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LDN[®]

Instructions for use
2-CAT (A-N) Research ELISA [™]

REF

BA E-5400

2x96



RUO

For Research use only-
Not for use in diagnostic
procedures

Adrenaline - Noradrenaline Research ELISA

1. Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of Adrenaline (Epinephrine) and Noradrenaline (Norepinephrine). Flexible test system for various biological sample types and volumes.

Adrenaline (Epinephrine) and Noradrenaline (Norepinephrine) are 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 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.

2. Advice on handling the test

2.1 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.

2.2 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 reclamation form and return it completely filled in to the manufacturer.

2.3 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.

2.4 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.

2.5 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.

2.6 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.

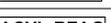
All reagents of this testkit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures.

All reagents, however, should be treated as potential biohazards in use and for disposal.

3. **Storage and stability**

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.

4.1 **Contents of the kit**

BA D-0032	 96	Microtiter Plate	1 x 96 wells	12 strips, 8 wells each, break apart
BA D-0090	 FOILS	Adhesive Foil	2 x 4	ready for use
BA E-0030	 WASH-CONC 	Wash Buffer Concentrate	2 x 20 mL	Concentrate. Dilute content with dist. water to a final volume of 1000 mL
BA E-0040	 CONJUGATE	Enzyme Conjugate	2 x 12 mL	ready for use, anti-rabbit IgG conjugated with peroxidase
BA E-0055	 SUBSTRATE	Substrate	2 x 12 mL	ready for use, containing a solution of TMB
BA E-0080	 STOP-SOLN	Stop Solution	2 x 12 mL	ready for use, containing 0.25 M H ₂ SO ₄
BA E-0131	  	Adrenaline-Metanephrine Microtiter Strips	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, blue coloured
BA E-0231	  	Noradrenaline-Normetanephrine Microtiter Strips	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, yellow coloured
BA E-5110	 ADR-AS	Adrenaline Antiserum	1 x 6 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA E-5210	 NAD-AS	Noradrenaline Antiserum	1 x 6 mL	from rabbit, ready for use, yellow coloured, yellow screw cap
BA R-0050	 ADJUST-BUFF	Adjustment Buffer	1 x 4 mL	ready for use
BA R-4617	 TE-BUFF	TE Buffer	1 x 4 mL	ready for use
BA R-5601	 STANDARD 	Standard A	1 x 4 mL	ready for use
BA R-5602	 STANDARD 	Standard B	1 x 4 mL	ready for use
BA R-5603	 STANDARD 	Standard C	1 x 4 mL	ready for use
BA R-5604	 STANDARD 	Standard D	1 x 4 mL	ready for use
BA R-5605	 STANDARD 	Standard E	1 x 4 mL	ready for use
BA R-5606	 STANDARD 	Standard F	1 x 4 mL	ready for use
BA R-5651	 CONTROL 	Control 1	1 x 4 mL	ready for use
BA R-5652	 CONTROL 	Control 2	1 x 4 mL	ready for use
BA R-6611	 ACYL-BUFF	Acylation Buffer	1 x 20 mL	ready for use
BA R-6612	 ACYL-REAG	Acylation Reagent	1 x 3 mL	ready for use
BA R-6614	 COENZYME	Coenzyme	1 x 4 mL	ready for use, S-adenosyl-L-methionine
BA R-6615	 ENZYME	Enzyme	4 x 1 mL	lyophilized, contains the enzyme COMT
BA R-6618	 EXTRACT-PLATE 	Extraction Plate	2 x 48 wells	coated with boronate affinity gel
BA R-6619	 HCL	Hydrochloric Acid	1 x 20 mL	ready for use, yellow coloured, contains 0.025 M HCl

4.2 **Additional materials and equipment required but not provided with the kit**

- Calibrated variable precision micropipettes (e.g. 1-10 µL / 10-100 µL / 100-1000 µL)
- Microtiter plate washing device
- ELISA reader capable of reading absorbance at 450 nm (reference filter 620 – 650 nm)
- Shaker (shaking amplitude 3mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Distilled water
- Vortex mixer

5. **Sample collection and storage**

Storage: up to 6 hours at 2 – 8 °C; for longer periods (up to 6 months) at - 20°C or – 80 °C.

Advice for the preservation of the biological sample: to prevent catecholamine degradation add EDTA (final concentration 1mM) and sodium metabisulfite (final concentration 4 mM) to the sample.

6. **Test procedure**

Allow reagents and samples to reach room temperature. Duplicate measurements are recommended.

