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Manual

IDK® Metanephrines LC-MS/MS Kit

For the in vitro determination of metanephrines in EDTA plasma

Valid from 2023-03-15

REF **KM2200**

Σ 300

+8 °C
+2 °C

IVD

CE

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1. INTENDED USE

The intended use of this device is assisting in diagnosis of adrenocortical carcinoma by determination of the levels of metanephrine, normetanephrine and 3-methoxytyramine (3-MT) in EDTA plasma, performed by automated quantitative LC-MS/MS assay technology. This kit is designed for laboratory professional use.

2. INTRODUCTION

Metanephrines are the methylation products of the three catecholamines epinephrine (adrenaline), norepinephrine (noradrenaline) and dopamine. These catecholamines are produced in the adrenal gland to regulate heartbeat, blood pressure and glucose homeostasis. They can also be released in excess by hormone-active tumors of the sympathoadrenal system, such as pheochromocytomas, neuroblastomas and paragangliomas, as well as tumors of the chromaffinic tissue of the adrenal gland.

Most of these tumors release epinephrine and norepinephrine, but a few tumors secrete mainly dopamine. This is why all three catecholamines are important to be determined when one of these tumors is suspected.

However, there are some difficulties with determining catecholamines since some tumors, for example, are biochemically silent or only periodically secrete these catecholamines leading to false negative results. False positives may also arise because of panic disorders or congestive heart failure.

Their metabolites metanephrine, normetanephrine and 3-methoxytyramine (3-MT) have superior diagnostic sensitivity and specificity compared to urinary and plasma catecholamines and are therefore recommended for the diagnosis of pheochromocytomas and paragangliomas.[1]

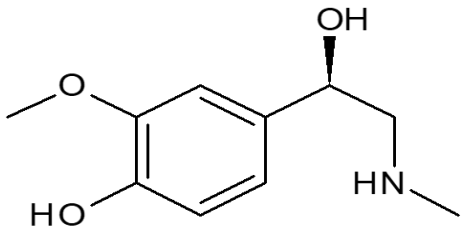


Fig 1: Metanephrine

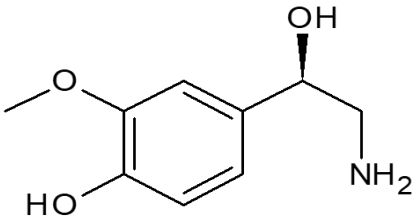


Fig 2: Normetanephrine

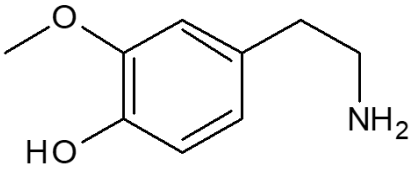


Fig 3: 3-Methoxytyramine

3. MATERIAL SUPPLIED

Cat. No.	Identifier	Kit components	Quantity
KM2200	AUTOWASH	Autosampler wash solution	1 x 1 000 ml
	BUF	Buffer (for reconstitution of INTSTD)	4 x 30 ml
	CAL1–6	Calibrators 1–6; lyophilised (see product specification for concentration)	2 vials (à 2 ml) per level
	CTRL1–3	Controls 1–3; lyophilised (see product specification for concentration)	3 vials (à 2 ml) per level
	ELUSOL	Elution solution	2 x 30 ml
	INTSTD	Internal standard; lyophilised	4 x 6 ml
	MOPHAA	Mobile phase A	1 x 250 ml
	MOPHAB	Mobile phase B	2 x 500 ml
	WASHSOL1	Wash solution 1	2 x 220 ml
	WASHSOL2	Wash solution 2	2 x 220 ml
	WASHSOL3	Wash solution 3	2 x 100 ml

For orders of single components, please use the catalogue number followed by the label without space as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

The following accessories are required for the *IDK*® Metanephrines LC-MS/MS application (not included in the kit):

- Precision pipettors and disposable tips to deliver 10–1 000 µl
- Vortex mixer
- Extraction manifold (e.g. Waters) or centrifuge for solid phase extraction columns
- Standard laboratory disposable plastic reagent tubes (e.g. 1.5 ml)
- LC-MS/MS system and LC-MS vials

The following accessories for the *IDK*® Metanephrines LC-MS/MS application can be ordered seperately at Immundiagnostik AG:

- 2 x 100 solid phase extraction columns (KM2200COL)
- UPLC column (KM2200SP)
- all single components

Please ask for our single component price list. Feel free to contact us for customized inquiries.

