

Manufactured for:

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## Instructions for use

# 2-CAT (Research/Norepi-/D) ELISA

Enzyme Immunoassay for the determination of  
Noradrenaline (Norepinephrine) and Dopamine.

A flexible test system for various biological types and volumes.

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

**REF**

**IB89155**



**RUO**

For Research use only-  
Not for use in diagnostic  
procedures

## Noradrenaline - Dopamine Research ELISA

### 1. **Intended use**

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

### 2. **Principle of the test**

Noradrenaline (Norepinephrine) and Dopamine are 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 calibrators, controls and unknowns 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.

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

<b>BA D-0032</b>	 96	<b>Microtiter Plate</b>	1 x 96 wells	12 strips, 8 wells each, break apart
<b>BA D-0090</b>	 FOILS	<b>Adhesive Foil</b>	2 x 4	ready for use
<b>BA E-0030</b>	 WASH-CONC 	<b>Wash Buffer Concentrate</b>	2 x 20 mL	Concentrate. Dilute content with dist. water to a final volume of 1000 mL
<b>BA E-0040</b>	 CONJUGATE	<b>Enzyme Conjugate</b>	2 x 12 mL	ready for use, anti-rabbit IgG conjugated with peroxidase
<b>BA E-0055</b>	 SUBSTRATE	<b>Substrate</b>	2 x 12 mL	ready for use, containing a solution of TMB
<b>BA E-0080</b>	 STOP-SOLN	<b>Stop Solution</b>	2 x 12 mL	ready for use, containing 0.25 M H <sub>2</sub> SO <sub>4</sub>
<b>BA E-0231</b>	  NAD  NMN	<b>Noradrenaline-Normetanephrine Microtiter Strips</b>	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, yellow coloured
<b>BA E-0331</b>	  DOP	<b>Dopamine Microtiter Strips</b>	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, green coloured
<b>BA E-5210</b>	 NAD-AS	<b>Noradrenaline Antiserum</b>	1 x 6 mL	from rabbit, ready for use, yellow coloured, yellow screw cap
<b>BA E-5310</b>	 DOP-AS	<b>Dopamine Antiserum</b>	1 x 6 mL	from rabbit, ready for use, green coloured, green screw cap
<b>BA R-0050</b>	 ADJUST-BUFF	<b>Adjustment Buffer</b>	1 x 4 mL	ready for use
<b>BA R-4617</b>	 TE-BUFF	<b>TE Buffer</b>	1 x 4 mL	ready for use
<b>BA R-5601</b>	 CALIBRATOR  A	<b>Calibrator A</b>	1 x 4 mL	ready for use
<b>BA R-5602</b>	 CALIBRATOR  B	<b>Calibrator B</b>	1 x 4 mL	ready for use
<b>BA R-5603</b>	 CALIBRATOR  C	<b>Calibrator C</b>	1 x 4 mL	ready for use
<b>BA R-5604</b>	 CALIBRATOR  D	<b>Calibrator D</b>	1 x 4 mL	ready for use
<b>BA R-5605</b>	 CALIBRATOR  E	<b>Calibrator E</b>	1 x 4 mL	ready for use
<b>BA R-5606</b>	 CALIBRATOR  F	<b>Calibrator F</b>	1 x 4 mL	ready for use
<b>BA R-5651</b>	 CONTROL  1	<b>Control 1</b>	1 x 4 mL	ready for use
<b>BA R-5652</b>	 CONTROL  2	<b>Control 2</b>	1 x 4 mL	ready for use
<b>BA R-6611</b>	 ACYL-BUFF	<b>Acylation Buffer</b>	1 x 20 mL	ready for use
<b>BA R-6612</b>	 ACYL-REAG	<b>Acylation Reagent</b>	1 x 3 mL	ready for use
<b>BA R-6614</b>	 COENZYME	<b>Coenzyme</b>	1 x 2 mL	ready for use, S-adenosyl-L-methionine
<b>BA R-6615</b>	 ENZYME	<b>Enzyme</b>	4 x 1 mL	lyophilized, contains the enzyme COMT
<b>BA R-6618</b>	 EXTRACT-PLATE 	<b>Extraction Plate</b>	2 x 48 wells	coated with boronate affinity gel
<b>BA R-6619</b>	 HCL	<b>Hydrochloric Acid</b>	1 x 20 mL	ready for use, yellow coloured, contains 0.025 M HCl

