Human/Mouse 3 CAT Research (Adrenaline + Noradrenaline + Dopamine) ELISA Kit

Enzyme Immunoassay for the quantification of 3 kinds of Human and mouse Catecholamine (Adrenaline + Noradrenaline + Dopamine) in various biological sample types including EDTA-Plasma, Cell culture supernatants and other biological samples types.

Catalog number: ARG80440

Package: 96 wells for Adrenaline
96 wells for Noradrenaline
96 wells for Dopamine

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PRINCIPLE OF THE ASSAY

This is an enzyme Immunoassay for the quantification of 3 kinds of Human and mouse Catecholamine (Adrenaline + Noradrenaline + Dopamine) in various biological sample types including EDTA-Plasma, Cell culture supernatants and other biological samples types. Adrenaline, Noradrenaline and Dopamine in controls, samples or standards are first extracted by using a cis-diol-specific affinity gel, acylated and derivatized enzymatically. This assay employs the competitive quantitative enzyme immunoassay technique. 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 3-CAT 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 3-CAT 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
Adrenaline-Metanephrine coated microplate	12 strips X 8 wells (blue)	4°C
Noradrenaline-Normetanephrine coated microplate	12 strips X 8 wells (yellow)	4°C
Dopamine coated microplate	12 strips X 8 wells (green)	4°C
Adhesive foil	12 pieces	RT
50X Wash Buffer	3 X 20 ml	4°C
Anti-rabbit IgG Peroxidase Conjugate	3 X 12 ml (Ready-to-use)	4°C
TMB substrate	3 X 12 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	3 X 12 ml (Ready-to-use)	4°C
Standard A-F	4 ml each (Ready-to-use)	4°C
Control 1	4 ml (Ready-to-use)	4°C
Control 2	4 ml (Ready-to-use)	4°C
Adrenaline Antiserum	6 ml (Ready-to-use)	4°C
Noradrenaline Antiserum	6 ml (Ready-to-use)	4°C
Dopamine Antiserum	6 ml (Ready-to-use)	4°C
Adjustment Buffer	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-Lmethionine)	4 ml (Ready-to-use)	4°C
Enzyme (COMT)	4 Vials (Lyophilized)	4°C
Extraction Plate (coated with boronate affinity gel)	2 X 48 wells (Ready-to-use)	4°C
Microtiter Plate for Enzyme Conversion	12 strips X 8 wells	4°C
Hydrochloric Acid	20 ml (Ready-to-use)	4°C

Concentrations of Controls:

	Adrenaline	Noradrenaline	Dopamine
Control 1	3 ± 1.2 ng/ml	1.2 ± 0.48 ng/ml	3 ± 1.2 ng/ml
Control 2	10 ± 4 ng/ml	4 ± 1.6 ng/ml	10 ± 4 ng/ml

	Concentration of standards					
Standard	A	B	C	D	E	F
Adrenaline (ng/ml)	0	0.5	1.5	5	20	80
Adrenaline (nmol/L)	0	2.7	8.2	27	109	437
Noradrenaline (ng/ml)	0	0.2	0.6	2	8	32
Noradrenaline (nmol/L)	0	1.2	3.5	12	47	189
Dopamine (ng/ml)	0	0.5	1.5	5	20	80
Dopamine (nmol/L)	0	3.3	9.8	33	131	522
Conversion	Adrenaline (ng/ml) x 5.46 = Adrenaline (nmol/L) Noradrenaline (ng/ml) x 5.91 = Noradrenaline (nmol/L) Dopamine (ng/ml) x 6.53 = Dopamine (nmol/L)					

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.
- 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 unopened reagents at 2- 8°C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 – 8 °C.
- The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant and used in the frame provided. Microtiter strips which are removed from the frame for usage

should be marked accordingly to avoid any mix-up. 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 (20 – 25 °C) 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.
- The 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 3-CAT 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 above).

• Avoid chaotropic chemicals like perchloric acid. The high salt content might reduce the recovery of Adrenaline, Noradrenaline and Dopamine. If your samples already contain high amounts of perchloric acid, neutralize them prior to the extraction step.

• Tissue samples can be homogenized in 0.01 N HCl in the presence of EDTA and sodium metabisulfite. Under these conditions, Adrenaline, Noradrenaline and Dopamine are 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 catecholamines.

