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Instructions for use

ADRENALINE high sensitive ELISA

REF **BA E-5100R**

2°C – 8°C

Σ 96

RUO For research use only – Not for use in diagnostic procedures

ADRENALINE high sensitive ELISA

1. Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of adrenaline (epinephrine). Flexible test system for various biological sample types and volumes.

Adrenaline (epinephrine) is 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 analyte compete for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antigen-antibody 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 standard curve prepared with known standard concentrations.

2. Procedural Cautions, Guidelines and Warnings

(1) This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
(2) The principles of Good Laboratory Practice (GLP) have to be followed.
(3) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable latex gloves and protective glasses where necessary.
(4) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
(5) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
(6) The microplate contains snap-off strips. Unused wells must be stored at 2 – 8 °C in the sealed foil pouch with desiccant and used in the frame provided.
(7) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
(8) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
(9) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
(10) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(11) A standard curve must be established for each run.
(12) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
(13) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
(14) Avoid contact with Stop Solution containing 0.25 M H_2SO_4. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
(15) TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them.
(16) For information on hazardous substances included in the kit please refer to Safety Data Sheet (SDS). The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
(17) Kit reagents must be regarded as hazardous waste and disposed according to national regulations.
(18) In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components must not be used for a test run. They must be stored properly until the manufacturer decides what to do with them. If it is decided that they are no longer suitable for measurements, they must be disposed of in accordance with national regulations.

3. Storage and stability

Store the unopened reagents at 2 – 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 2 months when stored at 2 – 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

4. Materials

4.1 Content of the kit

BA D-0032 96 **Microtiter Plate** – Ready to use
Content: 1 x 96 wells, empty in a resealable pouch

BA D-0090 FOILS **Adhesive Foil** – Ready to use
Content: Adhesive Foils in a resealable pouch
Volume: 1 x 4 foils

BA E-0030 WASH-CONC 50x **Wash Buffer Concentrate** – Concentrated 50x
Content: Buffer with a non-ionic detergent and physiological pH
Volume: 1 x 20 ml/vial, light purple cap

BA E-0040 CONJUGATE **Enzyme Conjugate** – Ready to use
Content: Goat anti-rabbit immunoglobulins, conjugated with peroxidase
Volume: 1 x 12 ml/vial, red cap

BA E-0055 SUBSTRATE **Substrate** – Ready to use
Content: Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide
Volume: 1 x 12 ml/black vial, black cap

BA E-0080 STOP-SOLN **Stop Solution** – Ready to use
Content: 0.25 M sulfuric acid
Volume: 1 x 12 ml/vial, light grey cap
Hazards identification:

H290 May be corrosive to metals.

BA E-0131 ADR MN **Adrenaline Microtiter Strips** – Ready to use
Content: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable blue pouch with desiccant

BA E-5110 ADR-AS **Adrenaline Antiserum** – Ready to use
Content: Rabbit anti-adrenaline antibody, blue coloured
Volume: 1 x 6 ml/vial, blue cap

BA E-6612 ACYL-REAG **Acylation Reagent** – Ready to use
Content: Acylation reagent in DMSO
Volume: 1 x 3 ml/vial, white cap

BA R-0050 ADJUST-BUFF **Adjustment Buffer** – Ready to use
Content: TRIS buffer
Volume: 1 x 4 ml/vial, green cap

BA R-4617 TE-BUFF **TE Buffer** – Ready to use
Content: TRIS-EDTA buffer
Volume: 1 x 4 ml/vial, brown cap

Standards and **Controls** – Ready to use

Cat. no.	Component	Colour/ Cap	Concentration ng/ml ADR	Concentration nmol/l ADR	Volume/ Vial
BA R-5601	STANDARD A	white	0	0	4 ml
BA R-5602	STANDARD B	light yellow	0.5	2.7	4 ml
BA R-5603	STANDARD C	orange	1.5	8.2	4 ml
BA R-5604	STANDARD D	dark blue	5	27	4 ml
BA R-5605	STANDARD E	light grey	20	109	4 ml
BA R-5606	STANDARD F	black	80	437	4 ml
BA R-5651	CONTROL 1	light green	Refer to QC-Report for expected value and acceptable range!		4 ml
BA R-5652	CONTROL 2	dark red			4 ml

