

Manufactured for:

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Instructions for use

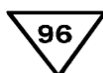
Epinephrine (Research) ELISA

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

For Research Use Only, Not for Use in Diagnostic Procedures.

REF

IB89539



RUO

For Research use only-
Not for use in diagnostic
procedures

Adrenaline Research ELISA

1. **Intended use**

Enzyme Immunoassay for the determination of Adrenaline (Epinephrine).
Flexible test system for various biological types and volumes.
For research use only, not for use in diagnostic procedures.

2. **Principle of the test**

Adrenaline (epinephrine) is 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 calibrators, controls and unknowns and the solid phase bound analyte 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.
Determination of unknowns is achieved by comparing their absorbance with a reference curve prepared with known calibrator concentrations.

3. **Procedural Notes**

3.1 **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 reference 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.

3.2 **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.

3.3 **Interference**

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of the biological being tested 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.

3.4 **Precautions**

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and biologicals with skin. No smoking, eating or drinking in areas where testing is conducted. Always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. 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.





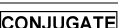

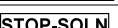








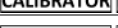

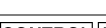
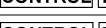
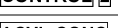


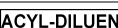
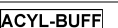





All reagents of this test kit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures.

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

4. **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.

5.1 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 	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-5110	 ADR-AS	Adrenaline Antiserum	1 x 6 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA E-5601	 CALIBRATOR A	Calibrator A	1 x 4 mL	ready for use
BA E-5602	 CALIBRATOR B	Calibrator B	1 x 4 mL	ready for use
BA E-5603	 CALIBRATOR C	Calibrator C	1 x 4 mL	ready for use
BA E-5604	 CALIBRATOR D	Calibrator D	1 x 4 mL	ready for use
BA E-5605	 CALIBRATOR E	Calibrator E	1 x 4 mL	ready for use
BA E-5606	 CALIBRATOR F	Calibrator F	1 x 4 mL	ready for use
BA E-5619	 HCL	Hydrochloric Acid	1 x 20 mL	ready for use, yellow coloured, contains 0.025 M HCl
BA E-5651	 CONTROL 1	Control 1	1 x 4 mL	ready for use
BA E-5652	 CONTROL 2	Control 2	1 x 4 mL	ready for use
BA R-0012	 ACYL-CONC	Acylation Concentrate	1 x 0.5 mL	Concentrate. Has to be diluted prior to use.
BA R-0050	 ADJUST-BUFF	Adjustment Buffer	1 x 4 mL	ready for use
BA R-0075	 ACYL-DILUENT	Acylation Diluent	1 x 4 mL	ready for use
BA R-6611	 ACYL-BUFF	Acylation Buffer	1 x 20 mL	ready for use
BA R-6613	 ASSAY-BUFF	Assay Buffer	1 x 6 mL	ready for use, contains 1 M HCl
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 COMT
BA R-6617	 EXTRACT-BUFF	Extraction Buffer	1 x 6 mL	ready for use
BA R-6618	 EXTRACT-PLATE 	Extraction Plate	2 x 48 wells	coated with boronate affinity gel

Calibrator	Concentration of the Calibrators (ng/mL)					
	A	B	C	D	E	F
Adrenaline	0	0.45	1.5	4.5	15	45

5.2 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

6. Collection and Handling of Unknowns

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 being tested: to prevent catecholamine degradation add EDTA (final concentration 1mM) and sodium metabisulfite (final concentration 4 mM) to the unknown.

7. **Test procedure**

Allow reagents and unknowns to reach room temperature. Duplicate determinations are recommended.

7.1 **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

Acylation Solution

The Acylation Concentrate has to be diluted 1 + 60 with Acylation-Diluent in a glass or polypropylene-vial.

