EAGLE
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Normetanephrine & Metanephrine (Plasma) ELISA Assay Kit

Catalog Number: NMN31-K02
2 x 96 Wells
For Research Use Only (RUO). Not for use in clinical, diagnostic or therapeutic procedures.
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1. Introduction and Principle of the Test

The Eagle Biosciences Normetanephrine & Metanephrine (Plasma) ELISA Assay Kit is intended for the quantitative determination of normetanephrine and metanephrine in plasma by enzyme immunoassay. The Normetanephrine and Metanephrine (Plasma) ELISA Kit is for research use only and not to be used in clinical, therapeutic or diagnostic procedures.

Normetanephrine and metanephrine are physiologically formed from the catechol-amines noradrenaline and adrenaline by the enzyme catechol-O-methyltransferase (COMT). Increased levels of normetanephrine and metanephrine can be found in pheochromocytoma, ganglioneuroma and other neurogenic tumors. The Normetanephrine & Metanephrine (Plasma) ELISA assay kit provides materials for the quantitative measurement of free normetanephrine and metanephrine in EDTA plasma. Normetanephrine and metanephrine are quantitatively acylated to their N-acyl-derivates.

The competitive Normetanephrine & Metanephrine (Plasma) ELISA Assay Kit uses the microtiter plate format. Metanephrine and normetanephrine, respectively, are bound to the solid phase of the microtiter plate. Acylated nor-/metanephrines from the sample and solid phase bound nor-/metanephrines 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. The antibody bound to the solid phase nor-/metanephrine is detected by anti-rabbit IgG / peroxidase. The substrate TMB / peroxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase catecholamine is inversely proportional to the catecholamine concentration of the sample.

2. Precautions

- The Normetanephrine and Metanephrine (Plasma) ELISA Assay Kit is for research use only and not to be used in clinical, therapeutic or diagnostic procedures.
- Some reagents contain sodium azide as preservative. Avoid skin contact.
- All reagents of human origin used in this Normetanephrine and Metanephrine (Plasma) ELISA kit are tested for HIV I/II antibodies, HCV and HBsAg and found to be negative. However, because no test method can offer complete assurance that infectious agents are absent, these reagents should be handled as potentially biohazardous materials.
- Material of animal origin used in the preparation of the Normetanephrine and Metanephrine (Plasma) ELISA Assay kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.
- Where required reagents show the respective hazard symbol on the label.

3. Storage and Stability

On arrival, store the Normetanephrine and Metanephrine (Plasma) ELISA Assay kit at 2-8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents. Do not use components beyond the expiration date shown on the kit labels. Do not mix various lots of any kit component within an individual assay.

4. Contents of the Kit

4.1 **Microtiter Strips** 2 x 12 strips
STRIPS-MN **STRIPS-NMN**
8 wells each, break apart
precoated with metanephrine (12 strips), colour-coded blue,
and normetanephrine (12 strips), colour-coded yellow

4.2 **Standards 1 – 6** **CAL 1 - 6** 7 vials
each 1.5 ml, lyophilized

Concentrations variable, see q.c. certificate
2 x Standard 1 for dilution of high level samples

4.3 **Control 1 & 2** **CON 1 & 2** 2 vials
each 1.5 ml, lyophilized
Range: See q.c. certificate

4.4 **Acylation Reagent** **ACYL-REAG** 3 vials
2.5 ml, lyophilised, dissolve with Solvent

4.5 **Acylation Buffer** **ACYL-BUFF** 1 vial
6 ml, ready for use

4.6 **Metanephrine Antiserum** **AS-MN** 1 vial
0.45 ml, concentrated, colour-coded blue
Rabbit-anti-N-acyl-metanephrine

4.7 **Normetanephrine Antiserum** **AS-NMN** 1 vial
4 ml, ready for use, coloured-coded yellow
Rabbit-anti-N-acyl-normetanephrine

4.8 **Enzyme Conjugate** **CONJ** 2 vials
13 ml, ready for use, anti-rabbit IgG-POD conjugate

4.9 **Wash Buffer** **WASH** 2 vials
20 ml, concentrated (50x)

4.10 **Substrate** **SUB** 2 vials
13 ml TMB solution, ready for use

4.11 **Stop Solution** **STOP** 2 vial
13 ml, ready for use
contains 0.3 M sulphuric acid

4.12 **Precipitation Tubes** **PRECI-TUBE** 100 pcs.

4.13 **Precipitator 1** **PRECI 1** 1 vial

4.14 **Precipitator 2** **PRECI 2** 1 vial
3.5 ml, ready for use, irritant

4.15 **Solvent** **SOLVENT** 2 vials
5.5 ml, ready for use
contains acetone, irritant, highly flammable

4.16 **Adhesive Foil** **FOIL** 4 pcs
ready for use

Additional materials and equipment required but not provided:

- Pipettes (25, 40, 50, 100 µl and 200 µl)
- Multichannel pipette or Microplate washing device
- Eppendorf Multipette (or similar devices)
- Microplate photometer (450nm)
- Centrifuge (4,000 x g)
- Vortex mixer
- Distilled water

5. Sample Collection and Storage

EDTA plasma should be used.

