Dopamine ELISA Kit

Enzyme Immunoassay for the quantification of Human/Mouse/Rat Dopamine in various biological sample types including EDTA-Plasma, Cell culture supernatants and other biological samples types.

Catalog number: ARG80450

Package: 96 wells

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PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. An antigen Dopamine has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Dopamine present compete for the fixed number of antibody binding site. After washing away any unbound substances, the antibody bound to the solid phase is detected by using TMB as a substrate. The reaction is monitored at 450 nm±2 nm. Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standards.

MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
Microtiter Plate	1 x 96 wells	4°C
Extraction Plate (coated with boronate affinity gel)	2 x 48 wells	4°C
Dopamine coated microtiter strips	12 x 8 wells	4°C
Standard A-F (0, 0.5, 1.5, 5, 20, 80 ng/ml)	6 x 4 ml (Ready-to-use)	4°C
Control 1 (3 ng/ml ± 40%)	4 ml (Ready-to-use)	4°C
Control 2 (10 ng/ml ± 40%)	4 ml (Ready-to-use)	4°C
Acylation Buffer	20 ml (Ready-to-use)	4°C
Acylation Reagent	3 ml (Ready-to-use)	4°C
TE Buffer	4 ml (Ready-to-use)	4°C

Coenzyme (S-adenosyl-L-methionine)	4 ml (Ready-to-use)	4°C
Enzyme (COMT)	4 vial (Lyophilized)	4°C
Enzyme-conjugated Antibody	12 ml (Ready-to-use)	4°C
Dopamine antiserum	6 ml (Ready-to-use)	4°C
Adjustment buffer	4 ml (Ready-to-use)	4°C
50X Wash buffer	20 ml	4°C
TMB substrate	12 ml (Ready-to-use)	4°C (Protect from light)
HCl	20 ml (Ready-to-use)	4°C
STOP solution	12 ml (Ready-to-use)	4°C
Adhesive foil	4 pieces	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: 620-650 nm as reference wave length)
- Pipettes and pipette tips
- Deionized or distilled water
- Orbital microplate shaker: 3 mm (0.1118 in) 600 ± 10 rpm or 19 mm (0.75 in) 170 ± 10 rpm.
- 37°C oven or incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- 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.
- 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. Once opened the reagents are stable for 1 months when stored at 2–8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.
- 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.
- If crystals are observed in the 50X Wash buffer, warm to RT or 37°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.
- 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.
- The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.

- Change pipette tips between the addition of different reagent or samples.
- 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.

SAMPLE COLLECTION & STORAGE INFORMATION

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.

Preparation: This Dopamine ELISA kit 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 above).
- 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 the sample prior to the extraction step.
- Tissue samples can be homogenized in 0.01 N HCl in the presence of EDTA (final concentration 1 mM) and sodium metabisulfite (final concentration 4 mM). 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 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 the test. Determine the sample volume needed to determine the Dopamine in your sample by testing different amounts of sample volume.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute **50X** Wash buffer into distilled water to yield 1X Wash buffer. E.g. dilute the 20 ml of 50X Wash Buffer into distilled water to a final volume of 1000 ml. The diluted wash buffer can be stored at 2 – 8 °C for 1 month.
- **Enzyme solution**: Reconstitute the Enzyme (COMT) vial with **1 ml** of **distilled water**, mix thoroughly. Add **0.3 ml** of **Coenzyme** followed by **0.7 ml** of **Adjustment Buffer.** The total volume of enzyme solution is **2 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!

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) and mix thoroughly before use. Standards, samples and controls should be assayed in duplicates.

- **Extraction and acylation**
  - If you have sample volumes between 1-100 µl follow 1.1
  - If you have sample volumes between 100-500 µl follow 1.2
  - If you have sample volumes between 500-750 µl follow 1.3

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

1.1. Pipette **10 µl standards, 10 µl controls and 1-100 µl samples** into the respective wells of **Extraction Plate.** Fill up each well with distilled water to a **final volume of 100 µl**.

1.2. Pipette **10 µl standards, 10 µl controls and 100-500 µl samples** into the respective wells of **Extraction Plate.** Fill up each well with distilled water to a **final volume of 500 µl.**

1.3. Pipette **10 µl standards, 10 µl controls and 500-750 µl samples** into the respective wells of **Extraction Plate.** Fill up each well with distilled water to a **final volume of 750 µl**.

2. Add **25 µl** of **TE buffer** into all wells. Cover the plate with adhesive foil and incubate for **1 hour at RT** on a microplate shaker (600 rpm).

