



Dopamine ELISA Kit

Catalog Number KA3838

96 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

Enzyme Immunoassay for the quantitative determination of Dopamine.

Principle of the Assay

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.

General Information

Materials Supplied

List of component

Component	Detail	Amount
Microtiter Plate	12 strips, 8 wells each, break apart	1 x 96 wells
Adhesive Foil	ready for use	1 x 4
Wash Buffer Concentrate	Concentrate. Dilute content with dist. water to a final volume of 1000 mL	1 x 20 mL
Enzyme Conjugate	ready for use, anti-rabbit IgG conjugated with peroxidase	1 x 12 mL
Substrate	ready for use, containing a solution of TMB	1 x 12 mL
Stop Solution	ready for use, containing 0.25 M H ₂ SO ₄	1 x 12 mL
Dopamine Microtiter Strips	12 strips, 8 wells each, break apart, pre-coated, green coloured	1 x 96 wells
Dopamine Antiserum	from rabbit, ready for use, green coloured, green screw cap	1 x 6 mL
Adjustment Buffer	ready for use	1 x 4 mL
TE Buffer	ready for use	1 x 4 mL
Standard A	ready for use	1 x 4 mL
Standard B	ready for use	1 x 4 mL
Standard C	ready for use	1 x 4 mL
Standard D	ready for use	1 x 4 mL
Standard E	ready for use	1 x 4 mL
Standard F	ready for use	1 x 4 mL
Control 1	ready for use	1 x 4 mL
Control 2	ready for use	1 x 4 mL
Acylation Buffer	ready for use	1 x 20 mL
Acylation Reagent	ready for use	1 x 3 mL
Coenzyme	ready for use, S-adenosyl-L-methionine	1 x 4 mL
Enzyme	lyophilized, contains the enzyme COMT	4 x 1 mL
Extraction Plate	coated with boronate affinity gel	2 x 48 wells
Hydrochloric Acid	ready for use, yellow coloured, contains 0.025 M HCl	1 x 20 mL

Storage Instruction

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.

Materials Required but Not Supplied

- ✓ 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 3 mm; approx. 600 rpm)
- ✓ Absorbent material (paper towel)
- ✓ Distilled water
- ✓ Vortex mixer

Precautions for Use

- ✓ 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.

- ✓ 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.

- ✓ 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.

- ✓ 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.

✓ **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.

✓ **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 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.

Assay Protocol

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

Reagent Preparation

- ✓ 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.

Note: The Enzyme Solution has to be prepared freshly prior to the assay (not longer than 10-15 minutes in advance). Discard after use!

Sample Preparation

- ✓ 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 1 mM) and sodium metabisulfite (final concentration 4 mM) to the sample.
- ✓ Sample preparation
The Dopamine 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 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.

Assay Procedure

✓ Extraction and acylation

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

- ✓ in case you have sample volumes between 1 – 100 μ L follow 1.1
- ✓ in case you have sample volumes between 100 – 500 μ L follow 1.2
- ✓ in case you have sample volumes between 500 – 750 μ L follow 1.3

Note: Within a run it is only possible to measure samples with the same volume!

1.1	1.2	1.3
<p>Sample volume 1 – 100 μL</p> <p>Pipette into the respective wells of the Extraction Plate:</p> <p>10 μL standards, 10 μL controls and 1 – 100 μL of the sample.</p> <p>Fill up each well with distilled water to a final volume of 100 μL (e.g. 10 μL standard plus 90 μL dist. water).</p>	<p>Sample volume 100 – 500 μL</p> <p>Pipette into the respective wells of the Extraction Plate:</p> <p>10 μL standards, 10 μL controls and 100 – 500 μL of the sample.</p> <p>Fill up each well with distilled water to a final volume of 500 μL (e.g. 10 μL standard plus 490 μL dist. water).</p>	<p>Sample volume 500 – 750 μL</p> <p>Pipette into the respective wells of the Extraction Plate:</p> <p>10 μL of Standards, 10 μL of controls and 500 – 750 μL of sample.</p> <p>Fill up each well with distilled water to a final volume of 750 μL (e.g. 10 μL standard plus 740 μL dist. water).</p>

2. Pipette 25 μ L of TE Buffer into all wells
3. Cover the plate with adhesive foil. 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 μ L of Acylation Buffer into all wells.
10. Pipette 25 μ L of Acylation Reagent 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 100 µL of Hydrochloric Acid into all wells.
18. Cover plate with adhesive foil. Shake 10 min at RT (20-25°C) on an o shaker (approx. 600 rpm).

Note: Do not decant the supernatant thereafter!

90 µL of the supernatant is needed for the subsequent enzymatic conversion

✓ 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 Reagent Preparation) 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

✓ Dopamine ELISA

1. Pipette 100 µL of standards, controls and samples from the Microtiter Plate (refer to Enzymatic Conversion) 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.

Data Analysis

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 standard refer to:

	Concentration of the standards (ng/mL)					
Standard	A	B	C	D	E	F
Dopamine	0	0.5	1.5	5	20	80

Note: 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 = $0.45 \text{ ng/mL} \times 0.013 = 0.006 \text{ ng/mL} = 6 \text{ pg/mL Dopamine}$

✓ 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.

✓ 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.

Note: In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm

Performance 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 Calculation of Results)

✓ 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				
Dopamine	Sample	Mean ± 3 SD (pg/mL)	SD (pg/mL)	CV (%)
	high	1438.6 ± 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)				
Dopamine	Sample	Mean ± 3 SD (pg/mL)	SD (pg/mL)	CV (%)
	high	2784.5 ± 1238.7	412.9	14.8
	medium	1003.7 ± 526.2	175.4	17.5
	low	74.7 ± 51.6	17.2	23.0

✓ Recovery

	Mean (%)	Range (%)	SD (og/mL)	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

Resources

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H