Calibrator	Concentration of the Calibrators (ng/mL)					
	A	B	C	D	E	F
Noradrenaline	0	0.2	0.6	2	8	32
Dopamine	0	0.5	1.5	5	20	80

## 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 biologicals:* 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 measurements 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

#### **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 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 unknowns. 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 (please refer to 6. *Collection and Handling of Unknowns*).
- Avoid chaotropic chemicals like perchloric acid. The high salt content might reduce the recovery of Adrenaline and Noradrenaline. 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, Dopamine and Noradrenaline are 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 catecholamines.
- It is advisable to perform a "Proof of Principle" to determine the recovery of the catecholamines in your unknowns. Prepare a stock solution of Dopamine and Noradrenaline. Add small amounts (to change the native matrix of the unknown being tested as less as possible) of the stock solutions to the matrix of the unknown and check the recovery.
- The used volume of unknowns determines the sensitivity of the test. Determine the volume needed to determine the catecholamines in your unknowns by testing different amounts of volume.

*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 the unknowns being tested:

- in case you have unknown volumes between 1 – 100 µL follow **1.1**
- in case you have unknown volumes between 100 – 500 µL follow **1.2**
- in case you have unknown volumes between 500 – 750 µL follow **1.3**




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

1.	<b>1.1</b> Unknown volume 1 – 100 µL	<b>1.2</b> Unknown volume 100 – 500 µL	<b>1.3</b> Unknown volume 500 – 750 µL
	Pipette into the respective wells of the Extraction Plate: <b>20 µL calibrators, 20 µL controls and 1 – 100 µL of the unknown.</b> Fill up each well with distilled water to a <b>final volume</b> of 100 µl (e.g. 20 µl calibrator plus 80 µl dist. water).	Pipette into the respective wells of the Extraction Plate: <b>20 µL calibrators, 20 µL controls and 100 – 500 µL of the unknown.</b> Fill up each well with distilled water to a <b>final volume</b> of 500 µl (e.g. 20 µl calibrator plus 480 µl dist. water).	Pipette into the respective wells of the Extraction Plate: <b>20 µL of calibrators, 20 µL of controls and 500 – 750 µL of unknown.</b> Fill up each well with distilled water to a <b>final volume</b> of 750 µl (e.g. 20 µl calibrator plus 730 µl dist. water).
<b>2.</b>	Pipette <b>25 µL</b> of <b>TE Buffer</b> into all wells		
<b>3.</b>	Cover the plate with adhesive foil. Shake <b>60 min</b> at <b>RT</b> (20-25°C) on a <b>shaker</b> (approx. 600 rpm).		
<b>4.</b>	Remove the foil and empty the plate. Blot dry by tapping the inverted plate on absorbent material.		
<b>5.</b>	Pipette <b>1 mL</b> of <b>Wash Buffer</b> into all wells.		
<b>6.</b>	Shake <b>5 min</b> at <b>RT</b> (20-25°C) on a <b>shaker</b> (approx. 600 rpm).		
<b>7.</b>	Blot dry by tapping the inverted plate on absorbent material.		
<b>8.</b>	<b>Wash one more time</b> as described (step 5, 6 and 7)!		
<b>9.</b>	Pipette <b>150 µL</b> of <b>Acylation Buffer</b> into all wells.		
<b>10.</b>	Pipette <b>25 µL</b> of <b>Acylation Reagent</b> into all wells.		
<b>11.</b>	Shake <b>20 min</b> at <b>RT</b> (20-25°C) on a shaker (approx. 600 rpm).		
<b>12.</b>	Empty the plate and blot dry by tapping the inverted plate on absorbent material.		
<b>13.</b>	Pipette <b>1 mL</b> of <b>Wash Buffer</b> into all wells.		
<b>14.</b>	Shake <b>5 min</b> at <b>RT</b> (20-25°C) on a shaker (approx. 600 rpm).		
<b>15.</b>	Blot dry by tapping the inverted plate on absorbent material.		
<b>16.</b>	<b>Wash one more time</b> as described (step 13, 14, 15).		
<b>17.</b>	Pipette <b>150 µL</b> of <b>Hydrochloric Acid</b> into all wells.		
<b>18.</b>	Cover plate with adhesive foil. Shake <b>10 min</b> at <b>RT</b> (20-25°C) on an o shaker (approx. 600 rpm).		
	<b>Do not decant the supernatant thereafter!</b>		
	<b>140 µL of the supernatant is needed for the subsequent enzymatic conversion</b>		

