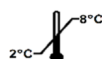


Instructions for use

2-CAT high sensitive ELISA

Please use only the valid version of the Instructions for Use provided with the kit

REF**BA E-5400R**

2 x 96

RUO

For research
use only –
Not for use
in diagnostic
procedures

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Please use only the valid version of the Instructions for Use provided with the kit

Related Products:

- ADRENALINE high sensitive ELISA
- NORADRENALINE high sensitive ELISA
- DOPAMINE high sensitive ELISA
- 3-CAT high sensitive 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 subsequent competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The derivatized standards, controls and samples compete with the solid phase bound analytes 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 resulting in a colour reaction. The reaction is monitored at a wavelength of 450 nm.

Quantification of unknown samples is achieved by comparing their absorbance with a reference 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 must 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) must be followed.
- (3) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective 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. For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water. Avoid repeated freezing and thawing of reagents and specimens.
- (5) 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. Microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up.
- (6) Standards, Controls and specimen samples should be assayed in duplicate.
- (7) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials, and devices are prepared for use at the appropriate time.
- (8) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
- (9) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
- (10) A standard curve must be established for each run.
- (11) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report provided with the kit.
- (12) 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.
- (13) For information about 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.
- (14) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
- (15) 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 kit and reagents at 2 – 8 °C until expiration date. Do not use kit and 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 of the ELISA plate has been opened, care should be taken to close it tightly again including the desiccant.

4. Materials

4.1 Contents 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	
Number:	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, 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	
Description:	Species is goat	
Hazard pictograms:		
	GHS07	
Signal word:	Warning	
Hazardous ingredients:	2-methyl-2H-isothiazol-3-one	
Hazard statements:	H317 May cause an allergic skin reaction.	
Precautionary statements:	P280 Wear protective gloves. P302+P352 IF ON SKIN: Wash with plenty of water. P333+P313 If skin irritation or rash occurs: Get medical advice/attention. P501 Dispose of contents/container to an authorised waste collection point.	
BA E-0055	 SUBSTRATE	Substrate – ready to use
Content:	Chromogenic substrate containing 3,3',5,5'-tetramethylbenzidine, substrate buffer and hydrogen peroxide	
Volume:	1 x 12 ml/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, grey cap	
BA E-0131	 ADR MN	Adrenaline Microtiter Strips – ready to use
Content:	1 x 96 wells (12x8) antigen precoated microwell plate in a resealable blue pouch with desiccant	
BA E-0231	 NAD NMN	Noradrenaline Microtiter Strips – ready to use
Content:	1 x 96 wells (12x8) antigen precoated microwell plate in a resealable yellow pouch with desiccant	
BA E-5110	 ADR-AS	Adrenaline Antiserum – ready to use
Content:	Rabbit anti-adrenaline antibody in buffer with proteins and non-mercury preservative, blue coloured	
Volume:	1 x 6 ml/vial, blue cap	
Description:	Species of antibody is rabbit, species of protein in buffer is bovine	
BA E-5210	 NAD-AS	Noradrenaline Antiserum – ready to use
Content:	Rabbit anti-noradrenaline antibody in buffer with proteins and non-mercury preservative, yellow coloured	
Volume:	1 x 6 ml/vial, yellow cap	
Description:	Species of antibody is rabbit, species of protein in buffer is bovine	

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	
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	COENZYM	Coenzyme – ready to use
Content:	S-adenosyl-L-methionine	
Volume:	1 x 4 ml/vial, purple cap	
BA R-6615	ENZYM	Enzyme – lyophilized
Content:	Catechol-O-methyltransferase	
Volume:	4 vials, pink cap	
Description:	Catechol-O-methyltransferase from pig liver	
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, green cap	

4.2 Calibration and Controls

Standards and Controls – ready to use

Cat. no.	Component	Colour/ Cap	Concentration [ng/ml]		Concentration [nmol/l]		Volume/ Vial
			ADR	NAD	ADR	NAD	
BA R-5601	STANDARD A	white	0	0	0	0	4 ml
BA R-5602	STANDARD B	yellow	0.5	0.2	2.7	1.2	4 ml
BA R-5603	STANDARD C	orange	1.5	0.6	8.2	3.5	4 ml
BA R-5604	STANDARD D	blue	5	2	27	12	4 ml
BA R-5605	STANDARD E	grey	20	8	109	47	4 ml
BA R-5606	STANDARD F	black	80	32	437	189	4 ml
BA R-5651	CONTROL 1	green	Refer to QC-Report for expected value and acceptable range.				4 ml
BA R-5652	CONTROL 2	red					4 ml