Drugs, alcohol and tobacco as well as stress influence the catecholamine release. This may lead to false positive results for metanephrine and normetanephrine. If clinically acceptable, medication (i.e. L-Dopa, alpha-blocker, antidepressants, MAO inhibitor, etc.) should be stopped five days before blood collection.

Patient should adhere to an at least four hours fasting; no tea, coffee, alcohol, nicotine and no strong physical activity. It is recommended to let the patient rest for 20 to 30 minutes after the venipuncture and before collecting the blood sample. The samples can be stored up to 6 hours at 2 - 8 °C. For a longer storage the samples must be frozen at -20 °C and are stable for at least 12 months. Repeated freezing and thawing should be avoided.

6.1. Preparation of Reagents

Standards and Controls
Dissolve standards and controls with 1.5 ml dist. water each, vortex shortly and leave on a roll mixer or similar shaker for minimum 20 minutes. Handle with care in order to minimize foam formation. The reconstituted standards and controls should be stored frozen at -20 °C and are stable until expiry date printed on vial label.

Acylation Reagent
Dissolve the content of one bottle in 2.5 ml Solvent and shake for minimum 15 minutes on a roll mixer or similar shaker. The Acylation Reagent has always to be prepared immediately before use and is stable for at least 3 hours. The two additional bottles are allowing a second and a third run of the test. If the whole kit is to be used in one run it is recommended to pool the dissolved contents of two vials of Acylation Reagent. After use the reagent has to be discarded.

Metanephrine Antiserum
Concentrate, has to be diluted 1 + 9 with dist. water before use. Diluted antiserum is stable for only one day. Therefore it is recommended to prepare the dilution freshly and only as much as necessary.

Wash Buffer
Dilute the content with dist. water to a total volume of 1,000 ml, mix shortly. The diluted wash buffer has to be stored at 2 - 8 °C for a maximum period of 4 weeks. For longer storage the diluted wash buffer should be stored frozen at -20 °C and is stable until expiry date printed on vial label.

All other reagents are ready for use.

6.2. Preparation of Samples (Precipitation and Acylation)

Allow all reagents to reach room temperature.
Duplicates are recommended.

The preparation of the standards, controls, and plasma samples is identical for both metanephrine and normetanephrine and has to be done only once.

1. Pipette each **200 µl dissolved Standards, Controls and Samples** into the respective marked **Precipitation Tubes**.
2. Pipette each **25 µl Precipitator 1** into all tubes.
3. Pipette each **25 µl Precipitator 2** into all tubes.
4. Mix tubes strongly and thoroughly (vortex). Strongly vortex each tube.
5. Centrifuge tubes for 15 minutes with at least 4,000 x g, preferable with a swing-out rotor.
   Attention: 4,000 x g is not identical to 4,000 x rpm (round per minute) and has to be adjusted for each centrifuge and rotor.
6. Pipette each **50 µl Acylation Buffer** into all tubes and continue with step 7 immediately.
7. Please note that solvent reacts with many plastic materials including plastic trays; solvent does not react with normal pipette tips and with glass devices. Solvent is volatile and evaporates quickly. Therefore, please do not use a tray with big surface together with a multichannel pipette for pipetting dissolved Acylation Reagent. Rather, use an Eppendorf multipette (or similar device), fill the syringe directly from the vial with dissolved Acylation Reagent and pipette tube by tube.

   Pipette each **40 µl dissolved Acylation Reagent** into one tube and immediately softly vortex the tube at medium speed for 2 to 4 seconds and then start with the next tube. Take care not to disturb the pellet at the bottom of the tube. Color changes to red.

8. Centrifuge tubes for 15 minutes with at least 4,000 x g, preferable with a swing-out rotor.

Take each 50 µl for the Metanephrine and Normetanephrine ELISA.

7. Assay Procedure

7.1 Metanephrine ELISA

1. Pipette each **50 µl acylated Standards, Controls and Samples** into the respective wells of the coated microtiter strips (blue).
2. Incubate for 1 hour at room temperature on an orbital shaker (medium shaking rate).
   Do not cover the wells or the plate; leave the plate open on the shaker.
3. Pipette each **25 µl Metanephrine Antiserum** into all wells. Colour changes to blue.
4. Cover the plate with adhesive foil and incubate for 2 hours at room temperature (20 - 25 °C) on an orbital shaker (medium shaking rate).
5. Discard or aspirate the contents of the wells, add each **300 µl diluted Wash Buffer**, again discard or aspirate the contents of the wells. Remove residual liquid by tapping the inverted plate on clean absorbent paper. Repeat the washing procedure 3 times.
   Alternatively use a microplate washing device.
6. Pipette each **100 µl Enzyme Conjugate** into all wells.
7. Incubate for 30 minutes at room temperature on an orbital shaker (medium shaking rate).
8. Washing: Repeat step 5.
9. Pipette each **100 µl Substrate** into all wells.
10. Incubate for 30 ± 5 minutes at room temperature (20 - 25 °C) on an orbital shaker (medium shaking rate).
11. Pipette **100 µl Stop Solution** into all wells, shake shortly.
12. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

