DRG® Dopamine Research ELISA (EIA-4824)

Revised 4 Mar. 2011 rm (Vers. 3.1) **RUO**

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

INTENDED USE AND PRINCIPLE OF THE TEST

Enzyme Immunoassay for 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 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 standards, controls and samples 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.

Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations.

ADVICE ON HANDLING THE TEST

1.1 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 norm 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.

The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.

1.2 Complaints

In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

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1.3 Warranty

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

1.4 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 upon request. The safety data sheets correspond to the standard: ISO 11014-1.

1.5 Interference

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples 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.

1.6 Precautions

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. 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 testkit 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.

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.

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CONTENTS OF THE KIT

96	**Microtiter Plate**	1 x 96 wells	12 strips, 8 wells each, break apart
FOILS	**Adhesive Foil**	1 x 4	ready for use
WASH-CONC 50x	**Wash Buffer Concentrate**	1 x 20 mL	Concentrate. Dilute content with dist. water to a final volume of 1000 mL
CONJUGATE	**Enzyme Conjugate**	1 x 12 mL	ready for use, anti-rabbit IgG conjugated with peroxidase
SUBSTRATE	**Substrate**	1 x 12 mL	ready for use, containing a solution of TMB
STOP-SOLN	**Stop Solution**	1 x 12 mL	ready for use, containing 0.25 M H_2SO_4
96 DOP	**Dopamine Microtiter Strips**	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, green coloured
DOP-AS	**Dopamine Antiserum**	1 x 6 mL	from rabbit, ready for use, green coloured, green screw cap
STANDARD A	**Standard A**	1 x 4 mL	ready for use
STANDARD B	**Standard B**	1 x 4 mL	ready for use
STANDARD C	**Standard C**	1 x 4 mL	ready for use
STANDARD D	**Standard D**	1 x 4 mL	ready for use
STANDARD E	**Standard E**	1 x 4 mL	ready for use
STANDARD F	**Standard F**	1 x 4 mL	ready for use
HCL	**Hydrochloric Acid**	1 x 20 mL	ready for use, yellow coloured, contains 0.025 M HCl
CONTROL 1	**Control 1**	1 x 4 mL	ready for use
CONTROL 2	**Control 2**	1 x 4 mL	ready for use
ACYL-CONC	**Acylation Concentrate**	1 x 0.5 mL	Concentrate. Has to be diluted prior to use.
ADJUST-BUFF	**Adjustment Buffer**	1 x 4 mL	ready for use
ACYL-DILUENT	**Acylation Diluent**	1 x 4 mL	ready for use
ACYL-BUFF	**Acylation Buffer**	1 x 20 mL	ready for use
ASSAY-BUFF	**Assay Buffer**	1 x 6 mL	ready for use, contains 1 M HCl
COENZYME	**Coenzyme**	1 x 2 mL	ready for use, S-adenosyl-L-methionine
ENZYME	**Enzyme**	4 x 1 mL	lyophilized, contains COMT
EXTRACT-BUFF	**Extraction Buffer**	1 x 6 mL	ready for use
EXTRACT-PLATE 48	**Extraction Plate**	2 x 48 wells	coated with boronate affinity gel

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1.7 Additional materials and equipment required but not provided with the kit

- Calibrated variable precision micropipettes (e.g. 1-10 µL / 10-100 µL / 100-1,000 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

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 1mM) and sodium metabisulfite (final concentration 4 mM) to the sample.

TEST PROCEDURE

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

1.8 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 Diluent

The Acylation Diluent has a freezing point of 18.5°C. To ensure that the Acylation Diluent is liquid when being used, it must be ensured that the Acylation Diluent has reached room temperature and forms a homogeneous, crystal-free solution before being used. Alternative the Acylation Diluent can be stored at room temperature (20 – 25°C) separate from the other kit components.

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!

1.9 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 catecholamine degradation by adding preservatives to the sample (see 5. 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 the catecholamines 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!

1.10 Extraction and acylation

The 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	**1.2**	**1.3**
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 of the sample**. Fill up each well with distilled water to a **final volume** of 100 µL (e.g. 10 µL standard plus 90 µL dist. water).	**Sample volume 100 – 500 µL** Pipette into the respective wells of the Extraction Plate: **10 µL standards,** **10 µL controls and** **100 – 500 µL of the sample**. Fill up each well with distilled water to a **final volume** of 500 µL (e.g. 10 µL standard plus 490 µL dist. water).	**Sample volume 500 – 750 µL** Pipette into the respective wells of the Extraction Plate: **10 µL of Standards,** **10 µL of controls and** **500 – 750 µL of sample**. Fill up each well with distilled water to a **final volume** of 750 µL (e.g. 10 µL standard 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 6.1*)* 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

1.11 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 to mix.

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

Dopamine	**100 µL**

1.12 Dopamine ELISA

1. Pipette **100 µL** of **standards, controls** and **samples** from the **Microtiter 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**. 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.

CALCULATION OF RESULTS

The calibration 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).

The use of a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima) is recommended.

The standards refer to:

	CONCENTRATION OF THE STANDARDS (NG/ML)					
STANDARD	**A**	**B**	**C**	**D**	**E**	**F**
DOPAMINE	0	0.9	3	9	30	90

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 0.9 ng/mL Dopamine.

Correction factor = 10/750 = 0.013

Concentration of the sample = 0.9 ng/mL x 0.013 = 0.01 ng/mL = 10 pg/mL Dopamine.

1.13 Quality control

It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC Report.

1.14 Calibration

The binding of the antisera and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the extinction values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. The extinction values also depend on the incubation times. 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

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