2-CAT (Adrenaline + Noradrenaline) ELISA Kit (For Human)

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

Catalog number: ARG80433

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PRINCIPLE OF THE ASSAY

This is an enzyme Immunoassay for the quantification of 2-CAT (Adrenaline + Noradrenaline) in plasma samples.

This assay employs the competitive quantitative enzyme immunoassay technique. Adrenaline and Noradrenaline in controls, samples or standards 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.

Component	Quantity	Storage information
Adrenaline-Metanephrine coated microplate	12 strips X 8 wells	4°C

Noradrenaline-Normetanephrine coated microplate	12 strips X 8 wells	4°C
Adhesive foil	2 X 4 pieces	RT
50X Wash Buffer	2 X 20ml	4°C
Anti-rabbit IgG-peroxidase conjugate	2 X 12ml (Ready-to-use)	4°C
TMB substrate	2 X 12ml (Ready-to-use)	4°C (Protect from light)
STOP solution	2 X 12ml (Ready-to-use)	4°C
Standard A-F	4ml each (Ready-to-use)	4°C
Adrenaline Antiserum	6ml (Ready-to-use)	4°C
Noradrenaline 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
TE Buffer	4ml (Ready-to-use)	4°C
Coenzyme (S-adenosyl-L-methionine)	4ml (Ready to use)	4°C
Enzyme (COMT)	4 X 1ml (Lyophilized)	
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
Hydrochloric Acid	20ml (Ready-to-use)	4°C
Microtiter Plate for Enzyme Conversion	12 strips X 8 wells	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 the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X 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.

A plasma volume between 100µl - 600µl is needed per single determination. If a plasma volume < 600µl is used, distilled water has to be added to a final volume of 600µl and this prediluted sample has to be used for the extraction procedure. The sample predilution has to be considered in the calculation of results.

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, Enzymatic Conversion

1. Pipette 30µl of standards, controls, urine samples and 600µl of plasma samples into the appropriate wells of the Extraction Plate.
2. Add 500µl of distilled water to wells with standards, controls and urine samples.
3. Add 25µl TE Buffer to all wells.
4. Add 50µl Extraction Buffer to all wells.
5. Cover plate and incubate for 60 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 5 mins at RT on a 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 150µl Acylation Buffer into all wells.
9. Add 25µl Acylation Reagent into all wells.
10. Incubate for 20 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 5 mins at RT on a shaker (600rpm). Blot dry by tapping the inverted plate on absorbent material.
12. Repeat wash as step 11.

13. Add 200µl Hydrochloric Acid into all wells.
14. Cover plate and incubate for 10 mins at RT on shaker (~600rpm)
15. Remove foil, **do not decant the supernatant!**
16. 190µl of supernatant is needed for the subsequent enzymatic conversion.
17. For enzymatic conversion, pipette 190µl of the extracted standards, controls and samples into the respective wells of the microtiter plate.
18. Add 50µl of Enzyme Solution to all wells.
19. Cover plate with adhesive foil. Shake 1 min at RT on a shaker.
20. Incubate for 2 hours at 37°C. Use 100µl for subsequent Adrenaline ELISA and 10µl for Noradrenaline ELISA procedures respectively.

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 100 µl of the extracted standards, controls and samples into the appropriate wells of Adrenaline Microtiter Strips.
3. Add 50 µl of Adrenaline Antiserum into all wells.
4. Cover plate with adhesive foil. Incubate for 1 min at RT on a shaker. Incubate for 15-20 hours (overnight) at 2-8°C.
5. 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.

6. Add 100 µl of Anti-rabbit IgG-peroxidase conjugate into wells.
7. Incubate for 30 mins at RT on a 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 450nm (with a reference wavelength between 620nm and 650nm) within 10 minutes

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 10 µl of the extracted standards, controls and samples into the appropriate wells of Noradrenaline Microtiter Strips.
3. Add 50 µl of Noradrenaline Antiserum into wells.
4. Cover plate with Adhesive foil and incubate for 1 min at RT on a shaker (600rpm).
5. Incubate for 15-20 hours (overnight) at 2-8°C.
6. 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.

7. Add 100 µl of Anti-rabbit IgG-peroxidase conjugate into wells.
8. Incubate for 30 mins at RT on a shaker (600rpm).
9. Aspirate each well and wash as step 6.
10. Add 100 µl of TMB substrate solution into each well. Incubate for 20-30 mins at RT with shaking (600rpm). Avoid exposure to light.
11. Add 100 µl of Stop Solution to each well and shake lightly to ensure homogeneous mixing.
12. 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. If <600 µl plasma volume was used, the concentration read from the standard curve has to be multiplied with a volume factor:

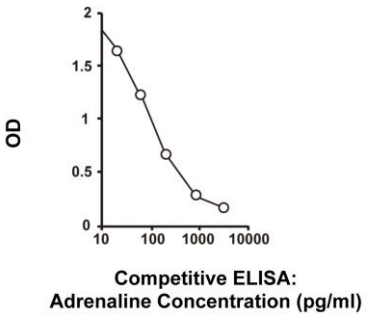
Volume factor = 600µl/used plasma volume (µl)

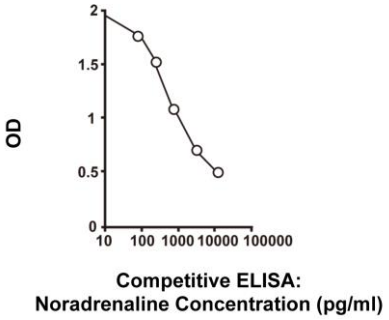
7. Refer to the table below for molar conversion:

	Concentration of standards					
Standard	A	B	C	D	E	F
Adrenaline (pg/ml)	0	20	60	200	800	3200
Adrenaline (pmol/L)	0	109	328	1092	4368	17472
Noradrenaline (pg/ml)	0	80	240	800	3200	12800
Noradrenaline (pmol/L)	0	473	1418	4728	18912	75648
Conversion	Adrenaline (ng/ml) x 5.46 = Adrenaline (nmol/L) Noradrenaline (ng/ml) x 5.91 = Noradrenaline (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

AD: 7 pg/ml

NAD: 35 pg/ml

Assay Range

AD: 20-3200 pg/ml; NAD: 80-12800 pg/ml

Specificity

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

Dopamine, 3-Methoxytyramine, 3-Methoxy-4-hydroxyphenylglycol, Tyramine, Phenylalanine, Caffeinic acid, L-Dopa, Homovanilic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 9.3-17.1%. [Adrenaline]

The CV value of intra-assay precision was 8.4-15.6%. [Noradrenaline]

Recovery

89.4-128.3% (Adrenaline)

104.8-125.6% (Noradrenaline)

Linearity

81-121% (Adrenaline)

84-123% (Noradrenaline)\