• It is advisable to perform a "Proof of Principle" to determine the recovery of the catecholamines in your samples. Prepare a stock solution of Adrenaline, Noradrenaline and 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 catecholamines 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 lyophilized "Enzyme (COMT)" with 1ml of distilled water and mix well. 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, 20 – 25 °C) and mix thoroughly before use. Standards, samples and controls should be assayed in duplicates. If a microplate shaker is not available, please optimize the shaking speed of user's shaker.

Sample Preparation, Extraction and Acylation, Enzymatic Conversion

1.1. **For sample volume 1-100 µl**: Pipette to the respective wells of the Extraction Plate: 30 µl of standards, 30 µl of controls and 1-100 µl of samples. Fill up each well to final volume of 100 µl with distilled water. (e.g. 30 µl of standard + 70 µl of distilled water)

1.2. **For sample volume 100-500 µl**: Pipette to the respective wells of the Extraction Plate: 30 µl of standards, 30 µl of controls and 100-500 µl of samples. Fill up each well to final volume of 500 µl with distilled water. (e.g. 30 µl of standard + 470 µl of distilled water)

1.3. **For sample volume 500-750µl**: Pipette to the respective wells of the Extraction Plate: 30 µl of standards, 30 µl of controls and 500-750 µl of samples. Fill up each well to final volume of 750 µl with distilled water. (e.g. 30 µl of standard +720 µl of distilled water)

2. Add 25 µl of TE Buffer to all wells.

3. Cover plate and incubate for 60 mins at RT on a microplate shaker (~600rpm)

4. Remove foil, discard and blot dry by tapping the inverted plate on absorbent material.

5. Wash each well with 1 ml of 1X wash buffer and shake for 5 mins at RT on a microplate shaker (600rpm). Blot dry by tapping the inverted plate on absorbent material.

6. Repeat wash as step 5. Discard and blot dry by tapping the inverted plate on absorbent material.
7. Add 150 µl of Acylation Buffer into all wells.
8. Add 25 µl of Acylation Reagent into all wells.
9. Incubate for 20 mins at RT on a microplate shaker (~600rpm).
10. Remove foil, discard and blot dry by tapping the inverted plate on absorbent material. Wash each well with 1 ml of 1X wash buffer and shake for 5 mins at RT on a microplate shaker (~600rpm). Blot dry by tapping the inverted plate on absorbent material.
11. Repeat wash as step 10. Discard and blot dry by tapping the inverted plate on absorbent material.
12. Add 200 µl of Hydrochloric Acid into all wells.
13. Cover plate and incubate for 10 mins at RT on a microplate shaker (~600rpm)
14. Remove foil, do not decant the supernatant!
15. 190 µl of supernatant is needed for the subsequent enzymatic conversion.
16. For enzymatic conversion, pipette 190 µl of the extracted standards, controls and samples into the respective wells of the enzymatic conversion microtiter plate.
17. Add 50 µl of Enzyme Solution (Refer to reagent preparation) to all wells. Cover plate with adhesive foil. Shake 1 min at RT on a microplate shaker (~600rpm).
18. Incubate for 2 hours at 37°C. Use 75 µl in each assay for subsequent Adrenaline ELISA, Noradrenaline ELISA and Dopamine ELISA procedures respectively.

Adrenaline, Noradrenaline and Dopamine ELISA Procedures

1. Remove microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **75 µl** of the **standards, controls and samples** from the **enzymatic conversion microtiter plate** into the appropriate wells of **pre-coated Microtiter Strips. (Adrenaline, Noradrenaline, Dopamine Microtiter strips)**
3. Add **50 µl** of respective **Antiserum** into all wells. **(Adrenaline, Noradrenaline, Dopamine Antiserum)**
4. Cover plate with adhesive foil. Shake for **1 min** at **RT** on a microplate shaker (~600rpm). Incubate for **15-20 hours (overnight) at 2-8°C**.
5. Remove the foil and discard. 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.
6. Add **100 µl** of **Anti-rabbit IgG-peroxidase conjugate** into wells.
7. Incubate for **30 mins at RT** on a microplate shaker (~600rpm).
8. Aspirate each well and **wash as step 5**.
9. Add **100 µl** of **TMB substrate solution** into each well. Incubate for **20-30 mins at RT** with shaking (600rpm). Avoid exposure to light.
10. Add **100 µl** of **Stop Solution** to each well and shake lightly to ensure homogeneous mixing.
11. **Read** the OD with a microplate reader at **450 nm** (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 and samples.