5. PREPARATION, STORAGE AND STABILITY OF REAGENTS

Note: Please unpack the kit components from the transport packaging immediately upon receipt and follow the instructions for storage conditions printed on the product labels.

All components should be stored protected from light, dry and at their given specified storage temperature. The test reagents stored in this way are usable until the indicated expiry date. The declared stated stabilities are only valid in case of no bacterial contamination.

Calibrators and controls

Handling:

Always remove the cap and rubber plug carefully (in order to avoid loss of content).

Reconstitute the calibrators and controls as follows:

- Reconstitute each calibrator and control with exactly 2 ml distilled or deionised water and incubate for 15 min at room temperature.
- Next, mix the component thoroughly to make sure that all dry material has dissolved; do not shake too vigorously to avoid foam formation.
- Handle the prepared component as a patient sample during the test procedure.

Stability and storage:

Before reconstitution:	2–8 °C	Until expiry date printed on the product label.
After reconstitution:	2–8 °C	2 weeks
After reconstitution:	-20 °C	1 month

Internal standard

Handling:

Always remove the cap and rubber plug carefully (in order to avoid loss of content).

Reconstitute the internal standard as follows:

- Reconstitute the content of one vial INTSTD with exactly 6 ml BUF and incubate for 15 min at room temperature. Put aside the used vial of BUF with the remaining 24 ml.
- Next, mix the component thoroughly to make sure that all dry material has dissolved; do not shake too vigorously to avoid foam formation.
- Finally, return the 6 ml reconstituted INTSTD into the BUF vial (with the remaining 24 ml) and mix toroughly.

Stability and storage:

Before reconstitution:	2–8 °C	Until expiry date printed on the product label.
After reconstitution:	After first opening the component can be used for 6 weeks if closed and stored at 2–8 °C.	

Mobile phases A and B

Handling:

The component is liquid and ready for use.

Stability and storage:

Store at 2–8 °C	After first opening the component can be used for 6 weeks if closed and stored at 2–8 °C or 4 weeks on the UHPLC.
Store at RT	Before first opening the component can be stored for 12 weeks at room temperature.

Wash solutions 1–3

Handling:

The components are liquid and ready for use.

Stability and storage:

Store at 2–8 °C	After first opening the component can be used for 6 weeks if closed and stored at 2–8 °C.

Elution solution

Handling:

The component is liquid and ready for use.

Stability and storage:

Store at 2–8 °C	After first opening the component can be used for 6 weeks if closed and stored at 2–8 °C.

Buffer

Handling:

The component is liquid and ready for use.

Stability and storage:

Store at 2–8 °C	After first opening the component can be used for 6 weeks if closed and stored at 2–8 °C.

Autosampler wash solution

Handling:

The component is liquid and ready for use.

Stability and storage:

Store at 2–8 °C	After first opening the component can be used for 6 weeks if closed and stored at 2–8 °C or 4 weeks on the UHPLC.

6. STORAGE, STABILITY AND PREPARATION OF SAMPLE

Storage and Stability

This assay is intended for EDTA plasma samples only. Avoid freeze-thaw cycles.

Plasma samples can be stored: 3 months at -20 °C.