6.1 **Preparation of reagents**

Wash Buffer

Dilute the 20 mL Wash Buffer Concentrate with distilled water to a final volume of 1000 mL.
Storage: up to 6 months 2–8°C

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**

The Research ELISA is a flexible test system for various biological sample types and volumes. It is not possible to give a general advice how to prepare the samples. However, the following basics should help the researcher to fit the protocol to his specific needs.

- Avoid excess of acid: excess of acid might exceed the buffer capacity of the extraction buffer. A pH > 7.0 during the extraction is mandatory.
- Prevent catecholamine degradation by adding preservatives to the sample (see 5. Sample collection and storage).
- Avoid chaotropic chemicals like perchloric acid. The high salt content might reduce the recovery of Adrenaline and Noradrenaline. If your samples already contain high amounts of perchloric acid, neutralize the sample prior to the extraction step.
- Tissue samples can be homogenised in 0.01 N HCl in the presence of EDTA and sodium metabisulfite. Under these conditions, Adrenaline and Noradrenaline are positively charged which reduces binding to proteins and optimizes solubility.
- Avoid samples that contain substances with a cis-diol structure. These will reduce the recovery of the catecholamines.
- It is advisable to perform a "Proof of Principle" to determine the recovery of the catecholamines in your samples. Prepare a stock solution of Adrenaline and Noradrenaline. Add small amounts (to change the native sample matrix as less as possible) of the stock solutions to the sample matrix and check the recovery.
- The used sample volume determines the sensitivity of the test. Determine the sample volume needed to determine the catecholamines in your sample by testing different amounts of sample volume.

If you need any support in establishing a protocol for your specific purposes, do not hesitate to contact the manufacturer directly!

6.3 Extraction and acylation

The Research ELISA offers a flexible test system for various biological sample types and volumes. Step 1 of the extraction procedure depends on the sample volume:

- in case you have sample volumes between 1 – 100 µL follow **1.1**
- in case you have sample volumes between 100 – 500 µL follow **1.2**
- in case you have sample volumes between 500 – 750 µL follow **1.3**



Within a run it is only possible to measure samples with the same volume!

1.	1.1 Sample volume 1 – 100 µL	1.2 Sample volume 100 – 500 µL	1.3 Sample volume 500 – 750 µL
	Pipette into the respective wells of the Extraction Plate: 20 µL standards, 20 µL controls and 1 – 100 µL of the sample. Fill up each well with distilled water to a final volume of 100 µl (e.g. 20 µl standard plus 80 µl dist. water).	Pipette into the respective wells of the Extraction Plate: 20 µL standards, 20 µL controls and 100 – 500 µL of the sample. Fill up each well with distilled water to a final volume of 500 µl (e.g. 20 µl standard plus 480 µl dist. water).	Pipette into the respective wells of the Extraction Plate: 20 µL of Standards, 20 µL of controls and 500 – 750 µL of sample. Fill up each well with distilled water to a final volume of 750 µl (e.g. 20 µl standard plus 730 µl dist. water).
2.	Pipette 25 µL of TE Buffer into all wells		
3.	Cover the plate with adhesive foil. Shake 60 min at RT (20-25°C) on a shaker (approx. 600 rpm).		
4.	Remove the foil and empty the plate. Blot dry by tapping the inverted plate on absorbent material.		
5.	Pipette 1 mL of Wash Buffer into all wells.		
6.	Shake 5 min at RT (20-25°C) on a shaker (approx. 600 rpm).		
7.	Blot dry by tapping the inverted plate on absorbent material.		
8.	Wash one more time as described (step 5, 6 and 7)!		
9.	Pipette 150 µL of Acylation Buffer into all wells.		
10.	Pipette 25 µL of Acylation Reagent into all wells.		
11.	Shake 20 min at RT (20-25°C) on a shaker (approx. 600 rpm).		
12.	Empty the plate and blot dry by tapping the inverted plate on absorbent material.		
13.	Pipette 1 mL of Wash Buffer into all wells.		
14.	Shake 5 min at RT (20-25°C) on a shaker (approx. 600 rpm).		
15.	Blot dry by tapping the inverted plate on absorbent material.		
16.	Wash one more time as described (step 13, 14, 15).		
17.	Pipette 150 µL of Hydrochloric Acid into all wells.		
18.	Cover plate with adhesive foil. Shake 10 min at RT (20-25°C) on an o shaker (approx. 600 rpm).		
	Do not decant the supernatant thereafter!		
	140 µL of the supernatant is needed for the subsequent enzymatic conversion		

6.4 Enzymatic conversion

1.	Pipette 140 µL of the extracted standards, controls and samples into the respective wells of the Microtiter Plate .		
2.	Add 50 µL of Enzyme Solution (refer to 6.1) to all wells.		
3.	Cover plate with Adhesive Foil . Shake 1 min at RT (20-25°C) on a shaker.		
4.	Incubate for 2 hours at 37°C . The following volumes of the supernatants are needed for the subsequent ELISA:		
	Adrenaline	90 µL	Noradrenaline
			90 µL