2. Using log-log, semi-log or 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. 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. The standards refer to:

Concentration of the standards (ng/ml)

Standard	A	B	C	D	E	F
Adrenaline	0	0.5	1.5	5	20	80
Noradrenaline	0	0.2	0.6	2	8	32
Dopamine	0	0.5	1.5	5	20	80

7. The concentration of the samples taken from the standard curve have to be multiplied by a correction factor:

Correction factor = 30 µl/Sample volume extracted

For example, 750 µl of sample is extracted and the concentration taken from the standard curve is 0.15ng/ml Noradrenaline.

Correction factor = 30/750 = 0.04

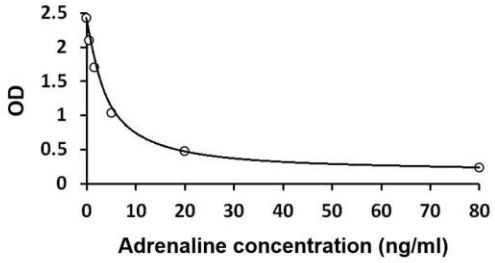
Concentration of Noradrenaline in the samples = 0.15 ng/ml X 0.04

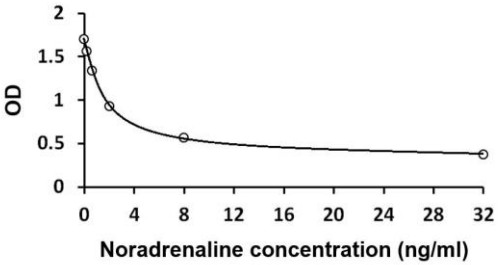
= 0.006 ng/ml = 6 pg/ml

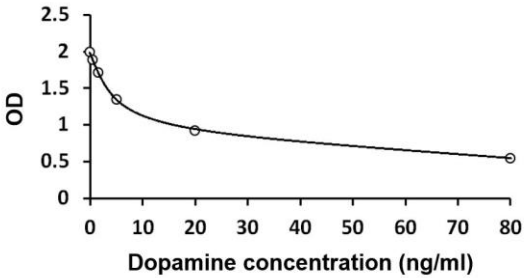
8. It is recommended to use control samples included in this kit. Use controls at both normal and pathological levels. The kit should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC Report.

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 (Limit of Detection)

Adrenaline: 0.25 ng/ml X Correction factor

Noradrenaline: 0.1 ng/ml X Correction factor

Dopamine: 0.25 ng/ml X Correction factor

Analytical Sensitivity (750 µl undiluted sample)

Adrenaline: 10 pg/ml

Noradrenaline: 4 pg/ml

Dopamine: 10 pg/ml

Functional Sensitivity (750 µl undiluted sample)

Adrenaline: 15 pg/ml

Noradrenaline: 6 pg/ml

Dopamine: 15 pg/ml

Specificity

	Cross-reactivity (%) for different assays		
Substance	Adrenaline	Noradrenaline	Dopamine
Derivatized Adrenaline	100	0.14	0.03
Derivatized Noradrenaline	0.2	100	0.87
Derivatized Dopamine	< 0.0007	0.2	100
Metanephrine	0.64	< 0.003	< 0.007
Normetanephrine	0.0009	0.48	0.008
3-Methoxytyramine	< 0.0007	< 0.003	0.55
3-Methoxy-4-hydroxyphenylglycol	0.03	0.01	< 0.007

Tyramine	< 0.0007	< 0.003	0.13
Caffeinic acid	< 0.0007	< 0.003	< 0.007
L-Dopa	< 0.0007	< 0.003	< 0.007
Homovanillic acid	< 0.0007	< 0.003	< 0.007
3-Methoxy-4-hydroxymandelic acid	< 0.0007	< 0.003	< 0.007
Phenylalanine	< 0.0007	< 0.003	< 0.007
Tyrosine	< 0.0007	< 0.003	< 0.007

Intra-assay precision

The CV value of intra-assay precision precision were as below:

	Intra-assay (%)	
	Human EDTA-Plasma	Cell Culture Medium
Adrenaline	9.3-17.1	11.2-16.3
Noradrenaline	8.4-15.6	8.4-12.2
Dopamine	10.8-21.5	14.8-23

Recovery

	Recovery (%)	
	Human EDTA-Plasma	Cell Culture Medium
Adrenaline	89.4-128.3	81.6-109.6
Noradrenaline	104.8-125.6	70.6-124.7
Dopamine	83.7-115.9	77.7-113.4