Acylation Concentrate	10 µL	20 µL	25 µL	50 µL
Acylation-Diluent	600 µL	1.2 mL	1.5 mL	3 mL

⚠ *The Acylation Solution has to be prepared freshly prior to the assay (not longer than 60 minutes in advance). Discard after use!*

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!*

7.2 **Unknown preparation**

The Adrenaline Research ELISA is a flexible test system for various biological types and volumes. It is not possible to give a general advice how to prepare the biologicals being tested. 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 unknown (see 6. Collection and Handling of Unknowns).
- Avoid chaotropic chemicals like perchloric acid. The high salt content might reduce the recovery of Adrenaline. If your unknowns already contain high amounts of perchloric acid, neutralize the unknown prior to the extraction step.
- Tissues 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 unknowns 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 unknowns. Prepare a stock solution of Adrenaline. Add small amounts (to change the native matrix as less as possible) of the stock solutions to the matrix of the unknown being tested and check the recovery.
- The used volume of the unknown being tested determines the sensitivity of this test. Determine the volume needed to determine the Adrenaline in your unknown by testing different volumes of unknowns being tested.

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

7.3 Extraction and acylation

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

- in case you have volumes of unknowns being tested between 1 – 100 µL follow **1.1**
- in case you have volumes of unknowns being tested between 100 – 500 µL follow **1.2**
- in case you have volumes of unknowns being tested between 500 – 750 µL follow **1.3**



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

1.	1.1 Unknown volume 1 – 100 µL	1.2 Unknown volume 100 – 500 µL	1.3 Unknown volume 500 – 750 µL
	Pipette into the respective wells of the Extraction Plate: 10 µL calibrators, 10 µL controls and 1 – 100 µL of the unknowns. Fill up each well with distilled water to a final volume of 100 µl (e.g. 10 µl calibrator plus 90 µl dist. water).	Pipette into the respective wells of the Extraction Plate: 10 µL calibrators, 10 µL controls and 100 – 500 µL of the unknowns. Fill up each well with distilled water to a final volume of 500 µl (e.g. 10 µl calibrator plus 490 µl dist. water).	Pipette into the respective wells of the Extraction Plate: 10 µL of calibrators, 10 µL of controls and 500 – 750 µL of unknowns. Fill up each well with distilled water to a final volume of 750 µl (e.g. 10 µl calibrator plus 740 µl dist. water).
2.	Pipette 50 µL of Assay Buffer into all wells.		
3.	Pipette 50 µL of Extraction 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 6, 7 and 8)!		
10.	Pipette 150 µL of Acylation Buffer into all wells.		
11.	Pipette 25 µL of Acylation Solution (refer to 7.1) 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 14, 15, 16).		
18.	Pipette 100 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!		
	90 µL of the supernatant is needed for the subsequent enzymatic conversion		

7.4 Enzymatic Conversion

1.	Pipette 90 µL of the extracted calibrators, controls and unknowns into the respective wells of the Microtiter Plate .
2.	Add 25 µL of Enzyme Solution (refer to 7.1) to all wells.
3.	Cover plate with Adhesive Foil . Shake 1 min at RT (20-25°C) on a shaker to mix.

4. Incubate for **2 hours** at **37°C**. The following volumes of the supernatants are needed for the subsequent ELISA:

Adrenaline	100 µL
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7.5 Adrenaline ELISA

1.	Pipette 100 µL of calibrators, controls and unknowns from the Microtiter Plate (refer to 7.4) into the respective pre-coated Adrenaline Microtiter Strips .
2.	Pipette 50 µL of the 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.

8. Results

The calibration curve from which the concentrations in the unknowns can be read off is obtained by plotting the absorbance readings (calculate the mean absorbance) measured for the calibrators (linear, y-axis) against the corresponding concentrations (logarithmic, x-axis).
The use of a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima) is recommended.

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

$$\text{Correction factor} = \frac{10 \mu\text{L (volume of calibrators extracted)}}{\text{volume of unknown } (\mu\text{L}) \text{ extracted}}$$

Example: 750µL of the unknown is extracted and the concentration taken from the calibration curve is 0.45 ng/mL Adrenaline.





$$\text{Correction factor} = 10/750 = 0.013$$

$$\text{Concentration of the unknown} = 0.45 \text{ ng/mL} \times 0.013 = 0.006\text{ng/mL} = 6 \text{ pg/mL Adrenaline}$$

8.1 Quality control

It is recommended to use controls according to state and federal regulations. The kit, or other commercially available, controls should fall within established confidence limits. The confidence limits of the kit controls are printed on the QC-Report.

Symbols:

	Storage temperature			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!

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