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Manual

IDK® Methylmalonic Acid LC-MS/MS Kit

For the in vitro determination of methylmalonic acid in plasma, serum and urine

Valid from 2023-03-15

REF **KM3110**

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 vitamin B_{12} deficiency by determination of the levels of Methylmalonic Acid in serum/plasma/urine, performed by automated quantitative LC-MS/MS assay technology. This kit is designed for laboratory professional use, only.

2. INTRODUCTION

Methylmalonic acid is an organic acid which blood level is usually elevated in cobalamin (vitamin B_{12}) deficiency. It is considered to be an early and sensitive biomarker for B_{12} deficiency.

High MMA serum values are found in people with renal insufficiency, hypovolemia (decreased volume of circulating blood) and intestinal bacterial overgrowth. In these cases MMA levels cannot be used to diagnose B_{12} deficiency, though a vitamin B_{12} deficiency might simultaneously exist. In the case of kidney disease (or hypovolemia) MMA levels in urine can be tested.

In patients with B_{12} deficiency who are taking (or have recently taken) antibiotics at the same time, MMA levels may be falsely normal.

In case of a B_{12} deficiency, its substitution will lower the elevated MMA level relatively quickly. Testing some time after starting treatment, for instance after one or two months, may serve as a confirmation of the B_{12} deficiency diagnosis. Furthermore, this testing can also be performed for people exhibiting a MMA value which is not distinctly above reference values.

Serum samples are generally used for MMA determination, as this matrix is used for parallel cobalamin level tests. Furthermore nutrition seems to have less influence on the MMA serum level than in case of urine. For determination in urine additional measurement of creatinine is necessary.

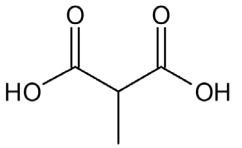


Fig 1: Methylmalonic Acid

Indications

- Vitamin B_{12} status assessment
- Defect of kidney functions
- Methylmalonic aciduria
- SIBO (small intestinal bacterial overgrowth)

3. MATERIAL SUPPLIED

Cat. No.	Identifier	Kit components	Quantity
KM3110	AUTOWASH	Autosampler wash solution;	1 x 1 000 ml
	PREC	Precipitation solution (contains internal standard)	3 x 55 ml
	CAL1–6	Calibrators 1–6; lyophilised (see product specification for concentration)	2 vials (à 500 µl) per level
	CTRL1–3	Controls 1–3; lyophilised (see product specification for concentration)	3 vials (à 500 µl) per level
	INTSTD	Internal Standard	3 x 6 ml
	MOPHAA	Mobile phase A	1 x 500 ml
	MOPHAB	Mobile phase B	1 x 300 ml
	SOLVENT	Solvent	3 x 30 ml

For reorders 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*® Methylmalonic Acid LC-MS/MS application (not included in the kit):

- Precision pipettors and disposable tips to deliver 10–1 000 µl
- Centrifuge 10 000 *g* (at least)
- Vortex mixer
- Vacuum centrifuge or nitrogen distributor
- 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*® Methylmalonic Acid LC-MS/MS application can be ordered seperately at Immundiagnostik AG:

- 500 ml Mobile Phase A with increased FA concentration (KM3110MOPHAA2)
- 100 ml Dilution solution for urine; ready to use (KM3110DILSOL)
- UPLC column (KM3110SP)
- 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 500 µl 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	1 week
After reconstitution:	-20 °C	2 weeks

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 each calibrator and control with exactly 6 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.

Stability and storage:

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

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.

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.

Precipitation 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 3 weeks if closed and stored at 2–8 °C.

Solvent

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 4 weeks if closed and stored at 2–8 °C.

*Dilution solution for urine**

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.

*(When the appropriate dilution solution for Urine have been ordered)

6. STORAGE, STABILITY AND PREPARATION OF SAMPLE

Serum and plasma

Storage and Stability

Use serum or plasma (EDTA- and Heparin-plasma). Avoid freeze-thaw cycles.

Samples can be stored: 3 days at room temperature (15–25 °C)
7 days at (2–8 °C)
1 month (-20 °C)

Sample preparation

1.	Pipet 100 µl sample, CAL or CTRL into a 1.5 ml reaction tube.
2.	Add 50 µl INTSTD and vortex shortly.
3.	Add 500 µl PREC.
4.	Mix for 30 s on a vortex mixer.
5.	Centrifuge for 5 min at 10 000 *g* (or more)
6.	Transfer 400 µl supernatant to a new 1.5 ml reaction tube and evaporate to dryness under nitrogen at 50 °C or in a vacuum centrifuge.
7.	Reconstitute in 200 µl SOLVENT.
8.	Inject 10 µl in the LC-MS/MS (see application note).

Urine

Storage and Stability

In the cases of patients with impaired renal functions, the analysis is performed from an urine sample. The stability of urine samples is identical to those of serum and plasma.

Samples can be stored: 3 days at room temperature (15–25 °C)
7 days at (2–8 °C)
1 month (-20 °C)

Sample preparation

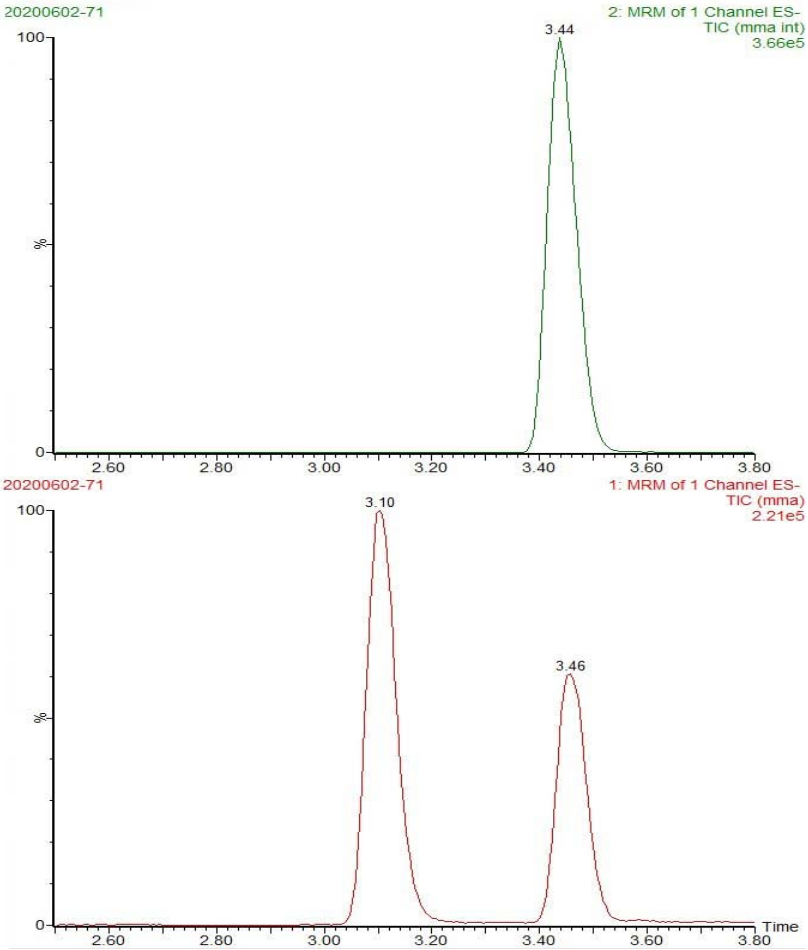
1.	Dilute 50 µl of the sample (urine) with 1 000 µl DILSOL in a 1.5 ml reaction tube and mix thoroughly.
2.	Pipet 100 µl of the diluted sample (step 1), CAL or CTRL into a new 1.5 ml reaction tube.
3.	Add 50 µl INTSTD and vortex shortly.
4.	Add 500 µl PREC.
5.	Mix for 30 s on a vortex mixer.
6.	Centrifuge for 5 min at 10 000 *g* (or more)
7.	Transfer 400 µl supernatant to a new 1.5 ml reaction tube and evaporate to dryness under nitrogen at 50 °C or in a vacuum centrifuge.
8.	Reconstitute in 200 µl SOLVENT.
9.	Inject 10 µl in the LC-MS/MS (see application note).

7. LC-MS/MS METHOD

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

8. EXAMPLES OF CHROMATOGRAMS

Methylmalonic Acid Internal Standard (top) vs Sample (bottom)



*recorded with the Waters LC-MS/MS TQS system

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 (Plasma, serum an urine)

Plasma, Serum: 0–350 nmol/l

Urine: < 3.6 mmol/mol Creatinine

We recommend each laboratory to establish its own reference range.

10. TESTCHARACTERISTICS

Measuring range with limit of quantification (LLOQ)

Analyte	nmol/l
Methylmalonic acid	37.8–2 000

Repeatability

Level	Measured value (nmol/l)	Standard Deviation (nmol/l)	CV (%)	N
Level I	211.3	14.1	6.7	25
Level II	350.0	12.5	3.6	25
Level III	1 114.7	27.1	2.4	25

11. 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.

12. DISPOSAL

Autosampler washing solution (AUTOWASH), precipitation solution (PREC), mobile phase A (MOPHAA), mobile phase B (MOPHAB) and solvent (SOLVENT) 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.

13. 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.

14. 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.

15. REFERENCES

1. L. Thomas, labor und diagnose: Indikation und Bewertung von laborbefunden für die medizinische Diagnostik, TH-Books Verlagsgesellschaft, Frankfurt/Main 2012, page 714 *
2. Norman, E.J. Urinary Methylmalonic Acid Test May have greater value than the total Homocysteine assay for screening elderly individuals for Cobalamin Deficiency, Clinical Chemistry 2004, 50 (8), 1482-1483.

Used symbols:


Temperature limitation

In Vitro Diagnostic Medical Device


Manufacturer

Lot number


Contains plasma derivatives or human blood

Consult specification data sheet

Unique Device Identification


Medicinal substance

Catalogue number

To be used with

Contains sufficient for <n> tests


Use by


Consult instructions for use


Do not re-use

Contains material of animal origin

Contains material of human origin