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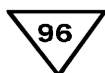
Internet: <http://www.ldn.de>

LDN[®]

Instructions for use
Adrenaline Plasma RIA **High Sensitive**

REF

BA R-4100



IVD



200 kBq

1. Principle of the test

High-sensitive Radioimmunoassay for the quantitative determination of adrenaline (epinephrine) in plasma

Adrenaline (epinephrine) is extracted from a plasma sample*) by using a cis-diol-specific affinity gel, acylated and then modified enzymatically.

The assay procedure follows the basic principle of radioimmunoassay, involving competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites. The amount of ¹²⁵I-labelled antigen bound to the antibody is inversely proportional to the analyte concentration of the sample. After the system is in equilibrium, the antibody bound radioactivity is precipitated with a second antibody in the presence of polyethylene glycol. The precipitate is counted in a gamma counter. Quantification of unknown samples is achieved by comparing their activity with a reference curve prepared with known standards.

*) Flexible sample volumes between 100 – 600 µl can be used with this assay.

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.

The radioactive material may be received, acquired, possessed, and used only by physicians, veterinarians in the practice of veterinary medicine, clinical laboratories or hospitals and only for in vitro clinical or laboratory tests not involving internal or external administration of the material, or the radiation there from, to human beings or animals. Its receipt, acquisition, possession, use, and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority. In no case the product must be administered to humans or animals.

All radioactive handling should be executed in a designated area, away from regular passage. A log book for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.

Any radioactive spills must be cleaned immediately in accordance with the radio safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.

3. **Storage and stability**

The reagents should be stored at 2 - 8 °C. Do not use components beyond the expiration date shown on the kit labels.

4.1 **Contents of the kit**

BA R-0030	PREC-REAG	Precipitating Reagent	1 x 55 mL	ready for use, goat anti-rabbit serum in PEG phosphate buffer. <i>Mix thoroughly before use!</i>
BA R-0050	ADJUST-BUFF	Adjustment Buffer	1 x 4 mL	ready for use
BA R-0120	¹²⁵I ADR MN	¹²⁵I – Adrenaline - Metanephrine	1 x 5.5 mL	activity < 200 kBq, ready for use, red coloured, blue screw cap
BA R-4110	AS ADR	Adrenaline Antiserum	1 x 5.25 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA R-4601	STANDARD A	Standard A	1 x 4 mL	ready for use
BA R-4602	STANDARD B	Standard B	1 x 4 mL	ready for use
BA R-4603	STANDARD C	Standard C	1 x 4 mL	ready for use
BA R-4604	STANDARD D	Standard D	1 x 4 mL	ready for use
BA R-4605	STANDARD E	Standard E	1 x 4 mL	ready for use
BA R-4606	STANDARD F	Standard F	1 x 4 mL	ready for use
BA R-4617	TE-BUFF	TE Buffer	1 x 4 mL	ready for use
BA R-4651	CONTROL 1	Control 1	1 x 4 mL	ready for use
BA R-4652	CONTROL 2	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	2 x 1 mL	lyophilized, contains the enzyme catechol-O-methyltransferase
BA R-6618	EXTRACT-PLATE 48	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)
- Polystyrene tubes and suitable rack
- Temperature controlled water bath, heating block or incubator (37 °C)
- Centrifuge capable of at least 3,000 x g
- Suitable device for aspirating or decanting
- Plate shaker (shaking amplitude 3mm; approx. 600 rpm)
- Gamma counter
- Vortex mixer
- Distilled water

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.

6. **Test procedure**

A plasma volume between **100 µl-600 µl** is needed per single determination.


If a plasma volume **< 600 µl** is used, dist. water has to be added to a **final volume of 600 µl** and this **prediluted sample** has to be used for the extraction procedure (please refer to point 6.2 of this protocol).

This sample predilution has to be considered in the calculation of results (please refer to point 7 of this protocol).


6.1 **Preparation of reagents**

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!*

Allow all reagents – with the exception of the Precipitating Reagent - to reach room temperature and mix thoroughly by gentle inversion before use. Number the assay tubes accordingly. Duplicate determinations are recommended.

