠ *The enzyme solution has to be prepared freshly prior to the assay (not longer than 10 - 15 minutes in advance). Discard after use!*

6.2 **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.



Do not decant the supernatant thereafter!

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

Adrenaline	100 µL
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Noradrenaline	20 µL
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6.3 Adrenaline ELISA

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| 1. | Pipette 25 µl of the Enzyme Solution (refer to 6.1) 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 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 20-30 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. |

6.4 Noradrenaline ELISA

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| 1. | Pipette 25 µl of the Enzyme Solution (refer to 6.1) 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 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 contents 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 20-30 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. |

7. Calculation of results

Standard	Concentration of the standards					
	A	B	C	D	E	F
Adrenaline (ng/mL)	0	1	4	16	64	256
Adrenaline (nmol/L)	0	5.46	21.8	87.4	349	1,398
Noradrenaline (ng/mL)	0	4	16	64	256	1,024
Noradrenaline (nmol/L)	0	23.6	94.6	378	1,513	6,052
Conversion:	Adrenaline (ng/mL) x 5.46 = Adrenaline (nmol/L) Noradrenaline (ng/mL) x 5.91 = Noradrenaline (nmol/L)					

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).

Urine samples and controls:

The concentrations of the **urine samples** and the **Controls 1 & 2** can be read directly from the standard curve.

Plasma samples:

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

7.1 Quality control

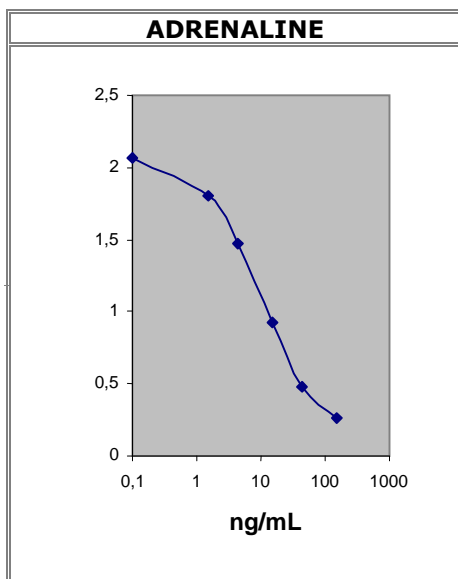
It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are printed on the QC Report.

7.2 Calibration

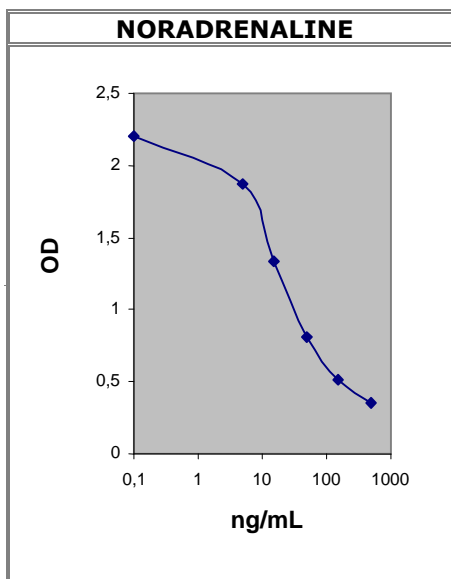
The binding of the antisera and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the extinction values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. The extinction values also depend on the incubation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

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

7.3 Typical calibration curves



⚠ Example. Do not use for calculation!



⚠ Example. Do not use for calculation!

8. Assay characteristics

Expected Reference Values		Adrenaline	Noradrenaline
	Urine	< 20 µg/day (110 nmol/day)	< 90 µg/day (535 nmol/day)
	Plasma	< 100 pg/mL	< 600 pg/mL

Analytical Sensitivity (Limit of Detection)	Mean signal (Zero-Standard) - 2SD		
		Adrenaline	Noradrenaline
	Urine	0.33 ng/mL	1.33 ng/mL
Plasma	11 pg/mL	44 pg/mL	

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)	
		Noradrenaline	Adrenaline
		Phenylalanine, Caffeinic acid, L-Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid	< 0.003

Precision							
Intra-Assay				Inter-Assay			
	Sample	Range (ng/mL)	CV (%)		Sample	Range (ng/mL)	CV (%)
Noradrenaline	1	24.4 ± 3.9	16.1	Noradrenaline	1	39.8 ± 3.4	8.5
	2	92.7 ± 9.0	9.8		2	135 ± 20	15.0
Adrenaline	1	2.5 ± 0.4	15.0	Adrenaline	1	8.8 ± 1.1	13.2
	2	11.7 ± 0.8	6.9		2	34.2 ± 5.2	15.4

Linearity			Range	Serial dilution up to	Range (%)
	Noradrenaline	Urine	20 - 339 ng/mL	1:16	85 - 123
		Plasma	318 - 2,436 pg/mL	1:8	84 - 123
	Adrenaline	Urine	4.6 - 81.4 ng/mL	1:16	86 - 124
		Plasma	92 - 545 pg/mL	1:8	81 - 121

Recovery			Mean (%)	Range (%)	spiking
	Noradrenaline	Urine	109	83 - 115	
		Plasma	97	85 - 108	
	Adrenaline	Urine	107	84 - 119	
		Plasma	92	80 - 113	

Method Comparison versus HPLC*	Noradrenaline	HPLC = 1.27 ELISA - 0.04	r = 0.96; n = 30
	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. 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.

9. Advice on handling the test

9.1 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, RILIBÄK, 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.

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.

9.2 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 complaint form and return it completely filled in to the manufacturer.

9.3 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.

9.4 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.

The appropriate safety data sheets of the individual products are available on the homepage. The safety data sheets correspond to the standard: ISO 11014-1.

9.5 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.

9.6 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 testkit 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.



Actual literature or any other information about the test are available on the homepage or contact the manufacturer directly.

Symbols:

	Contains sufficient for <n> tests		Manufacturer		Storage temperature
	Catalogue number		Batch code		Expiry date
	For in-vitro diagnostic use only!		Content		Consult instructions for use
	For research use only!		Caution		