Human 3-CAT (Adrenaline + Noradrenaline + Dopamine) ELISA Kit

Enzyme Immunoassay for the quantification of 3-CAT (Adrenaline + Noradrenaline + Dopamine) in plasma and urine samples.

Catalog number: ARG80437

TABLE OF CONTENTS

SECTION **Page**

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantification of 3-CAT (Adrenaline + Noradrenaline + Dopamine) in plasma and urine samples.

This assay employs the competitive quantitative enzyme immunoassay technique. Adrenaline, Noradrenaline and 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 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 coated microplate	12 strips X 8 wells	4°C
Noradrenaline coated microplate	12 strips X 8 wells	4°C
Dopamine coated microplate	12 strips X 8 wells	4°C
Adhesive foil	3 X 4 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
Standard A/B	4 ml (Ready-to-use) (For Dopamine determination in plasma, addition of Standard A/B is mandatory)	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	2 X 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)	6 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
Hydrochloric Acid	20 ml (Ready-to-use)	4°C
Control 1	4 ml (Ready-to-use)	4°C
Control 2	4 ml (Ready-to-use)	4°C

Concentrations of Controls:

	Adrenaline	Noradrenaline	Dopamine
Control 1	8 ± 3.2 ng/ml	40 ± 16 ng/ml	80 ± 32 ng/ml
Control 2	30 ± 12 ng/ml	150 ± 60 ng/ml	300 ± 120 ng/ml

Standards used for different samples:

Samples	Adrenaline	Noradrenaline	Dopamine
Urine	Standard A-F		Standard A-F
Plasma			Standard A-F + Standard A/B

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

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. Assay immediately (up to 48 hours at 2-8 °C, and up to 24 hours at RT), for longer storage, aliquot and store samples at ≤ -20 °C (up to 6 months). Avoid repeated freeze-thaw cycles. Avoid exposure to direct sunlight. If 24-hour urine is used please record the total volume of the collected urine. If the percentage of the final concentration of acid is too high, this will lead to incorrect results for the urine samples.

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 **0.3ml** of **Coenzyme** followed by **0.7ml** of **Adjustment buffer**. The total volume of Enzyme solution is 2ml. **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

1. Pipette **10 µl** of **standards, controls, urine samples** and **300µl of plasma samples** into the appropriate wells of the Extraction Plate.
   Note: for the determination of dopamine in plasma the additional Standard A/B is mandatory!
2. Add **250 µl** of **distilled water** to wells with **standards, controls and urine samples.**
3. Add **50 µl** of **Assay Buffer** to all wells.
4. Add **50 µl** of **Extraction Buffer** to all wells.
5. Cover plate and incubate for **30 mins at RT** on a microplate shaker (~600rpm)
6. 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 min at RT** 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** of **Acylation Buffer** into all wells.

9. Add **25 µl** of **Acylation Reagent** into all wells.
10. Incubate for **15 mins at RT** on a microplate shaker (~600rpm)
11. Remove foil, discard and blot dry by tapping the inverted plate on absorbent material. Wash each well with **1ml** of **1X wash buffer** and shake for **10 min at RT** on a microplate shaker (600rpm). Blot dry by tapping the inverted plate on absorbent material.
12. Add **175 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. Use **20 µl** for **Noradrenaline assay**

    Use **100 µl** for **Adrenaline assay.**

    Use **25 µl** for **Dopamine assay (for standards and urine samples)**

    Use **50 µl** for **Dopamine assay (for plasma samples).**

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** of **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** 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** on a microplate shaker (600rpm).
7. Remove the foil and discard. 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** 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** on a microplate shaker (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 O.D. with a microplate reader at **450 m** (with a reference wavelength between 620nm and 650nm) 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** of **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** 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** on a microplate shaker (600rpm).
7. Remove the foil and discard. 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** 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** on a microplate shaker (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 O.D. with a microplate reader at **450 m** (with a reference wavelength between 620nm and 650nm) within 10 minutes.

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** of **Enzyme solution** (refer to REAGENT PREPARATION section) into all wells of Dopamine Microtiter Strips.
3. Add **25 µl** of the **extracted standards, controls,** urine samples and **50 µl** of plasma samples into the appropriate wells of Dopamine Microtiter Strips.
4. Add **25 µl** of **Hydrochloric Acid** to the **standards, controls and urine samples** wells.
5. Incubate for **30 mins at RT** on a microplate shaker (~600rpm)
6. Add **50 µl** of **Dopamine Antiserum** into wells.
7. Cover plate with Adhesive foil and incubate for **2 hours at RT** on a microplate shaker (600rpm).
8. Remove the foil and discard. 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.
9. Add **100 µl** of **Anti-rabbit IgG-peroxidase conjugate** into wells.
10. Incubate for **30 mins at RT** on a microplate shaker (600rpm).
11. Aspirate each well and **wash as step 8**.
12. Add **100 µl** of **TMB substrate solution** into each well. Incubate for **20-30 mins at RT** on a microplate shaker (600rpm). Avoid exposure to light.
13. Add **100 µl** of **Stop Solution** to each well and shake lightly to ensure

homogeneous mixing.

14. Read the O.D. with a microplate reader at 450 m (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 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. The concentrations of undiluted samples and controls can be read directly from the standard curve.

6.

Measuring range		Adrenaline	Noradrenaline	Dopamine
	Urine (ng/ml)	0.7 - 200	2.5 - 1000	4.8 - 2000
	Plasma (pg/ml)	18 - 6667	93 - 33333	75 - 33333

7. Refer to the table below for molar conversion:

	Concentration of standards						
Standard	A	A/B	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
Dopamine (ng/ml)	0	4.5	10	40	150	500	2000
Dopamine (nmol/L)	0	29	65	261	980	3265	13060
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)						

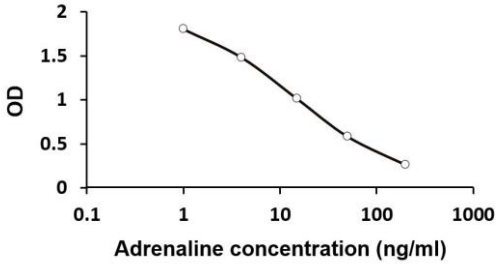
- For the determination of Dopamine in plasma, the addition of Standard A/B is mandatory. Concentration of Standard A/B is 4.5 ng/ml (29 nmol/L)
- Urine samples and Controls: The concentration of urine samples and controls can be read directly from the standard curve.
- Calculate the 24h excretion for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

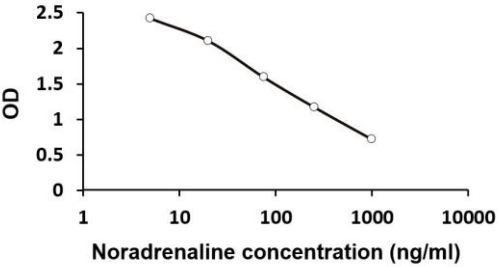
  For **Adrenaline and Noradrenaline** calculations, the read concentrations of **plasma samples** have to be **divided by 30.**

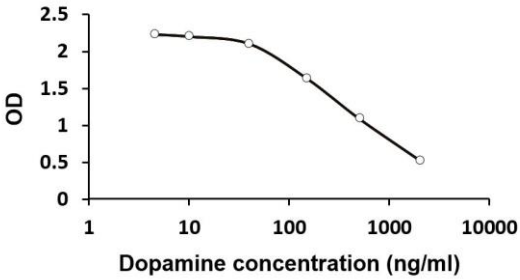
  For **Dopamine** calculations, the read concentrations of **plasma samples** have to be **divided by 60**.

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

LOD:

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

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

Dopamine: urine: 2.5 ng/ml; plasma: 49 pg/ml

LOQ:

Adrenaline: urine: 0.7 ng/ml; plasma: 18 pg/ml

Noradrenaline: urine: 2.5 ng/ml; plasma: 93 pg/ml

Dopamine: urine: 4.8 ng/ml; plasma: 75 pg/ml

Assay Range

Adrenaline: 1-200 ng/ml;

Noradrenaline: 5-1000 ng/ml;

Dopamine: 10-2000 ng/ml (urine), 4.5-2000 ng/ml (plasma)

Specificity

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

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

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

Dopamine: Derivatized Adrenaline, Derivatized 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 and inter-assay precision were as below:

	Intra-assay (%)		Inter-assay (%)	
	Urine	Plasma	Urine	Plasma
Adrenaline	14.3	16.1	13.9	12.9
Noradrenaline	13.4	12.7	16.9	9.8
Dopamine	15.1	26.3	15.6	20.4

Recovery

	Urine	Plasma
Adrenaline	94-120%	88-117%
Noradrenaline	91-113%	75-107%
Dopamine	101-124%	88-92%

Linearity

92-115% (Adrenaline); 100-125% (Noradrenaline); 83-132% (Dopamine)