### 7.4 Enzymatic conversion

<b>1.</b>	Pipette <b>140 µL</b> of the <b>extracted calibrators, controls</b> and <b>unknowns</b> into the respective wells of the <b>Microtiter Plate</b> .		
<b>2.</b>	Add <b>50 µL</b> of <b>Enzyme Solution</b> (refer to 7.1) to all wells.		
<b>3.</b>	Cover plate with <b>Adhesive Foil</b> . Shake <b>1 min</b> at <b>RT (20-25°C)</b> on a shaker.		
<b>4.</b>	Incubate for <b>2 hours</b> at <b>37°C</b> . The following volumes of the supernatants are needed for the subsequent ELISA:		
	<table style="width: 100%; border: none;"> <tr> <td style="border: 1px solid black; padding: 2px;"><b>Dopamine</b>     <b>90 µL</b></td> <td style="border: 1px solid black; padding: 2px;"><b>Noradrenaline</b>     <b>90 µL</b></td> </tr> </table>	<b>Dopamine</b> <b>90 µL</b>	<b>Noradrenaline</b> <b>90 µL</b>
<b>Dopamine</b> <b>90 µL</b>	<b>Noradrenaline</b> <b>90 µL</b>		

## 7.5 Dopamine and Noradrenaline ELISA

1.	Pipette <b>90 µL</b> of <b>calibrators, controls</b> and <b>unknowns</b> from the <b>Enzyme Plate</b> into the respective pre-coated <b>Microtiter Strips ( *<sup>1</sup> )</b> .
2.	Pipette <b>50 µL</b> of the respective <b>Antiserum ( *<sup>2</sup> )</b> into all wells.
3.	Cover the plate with <b>Adhesive Foil</b> . Incubate for <b>1 min</b> at <b>RT</b> (20-25°C) on a <b>shaker</b> .
4.	Incubate for <b>15 – 20 hours</b> (overnight) at <b>2 – 8 °C</b> .
5.	Remove the foil and discard or aspirate the contents of the wells and <b>wash</b> each well <b>4 times</b> thoroughly with 300 µL <b>Wash Buffer</b> . Blot dry by tapping the inverted plate on absorbent material.
6.	Pipette <b>100 µL</b> of <b>Enzyme Conjugate</b> into all wells.
7.	Cover the plate with <b>Adhesive Foil</b> and incubate <b>30 min</b> at <b>RT</b> (20-25°C) on a <b>shaker</b> (approx. 600 rpm).
8.	Remove the foil and discard or aspirate the contents of the wells and <b>wash</b> each well <b>4 times</b> thoroughly with 300 µL <b>Wash Buffer</b> . Blot dry by tapping the inverted plate on absorbent material.
9.	Pipette <b>100 µL</b> of <b>Substrate</b> into all wells.
10.	Incubate <b>20-30 min</b> at <b>RT</b> (20-25°C) on a <b>shaker</b> (approx. 600 rpm).  <b>Avoid exposure to direct sun light!</b>
11.	Pipette <b>100 µL</b> of <b>Stop Solution</b> into all wells.
12.	<b>Read</b> the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to <b>450 nm</b> and a reference wavelength between 620 nm and 650 nm.

 (\*<sup>1</sup>): **Dopamine Microtiter Strips, Noradrenaline Microtiter Strips**  
(\*<sup>2</sup>): **Dopamine Antiserum, Noradrenaline Antiserum**

## 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 calibrator 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.**

$$\text{Correction factor} = \frac{20 \mu\text{L (volume of calibrators extracted)}}{\text{volume of unknown } (\mu\text{L}) \text{ extracted}}$$

**Example:** 750µL of the unknown is extracted and the concentration taken from the calibration curve is 0.15 ng/mL Noradrenaline.






Correction factor = 20/750 = 0.027

Concentration of the unknown = 0.15 ng/mL x 0.027 = 0.004 ng/mL = 4 pg/mL Noradrenaline

### 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	<b>LOT</b>	Batch code	<b>IVD</b>	For in-vitro diagnostic use only!
	Consult instructions for use	<b>CONT</b>	Content	<b>CE</b>	CE labelled
	Caution	<b>REF</b>	Catalogue number	<b>RUO</b>	For research use only!

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