7.2 **Normetanephrine ELISA**

1. Pipette each **50 µl acylated Standards, Controls and Samples** into the respective wells of the coated microtiter strips (yellow).
2. Incubate for 1 hour at room temperature on an orbital shaker (medium shaking rate).
   Do not cover the wells or the plate; leave the plate open on the shaker.
3. Pipette each **25 µl Normetanephrine Antiserum** into all wells. Colour changes to orange.
4. Cover the plate with adhesive foil and incubate for 2 hours at room temperature (20 - 25 °C) on an orbital shaker (medium shaking rate).
5. Discard or aspirate the contents of the wells, add each **300 µl diluted Wash Buffer**, again discard or aspirate the contents of the wells. Remove residual liquid by tapping the inverted plate on clean absorbent paper. Repeat the washing procedure 3 times.
   Alternatively use a microplate washing device.
6. Pipette each **100 µl Enzyme Conjugate** into all wells.
7. Incubate for 30 minutes at room temperature on an orbital shaker (medium shaking rate).
8. Washing: Repeat step 5.
9. Pipette each **100 µl Substrate** into all wells.
10. Incubate for 30 ± 5 minutes at room temperature (20 - 25 °C) on an orbital shaker (medium shaking rate).
11. Pipette **100 µl Stop Solution** into all wells, shake shortly.
12. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

8. Calculation of Results

Concentrations of the standards: See q.c. certificate.

Conversion:
Metanephrine: 1 pg / ml = 5.07 pmol / l
Normetanephrine: 1 pg / ml = 5.46 pmol / l

On a semilogarithmic graph paper the concentration of the standards (x-axis, logarithmic) are plotted against their corresponding optical density (y-axis, linear). Alternatively, the optical density of each standard and

sample can be related to the optical density of the zero standard, expressed as the ratio OD/OD max , and then plotted on the y-axis.

A good fit is provided with 4 Parameter Logistic (alternatively Log-Logit or Cubic Spline). The concentration of the controls and samples can be read directly from the standard curve in pg / ml.

Quality Control

All kit controls must be found within the acceptable ranges as printed on the Q.C. Certificate. If the criteria are not met, the run is not valid and should be repeated.

9. Assay Characteristics

9.1 Normal Range

The reference ranges given below should only be taken as a guideline. It is recommended that each laboratory should establish its own normal values.

Metanephrine	Normetanephrine
< 90 pg/ml	< 190 pg/ml

9.2 Sensitivity

	Lower Detection Limit	Calculation
Metanephrine	< 7 pg/ml	OD_{Cal1} – 2xSD
Normetanephrine	< 7 pg/ml	OD_{Cal1} – 2xSD

9.3 Specificity

Substance	Metanephrine (%)	Normetanephrine (%)
Metanephrine	100	0.015
Normetanephrine	0.130	100
3-Methoxytyramine	0.003	0.076
Adrenaline	0.039	0.0003
Noradrenaline	0.0008	0.0030
Tyramine	0.0005	0.0043
Dopamine	< 0.0001	0.0006
Homovanillic Acid	< 0.0001	< 0.0001
Vanillic mandelic acid	< 0.0001	< 0.0001
L-Dopa	< 0.0001	< 0.0001
L-Tyrosine	< 0.0001	< 0.0001

9.4 Recovery

	Range (pg/ml)	Mean (%)	Range (%)
Metanephrine	20 – 900	94	82 - 117
Normetanephrine	34 – 1633	96	90 - 108

9.5 Linearity (Dilution with Standard 1)

	Range (pg/ml)	Highest Dilution	Mean (%)	Range (%)
Metanephrine	43 – 886	1 : 20	103	96 – 112
Normetanephrine	70 – 1613	1 : 20	93	86 - 105

9.6 Precision

	Range (pg/ml)	Intra-Assay-CV	Range (pg/mL)	Inter-Assay-CV
Metanephrine	157 – 403	7.9 – 7.8 %	118 – 276	8.8 – 8.6 %
Normetanephrine	193 – 757	8.4 – 4.1 %	246 – 551	9.3 – 9.2 %

9.7 Method Comparison

	Method	Correlation
Metanephrine	LC/MS	Y = 1.04 x LC/MS - 23; R = 0.991; N = 32
Normetanephrine	LC/MS	Y = 0.99 x LC/MS - 8; R = 0.984; N = 32

9.8 Calibration

The assay is calibrated by addition of defined stock solutions. The accuracy of the method was verified by comparing normal ranges (see 9.1) and other methods (see 9.7).

9.9 Limitations of Procedure

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire.

Samples showing concentration above the highest standard have to be diluted with Standard 1 and reassayed.

9.10 Interfering Substances

Hemolytic, lipemic, and icteric samples should not be used.

10. Literature

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Warranty Information

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