Sample preparation

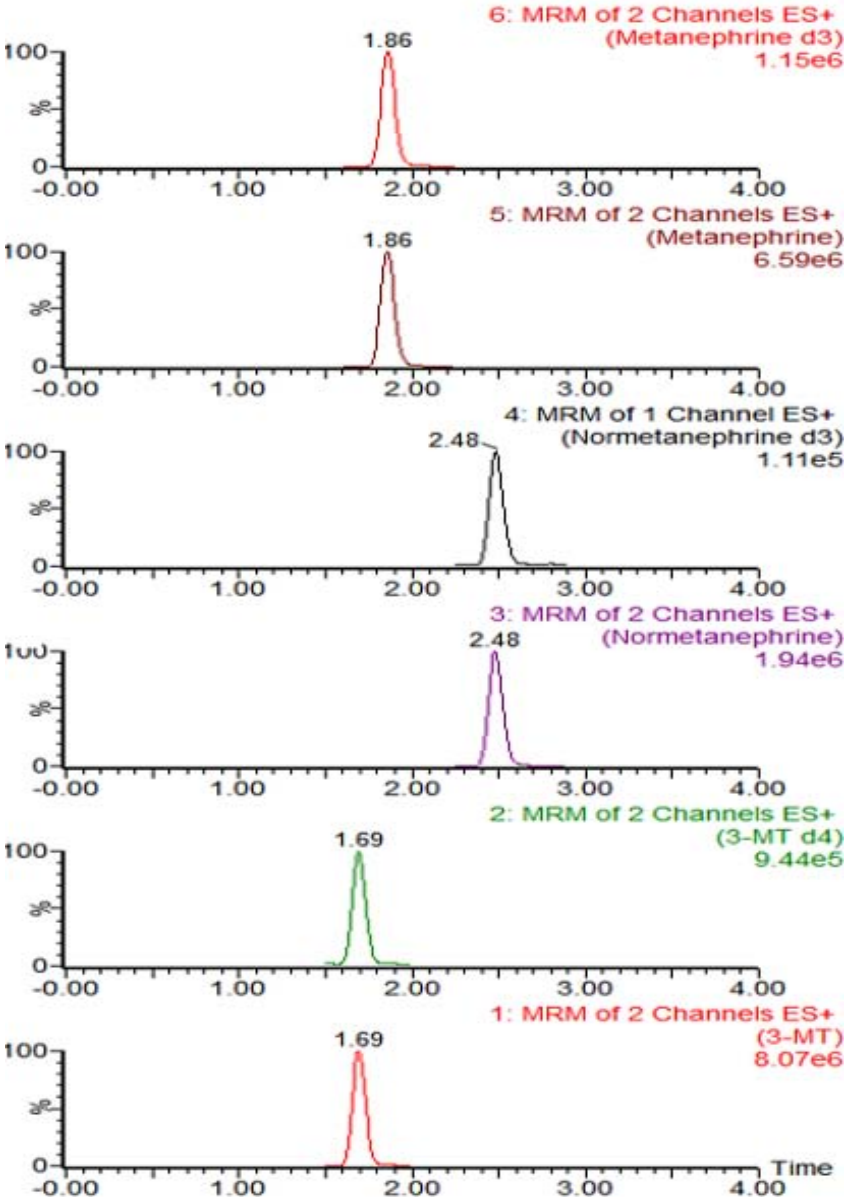
Note: The patient samples have to be centrifuged for 10 min at 3 000 *g* prior to spiking with INTSTD. Use the supernatant for the following test procedure.

1.	Pipet 500 µl sample, CAL or CTRL into a 1.5 ml reaction tube.
2.	Add 500 µl INTSTD and vortex shortly.
3.	Conditioning of the COL with 1 ml WASHSOL1. Use a extraction manifold/ centrifuge to aspirate/centrifuge into a waste tube/position. Discard the flowthrough.
4.	Repeat the conditioning with 1 ml WASHSOL2.
5.	Transfer 900 µl of the spiked sample, CAL or CTRL (step 2.) into the COL. Aspirate/centrifuge and discard the flowthrough.
6.	First wash step with 1 ml WASHSOL2, aspirate/centrifuge and discard the flowthrough.
7.	Second wash step with 1 ml WASHSOL1, aspirate/centrifuge and discard the flowthrough.
8.	Third wash step with 1 ml WASHSOL3, aspirate/centrifuge and discard the flowthrough.
9.	Change the waste position/tube to a new receiver position/tube.
10.	First elution step with 125 µl ELUSOL.
11.	Second elution step with 125 µl ELUSOL.
12.	Vortex the elution fraction for 10 s.
13.	Inject in the LC-MS/MS (see application note).

7. LC-MS/MS PARAMETERS AND CONDITIONS

Please refer to the application note or contact lcms@immundiagnostik.com for the LC-MS/MS method parameters.

8. EXAMPLES OF CHROMATOGRAMS



*recorded with the Waters UHPLC I-Class and the LC-MS/MS system Xevo TQS.