Conversion: adrenaline [ng/ml] x 5.46 = adrenaline [nmol/l]

noradrenaline [ng/ml] x 5.91 = noradrenaline [nmol/l]

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

4.3 Additional materials required but not provided in the kit

- Water (deionized, distilled, or ultra-pure)
- Absorbent material (paper towel)

4.4 Additional 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
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Vortex mixer
- Temperature controlled incubator (37 °C) or similar heating device

5. Sample collection, handling 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 all reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Number the Microtiter Plate, Extraction Plate and microwell plates (Microtiter Strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up). Duplicate determinations are recommended. 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. If the product is prepared in parts, unused wells in Extraction Plates should be covered to avoid contamination. After preparation, the used wells must be labelled to prevent double use. During the overnight incubation at 2 – 8 °C with the antiserum, the temperature should be uniform all over the ELISA plate to avoid any drift and edge-effect.

⚠ *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 and further notes

Wash Buffer

Dilute the 20 ml Wash Buffer Concentrate **WASH-CONC 50X** with water to a final volume of 1000 ml.

Storage: 2 months at 2 – 8 °C

Enzyme Solution

Reconstitute the content of the vial **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 **ADJUST-BUFF**. 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 and Noradrenaline 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 **ACYL-REAG** (BA E-6612) has a freezing point of 18.5 °C. To ensure that it 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 2-CAT 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 catecholamine degradation by adding preservatives to the sample (see *Sample collection, handling and storage*).
- Avoid chaotropic chemicals like perchloric acid. The high salt content might reduce the recovery of catecholamines. If your samples already contain high amounts of perchloric acid, neutralize them 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, catecholamines 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 this 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 2-CAT 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	1.2 Sample volume 100 – 500 µl	1.3 Sample volume 500 – 750 µl
	Pipette into the respective wells of the EXTRACT-PLATE 48 : 20 µl standards, 20 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. 20 µl standard plus 80 µl water (deionized, distilled, or ultra-pure)].	Pipette into the respective wells of the EXTRACT-PLATE 48 : 20 µl standards, 20 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. 20 µl standard plus 480 µl water (deionized, distilled, or ultra-pure)].	Pipette into the respective wells of the EXTRACT-PLATE 48 : 20 µl standards, 20 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. 20 µl standard plus 730 µl water (deionized, distilled, or ultra-pure)].
2.	Pipette 25 µl of TE-BUFF into all wells.		
3.	Cover the plate with FOILS . 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 ACYL-BUFF into all wells.		
10.	Pipette 25 µl of ACYL-REAG 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 HCL into all wells.		
18.	Cover plate with FOILS . Shake 10 min at RT (20 – 25 °C) on a 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 96 .		
2.	Add 50 µl of Enzyme Solution (refer to 6.1) to all wells.		
3.	Cover plate with FOILS . 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	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 Microtiter Strips (* ¹).
2.	Pipette 50 µl of the respective Antiserum (* ²) into all wells.
3.	Cover the plate with FOILS . 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 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-SOLN 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).

⚠ (*¹): **Adrenaline Microtiter Strips** **ADR MN**, **Noradrenaline Microtiter Strips** **NAD NMN**

(*²): **Adrenaline Antiserum** **ADR-AS**, **Noradrenaline Antiserum** **NAD-AS**

7. Calculation of results

The standard curve, which can be used to determine the concentration of the unknown samples, is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis) using a concentration of 0.001 ng/ml for Standard A (this alignment is mandatory because of the logarithmic presentation of the data). Use 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.

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

Example

750 µl 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 = $0.15 \text{ ng/ml} \times 0.027 = 0.004 \text{ ng/ml} = 4 \text{ pg/ml}$ noradrenaline

Conversion:

Adrenaline [ng/ml] $\times 5.46 =$ Adrenaline [nmol/l]

Noradrenaline [ng/ml] $\times 5.91 =$ Noradrenaline [nmol/l]

7.1 Quality control

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

8. Assay characteristics

Sensitivity (Limit of Detection)	
Adrenaline	0.25 ng/ml $\times C^*$
Noradrenaline	0.1 ng/ml $\times C^*$

C* = Correction factor (refer to 7.)

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

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

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

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
Noradrenaline	high	1,377.4 ± 483.6	161.2	11.7
	medium	502.6 ± 126.9	42.3	8.4
	low	32.7 ± 15.3	5.1	15.6

Precision				
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
Noradrenaline	high	2,027.8 ± 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 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:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Use-by date		Batch code		
	Consult instructions for use		Content		
	Caution		Catalogue number		Distributor
	Date of manufacture		Do not re-use		For research use only!