 *Pipetted liquids should not adhere to the wall of the RIA tubes. If necessary please centrifuge the tubes for 1 minute at 500xg to spin down adhering liquids.*

6.2 Sample preparation, extraction and acylation

1.	Pipette 30 µL of standards, controls and 600 µL of plasma samples (diluted or undiluted) into the respective wells of the Extraction Plate .		
2.	Add 500 µL of distilled water to the wells with standards and controls .		
3.	Pipette 25 µL of TE Buffer into all wells		
4.	Cover the plate with adhesive foil. Shake 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 distilled water into all wells.		
7.	Shake 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.	Shake 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 distilled water into all wells.		
15.	Shake 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 the plate with adhesive foil. Shake 10 min at RT (20-25°C) on an orbital shaker (approx. 600 rpm).		
	Do not decant the supernatant thereafter! The following volumes of the supernatants are needed for the subsequent RIA: <table border="1" data-bbox="204 1122 571 1167"> <tr> <td>Adrenaline</td><td>90 µL</td></tr> </table>	Adrenaline	90 µL
Adrenaline	90 µL		

6.3 Adrenaline RIA

1.	Pipette 90 µL of Hydrochloric Acid into the tubes for the NSB.
2.	Pipette 90 µL of the extracted standards, controls and samples into the respective tubes.
3.	Pipette 25 µL of Enzyme Solution (refer to 6.1) into all tubes (except totals).
4.	Mix thoroughly and incubate for 2 hours at 37 °C .
5.	Pipette 50 µL of the corresponding ¹²⁵I Adrenaline into the respective tubes .
6.	Pipette 50 µL of the corresponding Adrenaline Antiserum into the respective tubes (except totals and NSB) ; mix thoroughly.
7.	Cover the tubes. Incubate for 15 - 20 hours (overnight) at 2 - 8 °C
8.	Mix the chilled (2 - 8 °C) Precipitating Reagent thoroughly, pipette each 0.5 mL into all tubes (except totals), and mix on a vortex.
9.	Incubate for 15 minutes at 2 - 8 °C .
10.	Centrifuge for 15 minutes at 3,000 x g , if possible in a refrigerated centrifuge.
11.	Decant or aspirate the supernatant carefully (except totals). Blot the tubes dry and leave them upside for 2 minutes.
12.	Count all tubes for 1 minute in a gamma counter.

7. Calculation of results

The standards refer to:

Standard	Concentration of the standards					
	A	B	C	D	E	F
Adrenaline (pg/mL)	0	20	60	200	800	3 200
Adrenaline (pmol/L)	0	109	328	1 092	4 368	17 472
Conversion:	Adrenaline (ng/mL) x 5.46 = Adrenaline (nmol/L)					

Subtract the mean cpm of the non-specific binding NSB from the mean cpm of standards, controls and samples.

The calibration curve from which the concentrations in the samples can be read off, is obtained by plotting the percentage of (B-NSB)/(B0-NSB) measured for 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:

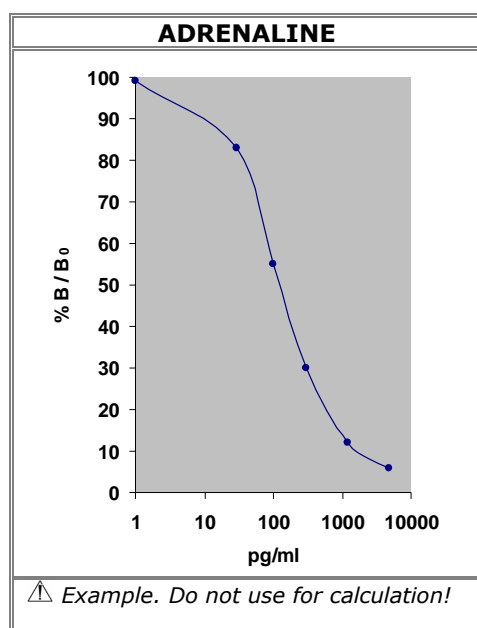
If only a plasma volume **< 600 µl** was used for the extraction, the concentration read from the standard curve has to be **multiplied** with a **volume-factor**:

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

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 Typical calibration curves















8. Assay characteristics

Expected Reference Values		Adrenaline			
	Plasma	< 100 pg/mL			
Analytical Sensitivity (600 µl undiluted plasma)		Mean signal (Zero-Standard) - 2SD			
		Adrenaline			
	Plasma	7 pg/mL			
Functional Sensitivity		Mean concentration < 20% CV			
		Adrenaline			
	Plasma	20 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 (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	
Recovery (human EDTA-plasma)		Mean (%)	Range (%)	SD (%)	CV (%)
Adrenaline		104.0	89.4 – 128.3	13.1	12.6

 **For updated 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		Batch code		For in-vitro diagnostic use only!
	Consult instructions for use		Content		CE labelled
	Caution		Catalogue number		For research use only!