Dopamine ELISA Assay Kit

Catalog Number:
DOP31-K01

For Research Use Only. Not for use in diagnostic procedures.
v. 1.0
INTENDED USE

The Eagle Biosciences Dopamine ELISA Assay Kit is intended for the quantitative determination of Dopamine in urine or plasma. The Dopamine ELISA Assay Kit is for research use only and not to be used in clinical, therapeutic or diagnostic procedures.

INTRODUCTION

Catecholamine is the name of a group of aromatic amines (noradrenaline, adrenaline, dopamine, and their derivatives) which act as hormones and neurotransmitter, respectively. Adrenaline and noradrenaline are formed from dopamine. They act on the cardiac musculature and the metabolism (adrenaline) as well as on the peripheral circulation (noradrenaline) and help the body to cope with acute and chronic stress.

An increased production of catecholamines can be found with tumours of the chromaffine system (pheochromocytoma, neuroblastoma, ganglio-neuroma). An increased or decreased concentration of the catechol-amines can also be found with hypertension, degenerative cardiac diseases, schizophrenia and manic-depressive psychosis. The measurement of dopamine and its derivatives is of special diagnostic value with children who are suspected to have a neuroblastoma.

PRINCIPLE OF THE ASSAY

The assay kit provides materials for the quantitative measurement of dopamine in plasma and urine. Dopamine is extracted using a cis-diol-specific affinity gel and acylated to N-acyl-dopamine and then converted enzymatically into N-acyl-3-methoxytyramine.

The competitive Dopamine ELISA kit uses the microtitre plate format. Dopamine is bound to the solid phase of the microtiter plate. Acylated dopamine from the sample and solid phase bound dopamine compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase dopamine is detected by anti-rabbit IgG / peroxidase. The substrate TMB / peroxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase dopamine is inversely proportional to the dopamine concentration of the sample.

PRECAUTIONS

- For research use only
- Some reagents contain sodium azide as preservative. Avoid skin contact.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.
- Some components of this kit are containing hazardous reagents.

STORAGE AND STABILITY

On arrival, store the kit at 2-8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.

Do not use components beyond the expiration date shown on the labels.

Do not mix various lots of any kit component within an individual assay.

CONTENTS OF THE KIT

Reagents for Sample Preparation

<table>
<thead>
<tr>
<th>4.1 Extraction Plate</th>
<th>2 plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>48 wells</td>
<td></td>
</tr>
<tr>
<td>Coated with boronate affinity gel</td>
<td></td>
</tr>
</tbody>
</table>
4.2 Extraction Buffer  1 vial  
6 ml, ready for use  
Colour coded purple

4.3 HCl  21 ml, ready for use  
0.025 M HCl  
Colour coded yellow orange

4.4 Standards (1-7) 7 vials  
Each 4 ml, ready for use  
Concentrations: 

<table>
<thead>
<tr>
<th>Standard</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine (ng/ml)</td>
<td>0</td>
<td>1.5</td>
<td>10</td>
<td>40</td>
<td>160</td>
<td>640</td>
<td>2,560</td>
</tr>
<tr>
<td>Dopamine (nmol/l)</td>
<td>0</td>
<td>9.8</td>
<td>65.3</td>
<td>261</td>
<td>1,045</td>
<td>4,179</td>
<td>16,717</td>
</tr>
</tbody>
</table>

4.5 Control 1&2  2 vials  
Each 4 ml, ready for use  
Concentrations: see q.c. certificate

4.6 Acylation Reagent  1 vial  
6 ml, ready for use, contains DMSO and DMF  
(please note that solvent reacts with many plastic materials including plastic trays; it does not react with normal pipette tips and with glass devices).

