Human 2 CAT (Adrenaline + Noradrenaline) ELISA Kit

Enzyme Immunoassay for the quantification of 2-CAT (Adrenaline + Noradrenaline) in plasma and urine samples.

Catalog number: ARG80431

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PRINCIPLE OF THE ASSAY

This assay employs the competitive quantitative sandwich enzyme immunoassay technique. Adrenaline and Noradrenaline 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 2-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 2-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. Once opened the reagents are stable for 1 month when stored at 2 – 8 °C.

Component	Quantity	Storage information
Adrenaline-Metanephrine coated microplate	12 strips X 8 wells	4°C
Noradrenaline-Metanephrine coated microplate	12 strips X 8 wells	4°C
Adhesive foil	2 X 4 pieces	RT
50X Wash Buffer	2 X 20 ml	4°C
Anti-rabbit IgG-peroxidase conjugate	2 X 12 ml (Ready-to-use)	4°C
TMB substrate	2 X 12 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	2 X 12 ml (Ready-to-use)	4°C
Standard A-F	4 ml each (Ready-to-use)	4°C
Adrenaline Antiserum	6 ml (Ready-to-use)	4°C
Noradrenaline 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
Assay Buffer	6 ml (Ready-to-use)	4°C
Coenzyme (S-adenosyl-L-methionine)	4 ml (Ready to use)	4°C
Enzyme (COMT)	4 vials (Lyophilized)	4°C
Extraction Buffer	6 ml (Ready to use)	4°C
Extraction Plate (coated with boronate affinity gel)	2 X 48 wells (Ready-to-use)	4°C
Control 1	4 ml (Ready-to-use)	4°C

(AD: 8 ng/ml ± 40% NAD: 40 ng/ml ± 40%)		
Control 2 (AD: 30 ng/ml ± 40% NAD: 150 ng/ml ± 40%)	4 ml (Ready-to-use)	4°C
Hydrochloric Acid	20 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read the plate between 620nm and 650nm as a reference wavelength)
- Pipettes and pipette tips
- Deionized or distilled water
- Microplate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- 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.
- 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.
- 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.
- 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.
- Incubation times do influence the results. All wells should be handled in the same order and time intervals.
- 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 urine or 24-hour urine (24-hour urine can be collected in a bottle containing 10-15ml of 6M HCl) can be used. Store at RT up to 24 hrs, store at 4°C up to 48 hrs. For longer 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: store diluted wash buffer at 2-8°C up to one month. (E.g.: 20 ml of 50X Wash buffer + 980 ml of distilled water)
- **Enzyme solution:** Reconstitute the lyophilized "Enzyme (COMT)" 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

All materials should be equilibrated to room temperature (20- 25°C) before use, each vial should be mixed thoroughly without foaming prior to use.

Sample Preparation, Extraction and Acylation

1. Pipette 10 µl of standards, controls, urine samples and 300 µl of plasma samples into the appropriate wells of the Extraction Plate.
2. Add 250 µl of distilled water to the wells with standards, controls and urine samples.
3. Add 50 µl Assay Buffer to all wells.
4. Add 50 µl Extraction Buffer to all wells.
5. Cover plate with Adhesive foil and incubate for 30 mins at RT (20-25°C) on a microplate shaker (~600rpm)
6. Remove foil, discard and blot dry by tapping the inverted plate on absorbent material. Wash each well with 1ml 1 x wash buffer and shake for 5 min at RT (20-25°C) on a microplate shaker (600rpm). Blot dry by tapping the inverted plate on absorbent material.
7. Repeat wash as step 6. Discard and blot dry by tapping the inverted plate on absorbent material.
8. Add 150 µl Acylation Buffer into all wells.
9. Add 25 µl Acylation Reagent into all wells.
10. Incubate for 15 mins at RT (20-25°C) on a microplate shaker (~600rpm)

11. Discard the solution and blot dry by tapping the inverted plate on absorbent material. Wash each well with 1ml 1 X wash buffer and shake for 10min at RT (20-25°C) on a microplate shaker (600rpm). Blot dry by tapping the inverted plate on absorbent material.
12. Add 150 µl Hydrochloric Acid into all wells.
13. Cover plate with Adhesive foil and incubate for 10 mins at RT (20-25°C) on a microplate shaker (~600rpm)
14. Remove foil, do not decant the supernatant!
15. Use 20 µl for Noradrenaline assay and 100 µl for Adrenaline assay.

Noradrenaline 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 (refer to REAGENT PREPARATION section) into all wells of Noradrenaline Microtiter Strips.
3. Add 20 µl of the extracted standards, controls and samples into the appropriate wells of Noradrenaline Microtiter Strips.
4. Incubate for 30 mins at RT (20-25°C) on a microplate shaker (~600rpm)
5. Add 50 µl of Noradrenaline Antiserum into wells.
6. Cover plate with Adhesive foil and incubate for 2 hours at RT (20-25°C). on a microplate shaker (600rpm).
7. Remove the foil. Discard or aspirate each well and wash, repeating the process 2 times for a total 3 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.

8. Add 100 µl of Anti-rabbit IgG-peroxidase conjugate into wells.
9. Incubate for 30 mins at RT (20-25°C) on a microplate 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 (20-25°C) 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 (optional: read the plate between 620nm and 650nm as a reference wavelength) within 10 minutes.

Adrenaline 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 (refer to REAGENT PREPARATION section) into all wells of Adrenaline Microtiter Strips.
3. Add 100 µl of the extracted standards, controls and samples into the appropriate wells of Adrenaline Microtiter Strips.
4. Incubate for 30 mins at RT (20-25°C) on a microplate shaker (~600rpm)
5. Add 50 µl of Adrenaline Antiserum into wells.
6. Cover plate with Adhesive foil and incubate for 2 hours at RT (20-25°C). on a microplate shaker (600rpm).
7. Remove the foil. Discard or aspirate each well and wash, repeating the process 2 times for a total 3 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.

8. Add 100 µl of Anti-rabbit IgG-peroxidase conjugate into wells.
9. Incubate for 30 mins at RT (20-25°C) on a microplate 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 (20-25°C) 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 (optional: read the plate between 620nm and 650nm as a reference wavelength) within 10 minutes

CALCULATION OF RESULTS

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

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

6. For diluted samples, the concentration read from the standard curve has to be multiplied with a volume factor:

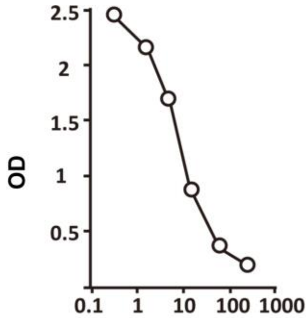
7. Refer to the table at below for molar conversion of Adrenaline and Noradrenaline:

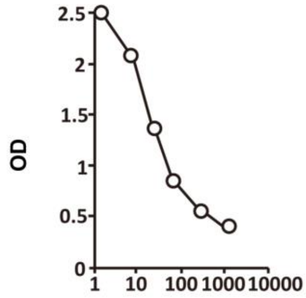
	Concentration of standards					
Standard	A	B	C	D	E	F
Adrenaline (ng/ml)	0	1	4	15	50	200
Adrenaline (nmol/L)	0	5.5	22	82	273	1092
Noradrenaline (ng/ml)	0	5	20	75	250	1000
Noradrenaline (nmol/L)	0	30	118	443	1478	5910
Conversion	Adrenaline (ng/ml) x 5.46 = Adrenaline (nmol/L) Noradrenaline (ng/ml) x 5.91 = Noradrenaline (nmol/L)					

- Urine samples: The concentration of urine samples 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: The read concentrations have to be divided by 30.

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

LOD:

AD: urine: 0.9 ng/ml; plasma: 10 pg/ml

NAD: urine: 1.7 ng/ml; plasma: 36 pg/ml

Assay Range

AD: 1-200 ng/ml; NAD: 5-1000 ng/ml

Measuring Range

AD: urine: 0.7-200 ng/ml; plasma: 18-6667 pg/ml

NAD: urine: 2.5-1000 ng/ml; plasma: 93-33333 pg/ml

Specificity

The cross-reactivity ratio was as the table at below:

Substance	Cross Reactivity (%)	
	Adrenaline	Noradrenaline
Derivatized Adrenaline	100	0.08
Derivatized Noradrenaline	0.13	100
Derivatized Dopamine	< 0.01	0.03
Metanephrine	0.18	< 0.01
Normetanephrine	< 0.01	0.16
3-Methoxytyramine	< 0.01	< 0.01
3-Methoxy-4-hydroxyphenylglycol	< 0.01	< 0.01
Tyramine	< 0.01	< 0.01
Phenylalanine	< 0.01	< 0.01
Caffeinic acid	< 0.01	< 0.01
L-Dopa	< 0.01	< 0.01
Homovanillic acid	< 0.01	< 0.01
Tyrosine	< 0.01	< 0.01
3-Methoxy-4-hydroxymandelic acid	< 0.01	< 0.01

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 12.95% and inter-assay precision was 11.75%. [Noradrenaline]

The CV value of intra-assay precision was 12.5% and inter-assay precision was 15.6%. [Adrenaline]

Recovery

75-113% (Noradrenaline)

88-120% (Adrenaline)