9. QUALITY CONTROL

Control samples should be analysed with each run. The results of the control samples are used to confirm the accuracy of the method. The test results may not be valid, if one or more values of the quality control sample are outside the acceptable range (see product specification).

Reference range

Metanephrine: 0.07–0.33 nmol/l (14–65 ng/l)

Normetanephrine: 0.23–1.07 nmol/l (42–196 ng/l)

3-MT: < 0.17 nmol (< 28 ng/l)

The indicated reference ranges are taken from scientific literature and were determined from healthy individuals. The blood was drawn in sitting position.[2]

We recommend each laboratory to establish its own reference range.

10. TESTCHARACTERISTICS

Measuring range with limit of quantification (LLOQ)

Analyte	nmol/l (ng/l)
3-MT	0.032–18 (5.4–3 010)
Metanephrin	0.041–25 (8.1–4 931)
Normetanephrin	0.093–25 (17.0–4 580)

Repeatability

Analyte	Level	Measured value [nmol/l (ng/l)]	Standard Deviation [nmol/l (ng/l)]	CV (%)
3-MT	Low	0.11 (18.4)	0.002 (0.3)	1.6
	High	2.99 (500.0)	0.018 (3.0)	0.6
	Patient	0.44 (73.6)	0.007 (1.2)	1.6
Meta-nephrine	Low	0.15 (29.6)	0.003 (0.6)	1.9
	High	3.82 (753.4)	0.034 (6.7)	0.9
	Patient	0.29 (57.2)	0.004 (0.8)	1.3

Analyte	Level	Measured value [nmol/l (ng/l)]	Standard Deviation [nmol/l (ng/l)]	CV (%)
Normeta-nephrine	Low	0.11 (20.2)	0.003 (0.5)	2.8
	High	3.51 (643.0)	0.039 (7.1)	1.1
	Patient	1.42 (260.1)	0.020 (3.7)	1.4

10. PRECAUTIONS

- Human material used in the kit components was tested and found to be negative for HIV, Hepatitis B and Hepatitis C. Still, all kit components should be treated as potentially infectious.
- The GHS symbols indicated on the individual components and specifications of the material safety data sheets (available on request from Immundiagnostik AG) must be noted. When working with these reagents, the legal protective precautions must be adhered to.
- As a precaution, it is recommended that the human material used is always considered potentially infectious.
- The test components contain organic solvents. Avoid contact with skin or mucous membranes.

11. DISPOSAL

Autosampler wash solution (AUTOWASH), elution solution (ELUSOL) and mobile phases (MOPHAA, MOPHAB) must be disposed as nonhalogenated solvents. The calibrators (CAL1–6) and controls (CTRL1–3) should be disposed as potentially infectious material in accordance with local regulations.

12. TECHNICAL HINTS

- Do not mix different lot numbers of any kit component.
- Reagents should not be used beyond the expiration date shown on the kit label.
- The assay should always be performed according the enclosed manual.
- Plugs and caps of different reagents should not be swapped.

- The individual components of the kit are designed for a maximum of the specified number of test runs. Any part of the components that has already been used must not be reused.

13. GENERAL NOTES ON THE TEST

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- *IDK®* is a trademark of Immundiagnostik AG.
- All reagents in the kit package are for *in vitro* diagnostic use only.
- The guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from wrong use.
- Serious incidents are to be reported to Immundiagnostik AG and the national regulatory authorities.
- Please contact Immundiagnostik AG if one or more components of the kit are damaged, missing (see material supplied) or precipitates are visible in the ready-to-use solutions.
- Warranty claims and complaints in respect of deficiencies must be lodged within 14 days after receipt of the product. The product shall be send to Immundiagnostik AG together with a written complaint.

14. REFERENCES

1. Weismann D, et al. (2015). Measurements of plasma metanephrines by immunoassay vs liquid chromatography with tandem mass spectrometry for diagnosis of pheochromocytoma. European Journal of Endocrinology, 172(3), 251-260.
2. De Jong, W.H.A., et al (2007). Plasma Free Metanephrine Measurement Using Automated Online Solid-Phase Extraction HPLC–Tandem Mass Spectrometry. Clinical Chemistry, 53(9), 1684-16933.

Used symbols:


Temperature limitation

Catalogue number

In Vitro Diagnostic Medical Device

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet