Metanephrine (Plasma) EIA

For the quantitative determination of free metanephrine
in EDTA or heparin plasma.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 17-METHU-E01-FST
Size: 96 Wells
Version: 20.0-r 2022-03-25 – ALPCO 2.0

26-G Keewaydin Drive, Salem, NH 03079 | P: (800) 592-5726 | F: (603) 898-6854 | ts@alpco.com | www.alpco.com

1. Introduction

1.1. Intended Use

Enzyme immunoassay for the quantitative determination of free metanephrine in EDTA or heparin plasma.

1.2. Principle of the Assay

Metanephrine (Metadrenaline) is first extracted using an ion exchange matrix followed by an acylation process. The subsequent competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated standards, controls, and samples and the solid phase bound analytes compete for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm.
Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standards.

2. Procedural Cautions, Guidelines, Warnings and Limitations

2.1. Procedural cautions, guidelines, and warnings

1. 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 must be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Reagents of this kit which contain serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg, and HCV by approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. The principles of Good Laboratory Practice (GLP) must be followed.
4. To reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves, and protective glasses where necessary.
5. All kit reagents and samples should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and samples.
6. For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
7. 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. Removed strips should be marked to avoid mix up.
8. Duplicate determination of sample is highly recommended.
9. Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials, and devices are prepared and ready at the appropriate time.
10. Incubation times do influence the results. All wells should be handled in the same order and time intervals.
11. To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard, and control.
12. A standard curve must be established for each run.
13. The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
14. Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.

15. Avoid contact with Stop Solution containing 0.25 M H_2SO_4. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
16. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them.
17. In case of any severe damage to test kit or components, inform ALPCO promptly. Do not use until acceptable use is confirmed by ALPCO.
18. For information on hazardous substances included in the kit please refer to Safety Data Sheet (SDS). The Safety Data Sheet for this product is made available directly on ALPCO's website.
19. Kit reagents must be regarded as hazardous waste and disposed according to national regulations.

2.2. Limitations

Any inappropriate handling of samples or modification of this test might influence the results. Commercially available synthetic normetanephrine or metanephrine is always a mixture of the D- and L- forms. This has important implications if synthetic metanephrines are used to enrich native samples. The antibody used in this kit has a specific D- and L- form recognition rate. Contact ALPCO for details in case synthetic metanephrine was used to enrich native samples.

2.2.1. Interfering substances

Samples containing precipitates or fibrin strands might cause inaccurate results. Hemolyzed samples (up to 0.5 mg/mL hemoglobin), icteric samples (up to 0.5 mg/mL bilirubin), and lipemic samples (up to 17 mg/mL triglycerides) have no influence on the assay results. If the concentrations cannot be established and there are doubts as to whether hemoglobin, bilirubin, or triglycerides fall below the concentrations listed above, the sample should not be used in the assay.

2.2.2. Drug interferences

Medications like antihypertensive agents, antidepressants, antipsychotics, sympathomimetics, and L-DOPA can influence plasma metanephrine levels. Caffeinated beverages, nicotine, and mood-enhancing drugs can also affect plasma metanephrine levels. In addition, stress and physical strain should be avoided shortly before sampling.

2.2.3. High-Dose-Hook effect

No hook effect was observed in this test.

3. Storage and stability

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 2 months when stored at 2 – 8°C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

4. Materials

4.1. Contents of the kit

FOILS	**Adhesive Foil** - Ready to use
Contents:	Adhesive Foils in a resealable pouch
Volume:	1 x 4 foils
WASH-CONC 50x	**Wash Buffer Concentrate -** Concentrated 50x
Contents:	Buffer with a non-ionic detergent and physiological pH

Volume: 1 x 20 mL/vial, purple cap

CONJUGATE **Enzyme Conjugate** - Ready to use
Contents: Goat anti-rabbit immunoglobulins conjugated with peroxidase
Volume: 1 x 12 mL/vial, red cap

SUBSTRATE **Substrate** - Ready to use
Contents: Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide
Volume: 1 x 12 mL/vial, black cap

STOP-SOLN **Stop Solution** - Ready to use
Contents: 0.25 M sulfuric acid
Volume: 1 x 12 mL/vial, grey cap

H290 May be corrosive to metals.

ADR MN **Metanephrine Microtiter Strips** - Ready to use
Contents: 1 x 96 wells (12 strips x 8 wells) antigen pre-coated microwell plate in a resealable blue pouch with desiccant

MN-AS **Metanephrine Antiserum** - Ready to use
Contents: Rabbit anti-metanephrine antibody in buffer with proteins and non-mercury preservative, blue colored
Description: Species of antibody is rabbit, species of protein in buffer is bovine
Volume: 1 x 6 mL/vial, blue cap

Standards and **Controls** - Ready to use

Component	Color/Cap	Concentration pg/mL	Concentration pmol/L	Volume/ vial
STANDARD A	white	0	0	4 mL
STANDARD B	yellow	36	183	4 mL
STANDARD C	orange	120	608	4 mL
STANDARD D	blue	360	1825	4 mL
STANDARD E	grey	1200	6084	4 mL
STANDARD F	black	3600	18252	4 mL
CONTROL 1	green	Refer to QC Report for expected value and acceptable range.		4 mL
CONTROL 2	red			4 mL

Conversion: Metanephrine (pg/mL) x 5.07 = Metanephrine (pmol/L)
Contents: Acidic buffer spiked with defined quantity of metanephrine

ADJUST-BUFF **Adjustment Buffer –** Ready to use
Contents: Tris-buffer
Volume: 1 x 10 mL/vial, yellow cap

ASSAY-BUFF **Assay Buffer –** Ready to use
Contents: 25% Organic Solvent
Volume: 1 x 30 mL/vial, orange cap

ACYL-CONC **Acylation Concentrate** - Concentrated

Contents: Acylation reagent in DMSO

Volume: 1 x 1.5 mL/vial, white cap

Hazards Identification: 

H302 Harmful if swallowed.
H319 Causes serious eye irritation.
H335 May cause respiratory irritation.

EXTRACT-PLATE 96 **Extraction Plate** – Ready to use

Contents: 1 x 96 well plate, precoated with ion-exchanger in a resealable pouch.

CLEAN-CONC 25x **Cleaning Concentrate** – Concentrated 25 X

Contents: Buffer with sodium acetate

Volume: 1 x 20 mL/vial, brown cap

ELUTION-BUFF **Elution Buffer** – Ready to Use

Contents: 0.1 M Sodium hydroxide, dark purple colored

Volume: 1 x 14 mL/vial, green cap

EQUA-REAG **Equalizing-Reagent** – Ready to Use

Contents: Human serum, negative for HIV I/II, HBsAg, and HCV

Volume: 1 x 14 mL/vial, white cap
Description: Species is human

4.2. Additional materials and equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 20 - 350 µL;3 mL
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 - 650 nm
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm*)
  *Shaking with different settings might influence test results
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)
- Vortex mixer
- Timer

5. Sample Collection and Storage

EDTA- or Heparin - Plasma

Whole blood should be collected into centrifuge tubes (Monovette ™ or Vacuette™) containing EDTA or heparin as anti-coagulant and centrifuged (according to manufacturer's instructions) immediately after collection.

Hemolyzed, icteric, and lipemic samples should not be used for this assay.
Storage: up to 3 days at 2 – 8°C, for longer periods (up to 6 month) at -20°C
Repeated freezing and thawing should be avoided.

6. Test Procedure

\Allow all reagents to reach room temperature and mix thoroughly by gentle inversion before use. Duplicate determinations are recommended. It is recommended to number the strips of the microwell and extraction plates before use to avoid any mix-up.

The binding of the antibodies, enzyme conjugates, and the activity of the enzyme used are temperature dependent; the absorption values may vary if a thermostat is not used. The higher the temperature, the higher the absorption values will be. Varying incubation times will have similar influences on the absorbance. The optimal temperature during the enzyme immunoassay is between 20 – 25°C.

If the product is prepared in parts, unused wells in each Reaction and Extraction Plates should be covered to avoid contamination. After preparation, the used wells must be labeled to prevent double use. During overnight incubation at 2 – 8°C with the antiserum, the temperature should be uniform all over the ELISA plate to avoid any drift and edge-effect.

⚠ *In case of ready overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm.*

6.1. Preparation of Reagents

Wash Buffer

Dilute the 20 mL Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 mL.
Storage: 2 months at 2 - 8°C

Cleaning Buffer
Dilute the 20 mL Cleaning Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 500 mL.
Storage: 2 months at 2 - 8°C

Acylation Solution
As the acylation solution is only stable for a maximum of 3 minutes. It should not be prepared before starting the assay. Therefore its preparation is described in the protocol in chapter 6.3, step 3.

Metanephrine 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.

Extraction Plate
In rare cases, residues of the cation exchanger can be seen in the wells as small, black dots or lines. These residues do not influence the quality of the product.

6.2. Preparation of samples

Extraction
The following extraction procedure can be run with 200 µL or 250 µL of plasma sample. The procedure for 200 µL sample is listed below, titled **200 µL Sample Procedure.** The procedure

for 250 µL sample is titled **250 µL Sample Procedure.**

200 µL Sample Procedure.

1. Pipette **20 µL** of **standards** and **controls** into the respective wells of the **Extraction Plate.**
2. Pipette **20 µL Standard A** to all wells containing plasma samples.
3. Add **200 µL** of **Equalizing Reagent** to the wells with **standards and controls.**
4. Pipette **200 µL** of **plasma samples** to the respective wells.
5. Incubate plate for 2 hours at RT (20 – 25°C) on a shaker (approx. 600 rpm)
6. Empty plate and blot dry by tapping the inverted plate on absorbent material.
7. Pipette **250 µL** of **Assay Buffer** into all wells. Incubate the plate for 5 min at RT (20 – 25°C) on a shaker (approx. 600 rpm). Empty plate and blot dry by tapping the inverted plate on absorbent material.
8. Wash the plate **3 x** by adding **350 µL** of **Cleaning Buffer, discarding** the content and blotting dry each time by tapping the inverted plate on absorbent material.
9. Pipette **100 µL** of **Elution Buffer** into all wells. *Please note: the color changes caused by the elution buffer can vary between standards and samples .*
10. Cover plate with adhesive foil. Incubate 15 min at RT (20 – 25°C) on a shaker (approx. 600 rpm). DO NOT decant the supernatant thereafter! The following volumes of the supernatant are needed for the subsequent ELISA. **Metanephrine 50 µL**

250 µL Sample Procedure.

1. Pipette **25 µL** of **standards** and **controls** into the respective wells of the **Extraction Plate.**
2. Pipette **25 µL Standard A** to all wells containing plasma samples.
3. Add **250 µL** of **Equalizing Reagent** to the wells with **standards and controls.**
4. Pipette **250 µL** of **plasma samples** to the respective wells.
5. Incubate plate for 2 hours at RT (20 – 25°C) on a shaker (approx. 600 rpm)
6. Empty plate and blot dry by tapping the inverted plate on absorbent material.
7. Pipette **250 µL** of **Assay Buffer** into all wells. Incubate the plate for 5 min at RT (20 – 25°C) on a shaker (approx. 600 rpm). Empty plate and blot dry by tapping the inverted plate on absorbent material.
8. Wash the plate **3 x** by adding **350 µL** of **Cleaning Buffer, discarding** the content and blotting dry each time by tapping the inverted plate on absorbent material.
9. Pipette **125 µL** of **Elution Buffer** into all wells. *Please note: the color changes caused by the elution buffer can vary between standards and samples .*
10. Cover plate with adhesive foil. Incubate 15 min at RT (20 – 25°C) on a shaker (approx. 600 rpm). DO NOT decant the supernatant thereafter! The following volumes of the supernatant are needed for the subsequent ELISA. **Metanephrine 50 µL**

6.3. Metanephrine ELISA

1. Pipette **25 µL** of **Adjustment Buffer** into all wells of the **Metanephrine Microtiter Strips.**

2.	Pipette **50 µL** of extracted standards, controls, and samples into the respective wells.
3.	Preparation of Acylation Solution: Pipette **80 µL** of **Acylation Reagent Concentrate** to 3 mL DI water mix thoroughly.
4.	Pipette **25 µL** of the freshly prepared **Acylation Solution** into all wells.
5.	Incubate for 15 min at RT (20 – 25°C) on a shaker (approx. 600 rpm).
6.	Pipette **50 µL** of the **Metanephrine Antiserum** into all wells.
7.	Cover the plate with **Adhesive Foil**, shake for 1 min at RT (20 – 25°C) on a shaker and incubate for 15 – 20 hours (overnight) at 2 – 8°C without shaking.
8.	Remove the foil. Discard or aspirate the content of the wells. Wash the plate **4 times** by adding **300 µL** of 1X working **Wash Buffer, discarding** the contents and **blotting dry each time** by tapping the inverted plate on absorbent material.
9.	Pipette **100 µL** of the **Enzyme Conjugate** into all wells.
10.	Incubate for **30 min** at **Room Temperature** (20 – 25°C) on a **shaker** (approx. 600 rpm).
11.	Discard or aspirate the content of the wells. Wash the plate **4 times** by adding **300 µL** of 1X working **Wash Buffer, discarding** the contents and **blotting dry each time** by tapping the inverted plate on absorbent material.
12.	Pipette **100 µL** of the substrate into all wells and incubate for **20-30 minutes** at **RT (**20 – 25°C) on a shaker (approx. 600 rpm). ***Avoid exposure to direct sunlight!***
13.	Add **100 µL** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
14.	**Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** (if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

Measuring range	**Metanephrine**
	15.1 – 3600 pg/L

The standard curve is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis) using a concentration of 0.001 pg/mL for Standard A. Use a non-linear regression for curve fitting (e.g. 4-parameter,marquardt).

This assay is a competitive assay. This means the Optical Density (OD) values decrease with increasing concentration of the analyte. OD values found below the standard curve correspond to high concentrations of the analyte in the sample and must be reported as greater than the top standard concentration. Samples found with concentrations higher than the highest standard (Standard F) may be diluted accordingly with the included Equalizing Reagent and re-assayed.

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

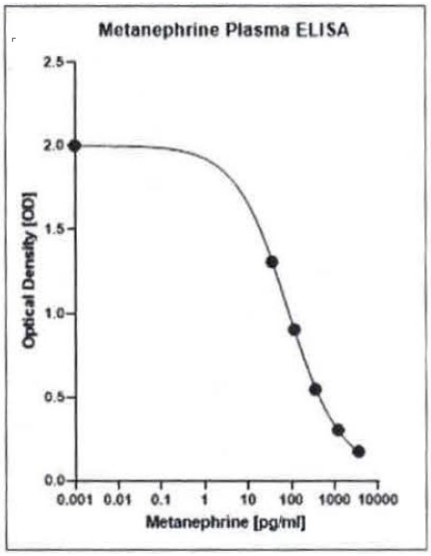
Conversion
Metanephrine (pg/mL) x 5.07 = Metanephrine (pmol/L)

7.1. Quality control

The confidence limits of the kit controls are indicated on the QC Report.

7.2. Typical standard curve

Example only, do not use for calculation!



8. Assay Characteristics

Analytical Sensitivity (Limit of Detection)		**Metanephrine**
	LOB (pg/mL)	9.9
	LOD (pg/mL)	14.9
	LOQ (pg/mL)	15.1

	Substance	**Cross-reactivity (%)**
		Metanephrine
Analytical Specificity (Cross-reactivity)	Metanephrine	100
	Normetanephrine	0.05
	3-Methoxytyramin	< 0.001
	Adrenaline	< 0.001
	Noradrenaline	< 0.001
	Dopamin	< 0.001
	Vanillic mandelic acid	< 0.001
	Homovanillic acid	< 0.001
	L-DOPA	< 0.001
	L-Tyrosine	< 0.001
	Tyramine	< 0.001
	Acetaminophen	< 0.001

Precision

	Intra-Assay			Inter-Assay		
	Sample	Mean (pg/mL)	CV (%)	Sample	Mean (pg/mL)	CV (%)
Metanephrine	1	66.3	11.4	1	67.8	17.6
	2	122	13.5	2	134	12.7
	3	308	10.6	3	319	11.0
	4	783	9.2	4	847	7.5

Lot-to-Lot			
Metanephrine (n=6)	Sample	Mean ± SD [pg/mL]	CV (%)
	1	97.7 ± 16.5	16.9
	2	870 ± 117	13.5

	Range [pg/mL]	Mean (%)	Range (%)
Recovery	33.0 - 2928	88	80-99

Linearity	Metanephrine	Serial dilution up to	Mean	Range (%)
		1:64	107%	101 -124

Method Comparison versus HPLC	Metanephrine	y = 0.91x +1.8	r = 0.96; n = 46

Metrological Traceability
The values assigned to the standards and controls of the Metanephrine ELISA are traceable to SI units by calibrated weighing with quality-controlled analyte.

Standards and Controls	
Metanephrine	Uncertainty [%]
	2.0 %

2-MET Plasma ELISA	
Metanephrine	Expanded Uncertainty [%] k =2*
	25.7 %

* This defines an interval about the measured result that will include the true value with a probability of 95%.