Conversion: Adrenaline (ng/ml) x 5.46 = Adrenaline (nmol/l)

Content: Acidic buffer with non-mercury stabilizer, spiked with defined quantity of adrenaline

BA R-6611 ACYL-BUFF **Acylation Buffer** – Ready to use

Content: Buffer with light alkaline pH for the acylation

Volume: 1 x 20 ml/vial, white cap

BA R-6614 COENZYME **Coenzyme** – Ready to use

Content: S-adenosyl-L-methionine

Volume: 1 x 4 ml/vial, purple cap

BA R-6615 ENZYME **Enzyme** – Lyophilized

Content: Catechol-O-methyltransferase

Volume: 4 vials, pink cap

BA R-6618 EXTRACT-PLATE 48 **Extraction Plate** – Ready to use

Content: 2 x 48 well plates coated with boronate affinity gel in a resealable pouch

BA R-6619 HCL **Hydrochloric Acid** – Ready to use

Content: 0.025 M Hydrochloric Acid; yellow coloured

Volume: 1 x 20 ml/vial, dark green cap

4.2 Additional materials and equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 1 – 750 µl; 1 ml
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 – 650 nm
- Shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Temperature controlled incubator (37 °C) or similar heating device
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)
- 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 1 mM) and sodium metabisulfite (final concentration 4 mM) to the sample.

6. Test procedure

Allow reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Duplicate determinations are recommended. It is recommended to number the strips of the microwell plate before usage to avoid any mix-up.

The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent. The higher the temperature, the higher the absorption values will be. Varying incubation times will have similar influences on the absorbance. 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.*

6.1 Preparation of reagents

Wash Buffer
Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1,000 ml.
Storage: 2 months at 2 – 8 °C

Enzyme Solution
Reconstitute the content of the vial labelled 'Enzyme' with 1 ml water (deionized, distilled, or ultra-pure) 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!*

Adrenaline Microtiter Strips
In rare cases residues of the blocking and stabilizing reagent can be seen in the wells as small, white dots or lines. These residues do not influence the quality of the product.

Acylation Reagent
The Acylation Reagent (BA E-6612) has a freezing point of 18.5 °C. To ensure that the Acylation Reagent is liquid when being used, it must be ensured that the Acylation Reagent has reached room temperature and forms a homogeneous, crystal-free solution before being used.

6.2 Sample preparation

The ADRENALINE high sensitive 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 adrenaline degradation by adding preservatives to the sample (see Sample collection and storage).
- Avoid chaotropic chemicals like perchloric acid. The high salt content might reduce the recovery of adrenaline. 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 is 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 adrenaline.
- It is advisable to perform a "Proof of Principle" to determine the recovery of the adrenaline in your samples. Prepare a stock solution of adrenaline. 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 this test. Determine the sample volume needed to determine the adrenaline 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 ADRENALINE high sensitive 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** Pipette into the respective wells of the Extraction Plate: **10 µl standards, 10 µl controls and 1 – 100 µl sample**. Fill up each well with water (deionized, distilled, or ultra-pure) to a **final volume** of 100 µl [e.g. 10 µl standard plus 90 µl water (deionized, distilled, or ultra-pure)].	**1.2** **Sample volume 100 – 500 µl** Pipette into the respective wells of the Extraction Plate: **10 µl standards, 10 µl controls and 100 – 500 µl sample**. Fill up each well with water (deionized, distilled, or ultra-pure) to a **final volume** of 500 µl [e.g. 10 µl standard plus 490 µl water (deionized, distilled, or ultra-pure)].	**1.3** **Sample volume 500 – 750 µl** Pipette into the respective wells of the Extraction Plate: **10 µl standards, 10 µl controls and 500 – 750 µl sample**. Fill up each well with water (deionized, distilled, or ultra-pure) to a **final volume** of 750 µl [e.g. 10 µl standard plus 740 µl water (deionized, distilled, or ultra-pure)].
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 **100 µl** of **Hydrochloric Acid** into all wells.		
18.	Cover plate with **Adhesive Foil**. Shake **10 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).		
⚠	***Do not decant the supernatant thereafter!*** **90 µl of the supernatant is needed for the subsequent enzymatic conversion**		

