Human Metanephrine ELISA Kit

Enzyme Immunoassay for the quantification of Metanephrine in Human urine.

Catalog number: ARG82792

Package: 96 wells

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MANUFACTURED BY:

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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]

PRINCIPLE OF THE ASSAY

This assay employs the competitive quantitative enzyme immunoassay technique. First, the target in urine are collection by hydrolysis procedure. Metanephrine 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 Metanephrine 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 Metanephrine 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	8 X 12 strips	4°C
Standards A to F (0, 20, 60, 200, 600, 2000 ng/mL)	4 mL each (ready to use)	4°C
Control 1 & 2	4 mL each (ready to use)	4°C
Hydrochloric Acid	30 mL (ready to use)	4°C
Acylation Concentrate	0.5 mL	4°C
Acylation Diluent	4 mL (ready to use)	4°C
Acylation Buffer	30 mL (ready to use)	4°C
Antibody Conjugate (Anti-Metaneohrine antibody)	12 mL (ready to use)	4°C
HRP Conjugate	12 mL (ready to use)	4°C
50X Wash Buffer	20 mL	4°C
TMB substrate	12 mL (ready to use)	4°C (protect from light)
STOP solution	12 mL (ready to use)	4°C
Reaction Tubes	2 X 50 tubes	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Water bath (Temperature controlled, 90°C) or similar heating device
- 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 assay can be performed with or without shaking. If a microplate shaker is used, it should have the following characteristics: shaking amplitude 3 mm; approx. 600 rpm.
- 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.

Urine: Spontaneous or 24-hour urine, collected in a bottle containing 10- 15 ml of 6 M HCl, should be used. Store at 2-8°C for up to 5 days, and store at -20°C for longer periods (up to 6 months). Avoid freeze-thaw cycles and direct sunlight.

Note:

1. Samples containing sodium azide should not be used in the assay.

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.
- Acylation solution: Before preparing the Acylation Solution make sure that the Acylation Diluent has reached room temperature (≥ 20°C) and forms a homogenous, crystal-free solution. Pipette 10 µL of Acylation Concentrate to 600 µL of Acylation Diluent and mix thoroughly. Prepare fresh and use immediately because the Acylation solution is only stable for a maximum of 60 minutes.

ASSAY PROCEDURE

Hydrolysis

1. Pipette 25 µL of Standards, Controls and urine samples into the respective Reaction Tubes.
2. Add 250 µL of Hydrochloric Acid to all tubes.
3. Mix thoroughly (vortex) and hydrolyze for 30 minutes at 90°C.
4. Cool down the tubes to room temperature (RT, 20-25°C).

Note: For the measurement of the free Metanephrine only, leave away step 3 and 4.

Acylation

1. Pipette 25 µL of Acylation Buffer into all tubes.
2. Add 25 µL of Acylation Solution to all tubes.
3. Mix thoroughly (vortex) and acylate for 15 minutes at RT.
4. Add 2.5 mL of distilled water to all tubes.
5. Take 25 µL of the acylated Standards, Controls and urine samples for the assay.

Metanephrine ELISA procedure

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

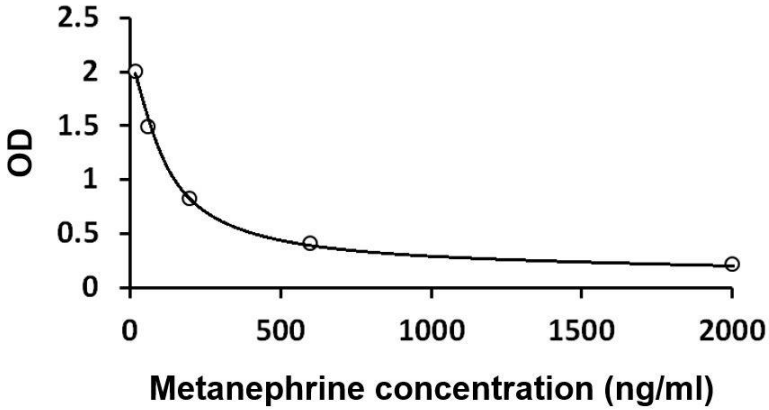
1. Add 25 µL of acylated Standards, Controls and urine samples into the appropriate wells of the Metanephrine Microtiter Strips.
2. Add 100 µL of Antibody Conjugate into all wells.
3. Incubate at RT for 30 minutes on a microplate shaker. Note: Without usage of a shaker, shack plate shortly by hand and incubate at RT for 1 hour.
4. 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.

5. Add **100 µL** of **HRP Conjugate** into each wells.
6. Incubate at **RT** for **30 minutes** on a microplate shaker.
7. Aspirate each well and **wash as step 4**.
8. Add **100 µL** of **TMB Substrate** to each well, including the blank wells. Incubate in the dark for **15 ± 2 minutes** at **RT** on a microplate shaker.
9. 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.
10. 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 Metanephrine 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 details. (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. Conversion: Metanephrine (ng/ml) x 5.07 = Metanephrine (nmol/l)
7. The amount of analyte excreted per day (µg/day) is calculated according to: concentration of the sample (µg/L) x volume of urine excreted per day (L/day)

   Example: The concentration of the sample read from the curve is 125 µg/L. The amount of urine collected during 24 hours is 1.3 L/day. Then the amount of analyte excreted during one day would be:

   125 µg/l x 1.3 l/day = 162.5 µg/day

8. Metanephrine expected reference value < 350 µg/day

QUALITY ASSURANCE

Sensitivity

The limit of detection (LOD) of Metanephrine is 8.6 ng/mL.

Specificity

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

Adrenaline, Noradrenaline, Normetanephrine, 3-Methoxytyramine, Dopamine, Vanillic mandelic acid, Homovanillic acid, L-DOPA, L-Tyrosin, Tyramine.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 9-12% and CV value of inter-assay precision was 16-17%.

Recovery

83-113%