

Instructions for Use

Dopamine Research ELISA

RUO

REF EIA-4824



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***Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.
Utilisez seulement la version valide des Instructions d'utilisation fournies avec le kit.***

Table of Contents

1	INTENDED USE AND PRINCIPLE OF THE TEST	2
2	PROCEDURAL CAUTIONS, GUIDELINES AND WARNINGS	2
3	STORAGE AND STABILITY	2
4	MATERIALS.....	3
5	SAMPLE COLLECTION AND STORAGE.....	5
6	TEST PROCEDURE	5
7	CALCULATION OF RESULTS.....	7
8	ASSAY CHARACTERISTICS.....	8
SYMBOLS USED.....		9

1 INTENDED USE AND PRINCIPLE OF THE TEST

Enzyme Immunoassay for the quantitative determination of dopamine. Flexible test system for various biological sample types and volumes.

Dopamine 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 °C to 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 H₂SO₄. 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 °C - 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 °C - 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

	96	Microtiter Plate - Ready to use
Content:	1 x 96 wells, empty in a resealable pouch	
	FOILS	Adhesive Foil - Ready to use
Content:	Adhesive Foils in a resealable pouch	
Volume:	1 x 4 foils	
	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	
	CONJUGATE	Enzyme Conjugate - Ready to use
Content:	Goat anti-rabbit immunoglobulins, conjugated with peroxidase	
Volume:	1 x 12 mL/vial, red cap	
	SUBSTRATE	Substrate - Ready to use
Content:	Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide	
Volume:	1 x 12 mL/black vial, black cap	
	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.
	DOP	Dopamine Microtiter Strips - Ready to use
Content:	1 x 96 well (12x8) antigen precoated microwell plate in a resealable green pouch with desiccant	
	DOP-AS	Dopamine Antiserum - Ready to use
Content:	Rabbit anti-dopamine antibody, green coloured	
Volume:	1 x 6 mL/vial, dark green cap	
	ACYL-REAG	Acylation Reagent – Ready to use
Content:	Acylation reagent in DMSO	
Volume:	1x3 mL/vial, white cap	
	ADJUST-BUFF	Adjustment Buffer - Ready to use
Content:	TRIS buffer	
Volume:	1 x 4 mL/vial, green cap	
	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

Component	Colour/ Cap	Concentration ng/mL	Concentration nmol/L	Volume/ Vial
		DOP	DOP	
STANDARD A	white	0	0	4 mL
STANDARD B	light yellow	0.5	3.3	4 mL
STANDARD C	orange	1.5	9.8	4 mL
STANDARD D	dark blue	5	33	4 mL
STANDARD E	light grey	20	131	4 mL
STANDARD F	black	80	522	4 mL
CONTROL 1	light green	Refer to QC-Report for expected value and acceptable range!		4 mL
CONTROL 2	dark red			4 mL

Conversion: Dopamine (ng/mL) x 6.53 = Dopamine (nmol/L)

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

ACYL-BUFF **Acylation Buffer** - Ready to use

Content: Buffer with light alkaline pH for the acylation

Volume: 1 x 20 mL/vial, white cap

COENZYME **Coenzyme**- Ready to use

Content: S-adenosyl-L-methionine

Volume: 1 x 4 mL/vial, purple cap

ENZYME **Enzyme** - Lyophilized

Content: Catechol-O-methyltransferase

Volume: 4 vials, pink cap

EXTRACT-PLATE 48 **Extraction Plate** - Ready to use

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

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 °C - 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. 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 °C - 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 °C - 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!*

Dopamine 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 Dopamine Research 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 dopamine 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 dopamine. 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, dopamine 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 dopamine.
- It is advisable to perform a "Proof of Principle" to determine the recovery of dopamine in your samples. Prepare a stock solution of dopamine. 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 dopamine 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 or your local distributor directly!

6.3 Extraction and acylation

The Dopamine Research 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
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 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)]. | 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)]. | Pipette into the respective wells of the Extraction Plate:

10 µL of 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)]. |
- Pipette **25 µL** of **TE Buffer** into all wells
 - Cover the plate with **Adhesive Foil**. Shake **60 min** at **RT** (20 °C - 25 °C) on a **shaker** (approx. 600 rpm).
 - Remove the foil and empty the plate. Blot dry by tapping the inverted plate on absorbent material.
 - Pipette **1 mL** of **Wash Buffer** into all wells.
 - Shake **5 min** at **RT** (20 °C - 25 °C) on a **shaker** (approx. 600 rpm).
 - Blot dry by tapping the inverted plate on absorbent material.
 - Wash one more time** as described (step 5, 6 and 7)!
 - Pipette **150 µL** of **Acylation Buffer** into all wells.
 - Pipette **25 µL** of **Acylation Reagent** into all wells.
 - Shake **20 min** at **RT** (20 °C - 25 °C) on a **shaker** (approx. 600 rpm).
 - Empty the plate and blot dry by tapping the inverted plate on absorbent material.
 - Pipette **1 mL** of **Wash Buffer** into all wells.
 - Shake **5 min** at **RT** (20 °C - 25 °C) on a **shaker** (approx. 600 rpm).
 - Blot dry by tapping the inverted plate on absorbent material.
 - Wash one more time** as described (step 13, 14, 15).
 - Pipette **100 µL** of **Hydrochloric Acid** into all wells.
 - Cover plate with **Adhesive Foil**. Shake **10 min** at **RT** (20 °C - 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 °C - 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:

Dopamine	100 µL
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6.5 Dopamine ELISA

1. Pipette **100 µL** of **standards, controls** and **samples** from the **Enzyme Plate** (refer to 6.4) into the respective pre-coated **Dopamine Microtiter Strips**.
2. Pipette **50 µL** of the respective **Dopamine Antiserum** into all wells.
3. Cover the plate with **Adhesive Foil**. Shake **1 min** at **RT** (20 °C - 25 °C) on a **shaker** (approx. 600 rpm).
4. Incubate for **15 – 20 h** (overnight) at **2 °C - 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 °C - 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 °C - 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 in 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, marguardt).

⚠ *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.**

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

Example

750 µL of the sample is extracted and the concentration taken from the standard curve is 0.45 ng/mL dopamine.

Correction factor = $10/750 = 0.013$

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

Conversion

Dopamine (ng/mL) x 6.53 = Dopamine (nmol/L)

7.1 Quality control

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

8 ASSAY CHARACTERISTICS

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)
		Dopamine
	Derivatized Adrenaline	0.03
	Derivatized Noradrenaline	0.87
	Derivatized Dopamine	100
	Metanephrine	< 0.007
	Normetanephrine	0.008
	3-Methoxytyramine	0.55
	3-Methoxy-4-hydroxyphenylglycol	< 0.007
	Tyramine	0.13
	Phenylalanine, Caffeinic acid, L-Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid	< 0.007

Sensitivity (Limit of Detection)	Dopamine
	0.25 ng/mL x C*

C* = Correction factor (refer to 7.)

Analytical Sensitivity (750 µL undiluted sample)	Dopamine
	3.3 pg/mL

Functional Sensitivity (750 µL undiluted sample)	Dopamine
	5 pg/mL

Precision				
Intra-Assay Human EDTA-Plasma				
	Sample	Mean ± 3 SD (pg/mL)	SD (pg/mL)	CV (%)
Dopamine	high	1,438.6 ± 465.6	155.2	10.8
	medium	565.9 ± 246.3	82.1	14.5
	low	56.4 ± 36.3	12.1	21.5
















Intra-Assay Cell Culture Medium (RPMI)				
	Sample	Mean ± 3 SD (pg/mL)	SD (pg/mL)	CV (%)
Dopamine	high	2,784.5 ± 1,238.7	412.9	14.8
	medium	1,003.7 ± 526.2	175.4	17.5
	low	74.7 ± 51.6	17.2	23.0

Recovery	Mean (%)	Range (%)	SD (%)	CV (%)
Dopamine				
Human EDTA-Plasma	97.7	83.7 – 115.9	11.8	12.1
Cell Culture Medium	98.6	77.7 – 113.4	12.1	12.2

⚠ 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 USED

Symbol	English	Deutsch	Italiano	Español	Français	Português
	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes	Conformidade Europeia
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation	Consultar as instruções de uso
	<i>In vitro</i> diagnostic medical device *	<i>In-vitro</i> -Dagnostikum *	Diagnostica in vitro	Diagnóstico in vitro	Diagnostic in vitro	Dispositivo médico para diagnóstico in vitro
	Catalogue number *	Katalognummer *	No. di Cat.	No de catálogo	Référence	Número de catálogo
	Batch code *	Chargen-bezeichnung *	Lotto no	Número de lote	No. de lot	Código do lote
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests	Suficiente para <n> determinações
	Temperature limit *	Temperaturgrenzwerte *	Temperatura di conservazione	Temperatura de conservación	Temperature de conservation	Limites de temperatura
	Use-by date *	Verwendbar bis *	Data di scadenza	Fecha de caducidad	Date limite d'utilisation	Prazo de validade
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant	Fabricante
	Distributor *	Vertriebspartner *	Distributore	Distribuidor	Distributeur	Distribuidor
	Date of manufacture *	Herstellungsdatum *	Data di produzione	Fecha de fabricación	Date de production	Data de fabricação
	Biological risks *	Biologische Risiken *	Rischi biologici	Riesgos biológicos	Risques biologiques	Riscos biológicos
	Caution *	Achtung *	Attenzione	Precaución	Attention	Cuidado
	Unique device Identifier *	eindeutige Produktidentifizierung *	Identificativo unico del dispositivo*	Identificación exclusiva del dispositivo *	Identifiant de dispositif unique*	Identificador único do dispositivo *
	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches	
<i>Content</i>	Content	Inhalt	Contenuto	Contenido	Conditionnement	Conteúdo
<i>Volume/No.</i>	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité	Volume / Quantidade