3. Remove the foil. Aspirate each well and wash, repeating the process 1 times for a **total 2 washes**. Wash by filling each well with **1 ml** of **1× Wash Buffe**r using a squirt bottle, manifold dispenser, or autowasher. Shake the

plate for **5 min at RT** on a microplate shaker (600 rpm). 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.

4. Add **150 µl** of **Acylation Buffer** into all wells.
5. Add **25 µl** of **Acylation Reagent** into all wells.
6. Shake **20 minutes at RT** on a microplate shaker (600 rpm).
7. **Wash** as according to step 3.
8. Add **100 µl** of **HCl** into all wells. Cover the plate with foil. Incubate for **10 min at RT** on a microplate shaker (600 rpm).
9. **Do not decant the supernatant thereafter!** Use **90 µl** from step 8 for the subsequent Enzymatic Conversion.

- **Enzymatic Conversion**

1. Add **90 µl** of the **extracted standards, controls and samples** from Extraction and acylation process in duplicate into the respective wells of **Microtiter plate**.
2. Add **25 µl** of **Enzyme solution** (refer to REAGENT PREPARATION) into all wells.
3. Cover the plate with adhesive foil and mix on a microplate shaker (600 rpm) for **1 min at RT**.
4. Incubate for **2 hours at 37°C**.
5. Use **100 µl** for the subsequent ELISA process.

- **Dopamine ELISA**

1. Add **100 µl** of **standards, controls and samples from the Microtiter plate** into the respective **Dopamine-coated Microtiter strips**.
2. Add **50 µl** of **Dopamine antiserum** into all wells.
3. Cover wells with adhesive foil and mix on a microplate **shaker** (600 rpm) for **1 min at RT.** Then incubate the plate for **15-20 hours at 2-8°C.**
4. Aspirate each well and wash, repeating the process 3 times for a **total 4 washes.** Wash by filling each well with **1× Wash Buffer (300 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.
5. Add **100 µl** of **Enzyme-conjugated Antibody** into all wells. Cover wells with adhesive foil and incubate for **30 minutes at RT** on a microplate shaker (600 rpm).
6. **Wash** as according to step 4.
7. Add **100 µl** of **TMB Substrate** to each well. Incubate for **20-30 minutes at room temperature** on a microplate shaker (600 rpm) in dark.
8. Add **100 µl** of **Stop Solution** to each well.
9. Read the OD with a microplate reader at **450 nm** within 10 min after adding Stop solution. (optional: read at 620-650nm as reference wave length)

   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.

CALCULATION OF RESULTS

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

2. Using semi-logarithmic 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.

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

For 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 ng/ml x 0.013 = 0.00585 ng/ml = 5.85 pg/ml dopamine

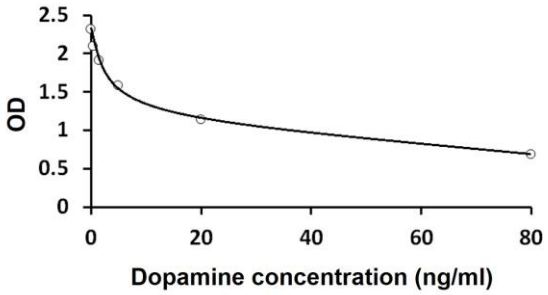
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. Refer to the table below for molar conversion:

	Concentration of standards					
Standard	A	B	C	D	E	F
Dopamine (ng/ml)	0	0.5	1.5	5	20	80
Dopamine (nmol/L)	0	3.3	9.8	33	131	522
Conversion	Dopamine (ng/ml) x 6.53 = Dopamine (nmol/L)					

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

The minimum detectable dose (MDD) of Dopamine ranged from 0-80 ng/ml.

The mean MDD was 0.25 ng/ml X correction factor

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 15.6% and inter-assay precision was 18.43%.

Recovery

Human EDTA-Plasma: 83.7-115.9%

Cell culture medium: 77.7-113.4%

Specificity:

	Substance	Cross Reactivity (%)
		Dopamine
Cross Reactivity	Derivatized Adrenaline	0.03
	Derivatized Noradrenaline	0.87
	Derivatized Dopamine	100
	Metanephrine	< 0.0007
	Normetanephrine	0.008
	3-Methoxytyramine	0.55
	3-Methoxy-4-hydrophenylcol	< 0.0007
	Tyramine	0.13
	Phenylalanine, Caffeinic acid, L-Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid	< 0.0007