4.7 Acylation Buffer  1 vial  
20 ml, ready for use  
Colour coded purple

4.8 Enzyme  3 vials  
Each 1.7 ml, lyophilized  
Catechol-O-methyltransferase

4.9 Coenzyme  1 vial  
1 ml, ready for use  
S-adenosyl-L-methionine

4.10 Enzyme Buffer  1 vial  
3.5 ml, ready for use

**Reagents for ELISA:**

4.11 Dopamine Antiserum  1 vial  
5.5 ml, ready for use, rabbit  
Colour coded green

4.12 MT-Strips  3x12 strips  
8 wells each, break apart, precoated with:  
Derivatized dopamine (12 strips), colour coded green
4.13  POD Conjugate  1 vial
       Each 12 ml, ready for use,
       Anti-rabbit IgG-POD conjugate/peroxidase

4.14  Wash Buffer     2 vials
       20 ml, concentrate
       Dilute content with dist. water to 500 ml total wash volume

4.15  Substrate      1 vial
       12 ml TMB solution, ready for use

4.16  Stop Solution   1 vial
       12 ml, ready for use
       Contains 0.3 M sulphuric acid

4.17  Adhesive Foil  10 pieces
       Ready for use

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED
- Pipettes 15, 20, 50, 120, 300, 700 μl
- Repeating dispenser for 10, 20, 50, 100, 150, 200, 250 μl und 1 ml
- Horizontal shaker
- Microplate washing device
- Microplate photometer
- Distilled water

SAMPLE COLLECTION

Plasma
EDTA plasma samples are required for the assay. Physical and psychical stress usually causes a
high increase of the catecholamine concentration. Therefore, it is recommended to let the
patient rest for 20 to 30 minutes after the venipuncture and before collecting the blood sample.
Haemolytic and especially lipemic samples should not be used for the assay, because false low
values will be obtained with such samples.
The plasma samples can be stored at 2 - 8 °C up to 6 hours. For a longer period (up to 1 week)
the samples should be stored at -20 °C.

Urine
The total volume of urine excreted during a 24-hours period should be collected and mixed in a
single bottle containing 10 - 15 ml of 6 M hydro-chloric acid as preservative. Avoid exposure to
direct sun light. Determine the total volume and take an aliquot for the measurement. For
patients with suspected kidney disorders the creatinine concentration should be tested, too.
Urine samples can be stored at -20 °C for at least 6 months.
PREPARATION OF REAGENTS AND SAMPLES

Preparation of Reagents

Wash Buffer
Dilute the content of the bottle with distilled water to a total volume of 500 ml. For further use the diluted wash buffer must be stored at 2 - 8 °C for a maximum period of 4 weeks.

Enzyme Mix
NOTE: The enzyme mix has to be prepared freshly prior to the assay (not longer than 10 - 15 minutes in advance). After use the reagent has to be discarded. Reconstitute the content of one vial labelled ENZYME with 1.7 ml distilled water. Add 0.3 ml COENZYME and 0.7 ml ENZYME-BUFF (total volume: 2.7 ml) and mix thoroughly. The two additional bottles of ENZYME are allowing a second and a third run of the test. If the whole kit is to be used in one run it is sufficient to prepare one bottle of enzyme mix.

Preparation of Samples
Allow reagents and samples to reach room temperature. Determinations in duplicates are recommended. Each 20 μl of Standards, Control 1 & 2 and urine samples are extracted. Each 300 μl of plasma samples are extracted.

1. Pipette each 20 μl Standard 1 - 7, 20 μl Control 1 & 2 and each 20 μl Urine Sample into the respective wells of the extraction plate. Add 250 μl of distilled water to these wells to correct for volume.
   Pipette each 300 μl Plasma Sample into the respective wells (no volume correction required).
2. Pipette each 50 μl Extraction Buffer into all wells.
3. Incubate 60 minutes at room temperature on an orbital shaker (400 - 600 r/min).
4. Decant the plate and remove residual liquid by tapping the inverted plate on a paper towel.
5. Pipette each 1 ml Wash Buffer into all wells and incubate for 5 minutes at room temperature on an orbital shaker (slow shaking).
6. Decant the plate and remove residual liquid by tapping the inverted plate on a paper towel.
7. Pipette each 150 μl Acylation Buffer into all wells.
8. Pipette each 50 μl Acylation Reagent into all wells and continue with step 9. immediately. (please note that solvent reacts with many plastic materials including plastic trays; it does not react with normal pipette tips and with glass devices)
9. Incubate the plate for 20 minutes at room temperature on an orbital shaker (400 - 600 r/min).
10. Decant the plate and remove residual liquid by tapping the inverted plate on a paper towel.
11. Pipette each 1 ml Wash Buffer into all wells and incubate for 5 minutes at room temperature on an orbital shaker (slow shaking).
12. Decant the plate and remove residual liquid by tapping the inverted plate on a paper towel.
13. Repeat the wash steps 11. and 12.
14. Pipette each 200 μl HCl (0.025 M) into all wells.
15. Incubate the plate with adhesive foil for 20 minutes at room temperature on an orbital shaker (400 - 600 r/min).