6.5 Adrenaline and Noradrenaline ELISA

1.	Pipette 90 µL of standards, controls and samples from the Enzyme Plate (refer to 6.4) into the respective pre-coated Mikrotiter Strips (*¹) .
2.	Pipette 50 µL of the respective Antiserum (*²) into all wells.
3.	Cover the plate with Adhesive Foil . Incubate for 1 min at RT (20-25°C) on a shaker .
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 . Blot dry 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 each well 4 times thoroughly with 300 µL Wash Buffer . Blot dry 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). ⚠ 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 and a reference wavelength between 620 nm and 650 nm.

⚠ (*¹): **Adrenaline Microtiter Strips, Noradrenaline Microtiter Strips**
 (*²): **Adrenaline Antiserum, Noradrenaline Antiserum**

7. Calculation of results

The calibration curve from which the concentrations in the samples can be read off, is obtained by plotting the absorbance readings (calculate the mean absorbance) measured for the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).

The use of a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima) is recommended.

The standards refer to:

Standard	Concentration of the standards (ng/mL)					
	A	B	C	D	E	F
Adrenaline	0	0.5	1.5	5	20	80
Noradrenaline	0	0.2	0.6	2	8	32

⚠ The concentrations of the samples taken from the standard curve have to be multiplied by a correction factor.

$$\text{Correction factor} = \frac{20 \mu\text{L (volume of standards extracted)}}{\text{sample volume } (\mu\text{L}) \text{ extracted}}$$

Example: 750µL of the sample is extracted and the concentration taken from the standard curve is 0.15 ng/mL Noradrenaline.

Correction factor = 20/750 = 0.027

Concentration of the sample = 0.15 ng/mL x 0.027 = 0.004 ng/mL = 4 pg/mL Noradrenaline

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 indicated 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

8. Assay characteristics

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)	
		Noradrenaline	Adrenaline
	Derivatized Adrenaline	0.14	100
	Derivatized Noradrenaline	100	0.20
	Derivatized Dopamine	0.2	< 0.0007
	Metanephrine	< 0.003	0.64
	Normetanephrine	0.48	0.0009
	3-Methoxytyramine	< 0.003	< 0.0007
	3-Methoxy-4-hydroxyphenylglycol	0.01	0.03
	Tyramine	< 0.003	< 0.0007
	Phenylalanine, Caffeinic acid, L-Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid	< 0.003	< 0.0007

Sensitivity (Limit of Detection)	Adrenaline	Noradrenaline
	0.25 ng/mL x C*	0.1 ng/mL x C*

C* = Correction factor (refer to 7.)

Analytical Sensitivity (750 µl undiluted sample)	Adrenaline	Noradrenaline
	6.6 pg/mL	2.6 pg/mL

Functional Sensitivity (750 µl undiluted sample)	Adrenaline	Noradrenaline
	10 pg/mL	4 pg/mL

Precision				
Intra-Assay Human EDTA-Plasma				
	Sample	Mean ± 3 SD (pg/mL)	SD (pg/mL)	CV (%)
Adrenaline	high	1329.3 ± 372.6	124.2	9.3
	medium	412.1 ± 129.6	43.2	10.5
	low	37.9 ± 19.5	6.5	17.1
Noradrenaline	high	1377.4 ± 483.6	161.2	11.7
	medium	502.6 ± 126.9	42.3	8.4
	low	32.7 ± 15.3	5.1	15.6
Intra-Assay Cell Culture Medium (RPMI)				
	Sample	Mean ± 3 SD (pg/mL)	SD (pg/mL)	CV (%)
Adrenaline	high	1649.6 ± 555.0	185	11.2
	medium	526.2 ± 186.6	62.2	11.8
	low	38.7 ± 18.9	6.3	16.3
Noradrenaline	high	2027.8 ± 712.5	237.5	11.7
	medium	716.5 ± 179.7	59.9	8.4
	low	46.0 ± 16.8	5.6	12.2

Recovery	Mean (%)	Range (%)	SD (%)	CV (%)
Adrenaline				
Human EDTA-Plasma	104.0	89.4 – 128.3	13.1	12.6
Cell Culture Medium	95.5	81.6 – 109.6	8.3	8.7
Noradrenaline				
Human EDTA-Plasma	116.5	104.8 – 125.6	8.0	6.9
Cell Culture Medium	96.7	70.6 – 124.7	17.1	17.7

 **For current literature, information about clinical significance or any other information please contact your local supplier.**

Symbols:

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