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

REF

LDN-BA E-4100



RUO

For Research use only-
Not for use in diagnostic
procedures

1. Principle of the test

Enzyme Immunoassay 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 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.

*) 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.














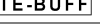
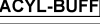
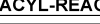
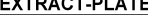

All reagents of this testkit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures.

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	1 x 4	ready for use
BA E-0030	 WASH-CONC  50x	Wash Buffer Concentrate	1 x 20 mL	Concentrate. Dilute content with dist. water to a final volume of 1000 mL
BA E-0040	 CONJUGATE	Enzyme Conjugate	1 x 12 mL	ready for use, anti-rabbit IgG conjugated with peroxidase
BA E-0055	 SUBSTRATE	Substrate	1 x 12 mL	ready for use, containing a solution of TMB
BA E-0080	 STOP-SOLN	Stop Solution	1 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-4110	 ADR-AS	Adrenaline Antiserum	1 x 6 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA R-0050	 ADJUST-BUFF	Adjustment Buffer	1 x 4 mL	ready for use
BA R-4601	 STANDARD 	Standard A	1 x 4 mL	ready for use
BA R-4602	 STANDARD 	Standard B	1 x 4 mL	ready for use
BA R-4603	 STANDARD 	Standard C	1 x 4 mL	ready for use
BA R-4604	 STANDARD 	Standard D	1 x 4 mL	ready for use
BA R-4605	 STANDARD 	Standard E	1 x 4 mL	ready for use
BA R-4606	 STANDARD 	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 	Control 1	1 x 4 mL	ready for use
BA R-4652	 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 2 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 in the kit

- Calibrated variable precision micropipettes (e.g. 1-10 µL / 10-100 µL / 100-1000µL)
- 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

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).

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 the 20 mL Wash Buffer Concentrate with distilled water to a final volume of 1000 mL.


Storage: up to 6 months 4-8°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, 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 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 Wash Buffer 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 5, 6 and 7)!
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 Wash Buffer 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 13, 14, 15).
18.	Pipette 200 µL of Hydrochloric Acid into all wells.
19.	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!
	190 µL of the supernatant is needed for the subsequent enzymatic conversion

6.4 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 (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: <table border="1" style="margin-left: 20px;"> <tr> <td>Adrenaline</td> <td>100 µL</td> </tr> </table>	Adrenaline	100 µL
Adrenaline	100 µL	

6.5 Adrenaline ELISA

1. Pipette 100 µL of standards, controls and samples from the Enzyme Plate (refer to 6.4) into the respective pre-coated Adrenaline Mikrotiter Strips .
2. Pipette 50 µL of the respective Adrenaline Antiserum 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.

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)					

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**:

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

7.1 Quality control

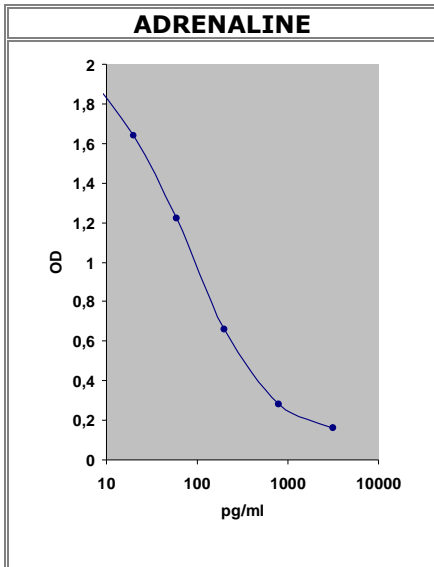
It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC Report.

7.2 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 curve



■ Example. Do not use for calculation!







8. Assay characteristics

Expected Reference Values	Plasma	Adrenaline < 100 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	
Analytical Sensitivity (750 µl undiluted sample)	Adrenaline 7 pg/mL	
Functional Sensitivity (750 µl undiluted sample)	Adrenaline 20 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	
Recovery		Mean (%)	Range (%)	SD (%)	CV (%)
Adrenaline					
Human EDTA-Plasma		104.0	89.4 – 128.3	13.1	12.6
Linearity			Range	Serial dilution up to	Range (%)
	Adrenaline		92 - 545 pg/mL	1:8	81 - 121
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.					

For actual 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!

Revision date: 07-July-2011

7/7

Li StarFish distribuisce: