Human 2-MET (Metanephrine + Normetanephrine) ELISA Kit

Enzyme Immunoassay for the quantification of 2-MET (Metanephrine + Normetanephrine) in Human plasma (EDTA, heparin).

Catalog number: ARG82791

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INTRODUCTION

Metanephrine (metadrenaline) is a metabolite of epinephrine (adrenaline) created by action of catechol-O-methyl transferase on epinephrine. An article in the Journal of the American Medical Association, 2002, indicated that the measurement of plasma free levels of the metanephrines group of molecules (including metanephrine and normetanephrine) is the best tool in the diagnosis of pheochromocytoma, an adrenal medullary neoplasm. [Provided by Wikipedia: Metanephrine]

Normetanephrine is a metabolite of norepinephrine created by action of catechol-O-methyl transferase on norepinephrine. It is excreted in the urine and found in certain tissues. It is a marker for catecholamine-secreting tumors such as pheochromocytoma. [Provided by Wikipedia: Normetanephrine]

PRINCIPLE OF THE ASSAY

This assay employs the competitive quantitative enzyme immunoassay technique. First, the target in plasma are collection by extraction procedure. 2MET (Metaphrine and Normetaphrine) in Controls, samples or Standards are then quantitatively acylated. The antigen has been pre-coated onto a microtiter plate. Acylated Controls, Standards or samples and the solid phase bound analytes compete for a number of antibody binding sites. When the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing procedure. HRP conjugated 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 inversely proportion to the amount of 2-MET 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 450 nm. The concentration of 2-MET in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Protein Coated microplate 1 (For Metanephrine)	8 X 12 strips	4°C
Protein Coated microplate 2 (For Normetanephrine)	8 X 12 strips	4°C
Standards A to F	4 mL each (ready to use)	4°C
Control 1 & 2	4 mL each (ready to use)	4°C
Diluent Buffer	10 mL (ready to use)	4°C
Assay Buffer	30 mL (ready to use)	4°C
Equalizing Reagent	14 mL (ready to use)	4°C
Acylation Concentrate	1.5 mL	RT
25X Cleaning Concentrate	20 mL	4°C
Elution Buffer	14 mL (ready to use)	4°C
Antibody Conjugate 1 (Anti-Metaneohrine antibody)	6 mL (ready to use)	4°C
Antibody Conjugate 2 (Anti-Normetaneohrine antibody)	6 mL (ready to use)	4°C
HRP Conjugate	12 mL (ready to use)	4°C
50X Wash Buffer	2 X 20 mL	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
Extraction Plate	1 X 96 plate	4°C
Adhensive foil	2 X 4 pieces	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Mixer or Ultra-Turrax
- Microplate shaker
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- The antibodies used in this test kit **only recognise the biologically relevant L-forms of Metanephrines**. Commercially available synthetic Normetanephrine or Metanephrine is always a mixture of the D- and Lform. The ratio between both forms differs widely from lot to lot. This has important implications if synthetic Metanephrines are used to enrich native samples. As only about 50% of the synthetic Metanephrines - the L-portion - will be detected by use of this kit, spiked samples will be underestimated. Therefore **native samples containing solely the L-form should be used.**
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (20-25°C).
- Remove the number of strips required and return unused strips to the pack and reseal.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 50X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.

- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Use a new adhesive plate cover for each incubation step.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Plasma: Collect blood with heparin or citrate and centrifuge at 1500 x g and 4°C for 15 minutes within 30 minutes of collection.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum/plasma sample.
3. Samples containing sodium azide should not be used in the assay.
4. Specimens stored for a longer time (up to 6 months) should be frozen only once at -80°C prior to assay. Thawed samples should be inverted several times prior to testing. Avoid freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 50X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 10 mL of 50X Wash Buffer into 490 mL of distilled water to a final volume of 500 mL) The 1X Wash Buffer is stable for up to 4 weeks at 2-8°C.
- 1X Cleaning Buffer: Dilute 25X Cleaning Buffer into distilled water to yield 1X Cleaning Buffer. (E.g., add 20 mL of 25X Cleaning Buffer into 480 mL of distilled water to a final volume of 500 mL) The 1X Cleaning Buffer is stable for up to 4 weeks at 2-8°C.
- Acylation solution: The Aclylation Concentrate should be stored at room temperature. Pipette 80 µL of Acylation Concentrate to 3 mL of distilled water and mix thoroughly. Prepare fresh and use immediately because the Acylation solution is only stable for a maximum of 3 minutes.

ASSAY PROCEDURE

Extraction

1. Pipette 20 µL of Standards and Controls into the respective Extraction Plate.
2. Add 200 µL of Equalizing Reagent to the wells with Standards and Controls.
3. Add 200 µL of plasma samples to the respective wells.
4. Add 20 µL of Standard A to all tubes containing plasma samples.
5. Incubate Extraction Plate at RT for 2 hours on a microplate shaker.
6. Empty plate and blot dry by tapping the inverted plate on absorbent material.
7. Add 250 µL of Assay Buffer into all wells. Incubate Extraction Plate at RT for 5 minutes on a microplate shaker.
8. Empty plate and blot dry by tapping the inverted plate on absorbent material.
9. Wash the plate 3 times by adding 350 µL of 1X Cleaning Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
10. Add 100 µL of Elution Buffer into all wells.
11. Cover plate with adhesive foil. Incubate 15 minutes at RT on a microplate shaker.
12. Remove the foil. Do not decant the supernatant thereafter!
13. Take 50 µL of the clear supernatant for Metanephrine ELISA assay or 25 µL of the clear supernatant for Normetanephrine ELISA assay.

2-MET ELISA procedure

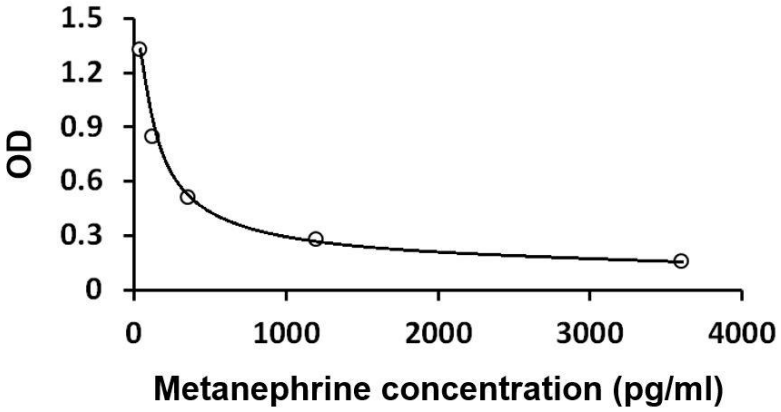
All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

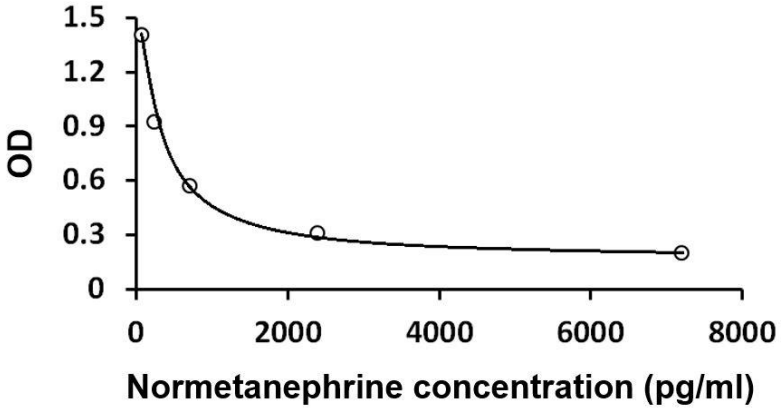
1. Add **25 µL** of **Diluent Buffer** into the appropriate wells of Normetanephrine Microtiter Strips.
2. Add **50 µL** (For Metanephrine) or **25 µL** (For Noretanephrine) of extracted **samples, Control and Standards** into the appropriate wells.
3. Add **25 µL** of **Acylation Solution** into wells. **NOTE: The acylation solution is only stable for 3 minutes.**
4. Seal the plate with the provided foil and incubate at **RT** for **15 minutes** on a microplate shaker.
5. Add **50 µL** of **Antibody Conjugate** into all wells.
6. Seal the plate with the provided foil and incubate at **RT** for **1 minutes** on a microplate shaker. Then Incubate for **15-20 hours (overnight)** at **2-8°C** or incubate for **2 hours** at **RT** on a microplate shaker.
7. 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.
8. Add **100 µL** of **HRP Conjugate** into each wells.
9. Seal the plate with the provided foil and incubate at **RT** for **30 minutes** on a microplate shaker.
10. Aspirate each well and **wash as step 3**.

11. Add 100 µL of TMB Substrate to each well, including the blank wells. Incubate in the dark for 20-30 minutes at RT without shacking.
12. Immediately Add 100 µL of Stop Solution to each well, including the blank wells. The color of the solution should change from blue to yellow.
13. Read the OD with a microplate reader at 450 nm immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance within 10 minutes after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD VALUES

The following figures demonstrate typical results with the Human Normetanephrine ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.





CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of Controls, 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. 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.
4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (https://www.arigobio.com/elisa-analysis)
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.
6. Refer to the table below for molar conversion:

	Concentration of standards					
Standard	A	B	C	D	E	F
Metanephrine (pg/ml)	0	36	120	360	1200	3600
Metanephrine (pmol/L)	0	183	608	1825	6084	18252
Conversion	Metanephrine (pg/ml) x 5.07 = Metanephrine (pmol/L)					

	Concentration of standards					
Standard	A	B	C	D	E	F
Normetanephrine (pg/ml)	0	72	240	720	2400	7200
Normetanephrine (pmol/L)	0	393	1310	3931	13104	39312
Conversion	Normetanephrine (pg/ml) x 5.46 = Normetanephrine (pmol/L)					

QUALITY ASSURANCE

Sensitivity

The limit of detection (LOD) of Metanephrine is 14.9 pg/mL.

The limit of detection (LOD) of Normetanephrine is 17.9 pg/mL.

Specificity

	Substance	Cross Reactivity (%)	
		Metanephrine	Normetanephrine
Analytical Specificity	Derivatized Metanephrine	100	0.72
	Derivatized Normetanephrine	005	100
	3-Methoxytyramin	< 0.01	6.5
	Adrenaline	< 0.01	< 0.01
	Noradrenaline	< 0.01	< 0.01
	Dopamin	< 0.01	< 0.01
	Vanillic mandelic acid	< 0.01	< 0.01
	Homovanillic acid	< 0.01	< 0.01
	L-DOPA	< 0.01	< 0.01
	L-Tyrosin	< 0.01	< 0.01
	Acetaminophen	< 0.01	< 0.01

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 9.2-13.5% and CV value of inter-assay precision was 7.5-17.6%. (Metanephrine)

The CV value of intra-assay precision was 8.2-10.5% and CV value of inter-assay precision was 5.0-10.6%. (Normetanephrine)

Recovery

Metanephrine 80-99%

Normetanephrine 105-114%