Caution: Do not decant the supernatant thereafter

Take each 50 μl of the supernatant for the dopamine assay
TEST PROCEDURE ELISA
Allow reagents to reach room temperature. Duplicates are recommended.

1. Pipette each 10 μl of freshly prepared Enzyme Mix into all wells (colour code green).
2. Pipette each 50 μl prepared Standards, Controls and Patient Samples into the respective wells (colour coded green).
3. Incubate the plate with adhesive foil for 30 minutes at room temperature (20 – 25 ºC) on an orbital shaker (400 - 600 r/min).
4. Pipette each 50 μl Dopamine-Antiserum (colour coded green) into all wells.
5. Cover the plate with adhesive foil, shake for 10 seconds and incubate for 12 – 20 hours (overnight) at 2-8 ºC.
6. Discard or aspirate the contents of the wells and wash thoroughly with each 250 μl Wash Buffer. Remove residual liquid by tapping the inverted plate on clean absorbent paper. Repeat the washing procedure 4 times.
7. Pipette each 100 μl POD-Conjugate into all wells.
8. Incubate for 30 minutes at room temperature on an orbital shaker (400 - 600 r/min).
10. Pipette each 100 μl Substrate into all wells.
11. Incubate 25 to 35 minutes at room temperature (20 – 25 ºC) on an orbital shaker (400 - 600 r/min).
12. Pipette 100 μl Stop Solution into all wells.
13. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

CALCULATION OF THE RESULTS
On a semilogarithmic graph paper the concentration of the standards (x-axis, logarithmic) are plotted against their corresponding optical density (y-axis, linear). Alternatively, the optical density of each standard and sample can be related to the optical density of the zero standard, expressed as the ratio OD/ODmax, and then plotted on the y-axis. A good fit is provided with 4 Parameter Logistic (alternatively Log-Logit or Cubic Spline).
The concentration of the controls and urine samples can be read off the standard curve directly without any further conversion.
The read concentrations of dopamine in plasma samples have to be divided by 15 due to the use of 300 μl plasma sample in relation to 20 μl standard.

Typical standard curve:
ASSAY CHARACTERISTICS

**Reference Ranges**
The reference ranges given below should only be taken as a guideline. It is recommended that each laboratory should establish its own normal values.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Reference Ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>&lt;600 μg/day</td>
</tr>
<tr>
<td>Plasma</td>
<td>&lt;100 pg/ml</td>
</tr>
</tbody>
</table>

**Sensitivity**
The lower limit of detection was determined by taking the 2fold standard deviation of the absorbance of the Zero Reference and reading the corresponding value from the standard curve.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Sensitivity (Urine)</th>
<th>Sensitivity (Plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine</td>
<td>0.44 ng/ml</td>
<td>29 pg/ml</td>
</tr>
</tbody>
</table>

**Specificity (Cross Reactivity)**
Structural related components were tested for possible interference with the antiserum against dopamine used in the ELISA method.

<table>
<thead>
<tr>
<th>Components</th>
<th>Cross Reactivity (%) Dopamine-Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenaline</td>
<td>&lt;0.020</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>0.23</td>
</tr>
<tr>
<td>Dopamine</td>
<td>100</td>
</tr>
<tr>
<td>Metanephrine</td>
<td>&lt;0.020</td>
</tr>
<tr>
<td>Normetanephrine</td>
<td>&lt;0.020</td>
</tr>
<tr>
<td>3-Methoxytyramine</td>
<td>0.28</td>
</tr>
<tr>
<td>L-Dopa</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Tyramine</td>
<td>0.011</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Homovanillic acid</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Vanillic mandelic acid</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**Recovery**
Increasing amounts of dopamine were added to an urine and to a plasma sample. Each spiked sample was assayed. The analytical recovery was estimated at different concentrations by using the theoretically expected and the actually measured values. Concentrations in ng/ml
**Linearity**
The linearity of the ELISA method was investigated using different dilutions of an urine and a plasma sample. Concentrations in ng/ml

<table>
<thead>
<tr>
<th>Urine</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>added</td>
<td>measured</td>
</tr>
<tr>
<td>0.00</td>
<td>26.8</td>
</tr>
<tr>
<td>9.1</td>
<td>30.4</td>
</tr>
<tr>
<td>20.8</td>
<td>48.5</td>
</tr>
<tr>
<td>32.4</td>
<td>52.1</td>
</tr>
<tr>
<td>51.0</td>
<td>88.6</td>
</tr>
<tr>
<td>68.5</td>
<td>126.4</td>
</tr>
<tr>
<td>187.5</td>
<td>204.3</td>
</tr>
<tr>
<td>390.5</td>
<td>377.1</td>
</tr>
<tr>
<td>mean recovery: 98</td>
<td></td>
</tr>
</tbody>
</table>

**Reproducibility**

**Intra-Assay**
The reproducibility of the ELISA method was investigated by determining the intra-assay-coefficients of variation (cv) by repeated measurements of two samples with different concentrations. Concentrations in ng/ml

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>Mean value</th>
<th>Sd</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>25.1</td>
<td>2.84</td>
<td>11.3</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>146</td>
<td>11.6</td>
<td>7.9</td>
</tr>
</tbody>
</table>
Pipetting Scheme Sample Preparation

<table>
<thead>
<tr>
<th></th>
<th>Standards</th>
<th>Controls</th>
<th>Urine</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 1 - 7 µl</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 1&amp;2 µl</td>
<td></td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient Urine µl</td>
<td></td>
<td></td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Patient Plasma µl</td>
<td></td>
<td></td>
<td></td>
<td>300</td>
</tr>
<tr>
<td>Dist. Water µl</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>Extraction Buffer µl</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Incubate 60 minutes at RT (shake: 400 - 600 r/min)
Decant plate and remove residual liquid

Wash Buffer ml | 1 | 1 | 1 | 1 | 1

Incubate 5 minutes at RT (slow shaking)
Decant plate and remove residual liquid

Acylation Buffer µl | 150 | 150 | 150 | 150
Acyl. Reagent µl    | 50   | 50  | 50  | 50

Immediately shake 20 minutes at RT (shake: 400 - 600 r/min)
Decant plate and remove residual liquid

Wash Buffer ml | 1 | 1 | 1 | 1 | 1

Incubate 5 minutes at RT (slow shaking)
Decant plate and remove residual liquid

Wash Buffer ml | 1 | 1 | 1 | 1 | 1

Incubate 5 minutes at RT (slow shaking)
Decant plate and remove residual liquid

HCl µl | 200 | 200 | 200 | 200

Incubate 20 minutes with adhesive foil at RT (shake: 400 - 600 r/min)

Caution: Do not decant the supernatant thereafter

For the ELISA take each 50 µl for Dopamine
Pipetting Scheme ELISA

<table>
<thead>
<tr>
<th>Standards</th>
<th>Controls</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine (green)</td>
<td>µl</td>
<td>10</td>
</tr>
<tr>
<td>Enzyme Mix (Fresh)</td>
<td>µl</td>
<td>50</td>
</tr>
<tr>
<td>Standard 1 - 7</td>
<td>µl</td>
<td>50</td>
</tr>
<tr>
<td>Controls 1&amp;2</td>
<td>µl</td>
<td>50</td>
</tr>
<tr>
<td>Samples</td>
<td>µl</td>
<td>50</td>
</tr>
<tr>
<td>Cover with adhesive foil; shake 30 min at room temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine Antiserum</td>
<td>µl</td>
<td>50</td>
</tr>
</tbody>
</table>

Cover the plate with adhesive foil
Shake for 10 seconds
Incubate for 12 – 20 hours (overnight) at 2-8°C
4 x washing

| POD-Conjugate | µl | 100 | 100 | 100 |

Incubate for 30 minutes at room temperature on an orbital shaker
4 x washing

| Substrate | µl | 100 | 100 | 100 |

Incubate 25 to 35 minutes at room temperature on an orbital shaker

| Stop Solution | µl | 100 | 100 | 100 |

Reading of absorbance at 450 nm

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.