Human Dopamine ELISA Kit

Enzyme Immunoassay for the quantification of Dopamine in plasma and urine samples.

Catalog number: ARG80447

Package:96wells

For research use only. Not for use in diagnostic procedures.

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PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantification Dopamine in plasma and urine samples.
This assay employs the competitive quantitative enzyme immunoassay technique. Dopamine are first extracted by using a cis-diol-specific affinity gel, acylated and derivatized enzymatically.
The antigen has been pre-coated onto a microtiter plate. Extracted and derivatized controls, standards or samples and the solid phase bound analytes 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. Anti-rabbit IgG conjugated to Peroxidase is added to each well and incubated. After washing away any unbound antibody conjugate, a substrate solution (TMB) is added to the wells and color develops in inverse-proportion to the amount of Dopamine present in the samples. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of Dopamine in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Dopamine coated microplate	12 strips X 8 wells	4°C

Adhesive foil	4 pieces	RT
50X Wash Buffer	20ml	4°C
Anti-rabbit IgG-peroxidase conjugate	12ml (Ready-to-use)	4°C
TMB substrate	12ml (Ready-to-use)	4°C (Protect from light)
STOP solution	12ml (Ready-to-use)	4°C
Standard A-F	4ml each (Ready-to-use)	4°C
Dopamine Antiserum	6ml (Ready-to-use)	4°C
Adjustment Buffer	4ml (Ready-to-use)	4°C
Acylation Buffer	20ml (Ready-to-use)	4°C
Acylation Reagent	3ml (Ready-to-use)	4°C
Assay Buffer	6ml (Ready-to-use)	4°C
Coenzyme (S-adenosyl-L-methionine)	4ml (Ready to use)	4°C
Enzyme (COMT)	2 X 1ml (Lyophilized)	4°C
Extraction Buffer	6ml (Ready to use)	4°C
Extraction Plate (coated with boronate affinity gel)	2 X 48 wells (Ready-to-use)	4°C
Control 1	4ml (Ready-to-use)	4°C
Control 2	4ml (Ready-to-use)	4°C
Standard A/B	4ml (Ready-to-use)	4°C
Hydrochloric Acid	20ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down all vials before use.
- If crystals are observed in the 50X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

Plasma - Collect plasma using EDTA as an anticoagulant. Do not use haemolytic or lipemic samples. Assay immediately (up to 6 hours at 2-8 °C), or aliquot and store samples at ≤ -20 °C (up to 6 months). Avoid repeated freeze-thaw cycles. Avoid exposure to direct sunlight.

Urine – Spontaneous or 24-hour urine, collected in a bottle containing 10-15ml of 6M HCl. For linger storage, aliquot and store samples at ≤ -20 °C (up to 6 months). Avoid repeated freeze-thaw cycles. Avoid exposure to direct sunlight.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer. Storage: up to 6 months at 4-8°C.
- **Enzyme solution:** Reconstitute the lyophilized "Enzyme" with 1ml of distilled water and mix well. Add 0.3ml coenzyme followed by 0.7ml Adjustment buffer. The total volume of Enzyme solution is 2ml. Prepare fresh prior to assay (not more than 10-15 minutes in advance). Discard unused Enzyme solution.

ASSAY PROCEDURE

Sample Preparation, Extraction and Acylation

1. Pipette 10µl of standards, controls, urine samples and 300µl of plasma samples into the appropriate wells of the Extraction Plate.
2. Add 250µl of distilled water to wells with standards, controls and urine samples.
3. Add 50µl Assay Buffer to all wells.
4. Add 50µl Extraction Buffer to all wells.
5. Cover plate and incubate for 30 mins at RT on shaker (~600rpm)
6. Remove foil, discard and blot dry by tapping the inverted plate on absorbent material. Wash each well with 1ml wash buffer and shake for 5min at RT on a shaker (600rpm). Blot dry by tapping the inverted plate on absorbent material.
7. Repeat wash. Discard and blot dry by tapping the inverted plate on absorbent material.