6.4 Enzymatic Conversion

1.	Pipette **90 µl** of the **extracted standards, controls** and **samples** into the respective wells of the **Microtiter Plate.**
2.	Add **25 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** (approx. 600 rpm) to mix.
4.	Incubate for **2 h** at **37 °C**. The following volumes of the supernatants are needed for the subsequent ELISA: **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 Microtiter Strips**.
2.	Pipette **50 µl** of the respective **Adrenaline Antiserum** into all wells.
3.	Cover the plate with **Adhesive Foil**. Shake **1 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
4.	Incubate for **15 – 20 h** (overnight) at **2 – 8 °C**.
5.	Remove the foil. Discard or aspirate the content of the wells. Wash the plate **4 x** by adding **300 µl** of **Wash Buffer**, **discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
6.	Pipette **100 µl** of **Enzyme Conjugate** into all wells.
7.	Incubate **30 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
8.	Discard or aspirate the content of the wells. Wash the plate **4 x** by adding **300 µl** of **Wash Buffer**, **discarding** the content and **blotting dry each time** 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 sunlight!***
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** (if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

The standard curve from which the concentrations of 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).
Use a non-linear regression for curve fitting (e.g. 4-parameter, marquardt).

⚠ *This assay is a competitive assay. This means: the OD-values are decreasing with increasing concentrations of the analyte. OD-values found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.*

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

$$\text{Correction factor} = \frac{\text{10 µl (volume of standards extracted)}}{\text{sample volume (µl) extracted}}$$

Example
750 µl of the sample is extracted and the concentration taken from the standard curve is 0.45 ng/ml adrenaline.
Correction factor = 10/750 = 0.013
Concentration of the sample = 0.45 ng/ml x 0.013 = 0.006 ng/ml = 6 pg/ml adrenaline

Conversion
Adrenaline (ng/ml) x 5.46 = Adrenaline (nmol/l)

7.1 Quality control

The confidence limits of the kit controls are indicated on the QC-Report.

8. Assay characteristics

	Substance	Cross Reactivity (%)
		Adrenaline
Analytical Specificity (Cross Reactivity)	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

Sensitivity (Limit of Detection)	**Adrenaline**
	0.25 ng/ml x C*

C* = Correction factor (refer to 7.)

Analytical Sensitivity (750 µl undiluted sample)	**Adrenaline**
	3.3 pg/ml

Functional Sensitivity (750 µl undiluted sample)	**Adrenaline**
	5 pg/ml

Precision				
Intra-Assay Human EDTA-Plasma				
	Sample	Mean ± 3 SD (pg/ml)	SD (pg/ml)	CV (%)
Adrenaline	high	1,329.3 ± 372.6	124.2	9.3
	medium	412.1 ± 129.6	43.2	10.5
	low	37.9 ± 19.5	6.5	17.1

Intra-Assay Cell Culture Medium (RPMI)				
	Sample	Mean ± 3 SD (pg/ml)	SD (pg/ml)	CV (%)
Adrenaline	high	1,649.6 ± 555.0	185	11.2
	medium	526.2 ± 186.6	62.2	11.8
	low	38.7 ± 18.9	6.3	16.3

Recovery Adrenaline	Mean (%)	Range (%)	SD (%)	CV (%)
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

⚠ ***For literature or any other information please contact your local supplier.***

⚠ ***The liability of the manufacturer shall be limited to the replacement of defective products. The manufacturer takes no liability for any damages or expenses arising directly or indirectly from the use of this product.***

Symbols:

+2 +8 °C	Storage temperature		Manufacturer	Σ	Contains sufficient for <n> tests
	Use-by date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number		Distributor
	Date of manufacture			RUO	For research use only!