8. Add 150µl Acylation Buffer into all wells.
9. Add 25µl Acylation Reagent into all wells.
10. Incubate for 15 mins at RT on shaker (~600rpm)
11. Remove foil, discard and blot dry by tapping the inverted plate on absorbent material. Wash each well with 1ml wash buffer and shake for 10min at RT on a shaker (600rpm). Blot dry by tapping the inverted plate on absorbent material.
12. Add 175µl Hydrochloric Acid into all wells.
13. Cover plate and incubate for 10 mins at RT on shaker (~600rpm)
14. Remove foil, **do not decant the supernatant!**
15. Use 25µl for Dopamine assay (for standards and urine samples) and 50µl for Dopamine assay (for plasma samples).

Dopamine ELISA procedure

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 25 µl Enzyme solution into all wells of Dopamine Microtiter Strips.
3. Add 25 µl of the extracted standards, controls, urine samples and 50µl plasma samples into the appropriate wells of Dopamine Microtiter Strips.
4. Incubate for 30 mins at RT on shaker (~600rpm)
5. Add 50 µl of Dopamine Antiserum into wells.
6. Cover plate with Adhesive foil and incubate for 2 hours at RT on a shaker (600rpm).
7. Remove the foil and discard. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1×

Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

8. Add 100 µl of Anti-rabbit IgG-peroxidase conjugate into wells.
9. Incubate for 30 mins at RT on a shaker (600rpm).
10. Aspirate each well and wash as step 7.
11. Add 100 µl of TMB substrate solution into each well. Incubate for 20-30 mins at RT with shaking (600rpm). Avoid exposure to light.
12. Add 100 µl of Stop Solution to each well and shake lightly to ensure homogeneous mixing.
13. Read the OD with a microplate reader at 450nm (with a reference wavelength between 620nm and 650nm) within 10 minutes

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter

Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. The concentrations of undiluted samples and controls can be read directly from the standard curve.

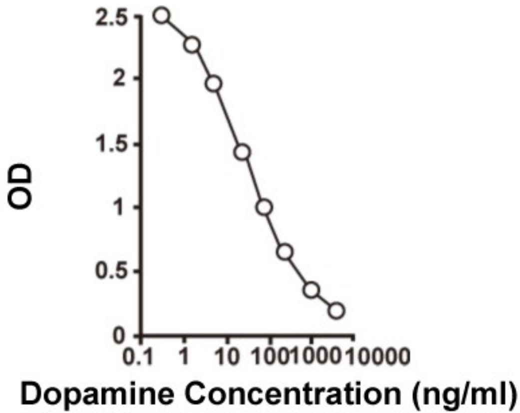
6. Refer to the table below for molar conversion:

	Concentration of standards					
Standard	A	B	C	D	E	F
Dopamine (ng/ml)	0	10	40	150	500	2000
Dopamine (nmol/L)	0	65	261	980	3265	13060
Conversion	Dopamine (ng/ml) x 6.53 = Dopamine (nmol/L)					

- For the determination of Dopamine in plasma, the addition of Standard A/B is mandatory. Concentration of Standard A/B is 4.5ng/ml (29nmol/L)
- Urine samples and Controls: The concentration of urine samples and controls can be read directly from the standard curve. Calculate the 24h excretion for each urine sample: µg/24h = µg/L X L/24h
- Plasma samples: For Adrenaline and Noradrenaline calculations, the read concentrations have to be divided by 30. For Dopamine calculations, the read concentrations have to be divided by 60.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

urine: 4.5ng/ml; plasma: 25pg/ml

Assay Range

DOP: 10-2000ng/ml

Specificity

No significant cross-reactivity was found for the following factors:

Adrenaline, Noradrenaline, Metanephrine, Normetanephrine, 3-Methoxytyramine, 3-Methoxy-4-hydroxyphenylglycol, Tyramine, Phenylalanine, Caffeinic acid, L-DPOA, Homovanillic acid, 3-Methoxy-4-Hydroxymandelic acid.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 12.65% and inter-assay precision was 17%. [Dopamine]

Recovery

85-104% (Dopamine-urine), 74-110% (Dopamine-plasma)

Linearity

84-106% (Dopamine-urine), 74-131 (Dopamine-plasma)