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Manual

IDK® Vitamin B_1 & B_6 LC-MS/MS Kit

For the in vitro determination of vitamin B_1 & B_6 in whole blood

Valid from 2023-03-16

REF **KM5100**

Σ 300

+8 °C
+2 °C

IVD

CE

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1. INTENDED USE

The intended use of this device is assessment of vitamin profiles by determination of the levels of vitamin B_1 & B_6 in whole blood, performed by automated quantitative LC-MS/MS assay technology. This kit is designed for laboratory professional use.

2. INTRODUCTION

Water-soluble B vitamins are important cofactors in cell metabolism. Two water-soluble vitamins with clinical relevance are vitamins B_1 and B_6.

Thiamine pyrophosphate (TPP) is the biologically active form of vitamin B_1 and is required for various metabolic functions. TPP is formed by phosphorylation of thiamine. Prolonged deficiency can cause Beriberi, a debilitating neurological disease.[1]

Vitamin B_6 is a vitamin group of pyridoxine, pyridoxal and pyrodoxamine as well as their phosporylated forms which are converted in the biologically active form pyridoxal 5-phosphate (PLP). PLP (here referred to as vitamin B_6) is a coenzyme for a number of transamination reactions. PLP plays critical roles in chronic disease and pro-inflammatory response.[2] Additionally, both vitamin B_1 and B_6 have also been linked to increased survival rates in the elderly.[3]

Thiamine deficiency may give rise to manifestations in the cardiovascular and nervous systems. There are two forms; dry Beriberi, which consists of sensorimotor neuropathy and Wernicke-Korsakoff syndrome, and wet Beriberi, which consists of edema and cognitive heart failure, but little CNS manifestations. The clinical picture of Wernicke's encephalopathy, with or without Korsakoff syndrome, is frequently encountered in alcoholics, with predominant oculomotor and cerebellar symptomatology, although it can also be seen in other conditions, such as hyperemesis, dialyses, and post-gastrointestinal surgery.

Pyridoxine deficiency on a nutritional basis has been recognized as a rare cause of serve and even fatal convulsions in neonates and infants. Pyridoxine dependency develops during fetal life as a genetic disorder and causes both intrauterine and postnatal seizures.

Neurologic disorders reflecting both pyridoxine deficiency and pyridoxine toxicity have been recognized. Both overdose and deficiency may cause peripheral neuropathy. Pyridoxine deficiency causes injury of motor and sensory axons, whereas an overdose of pyridoxine causes a pure sensory neuropathy or neuronopathy with sensory ataxia.

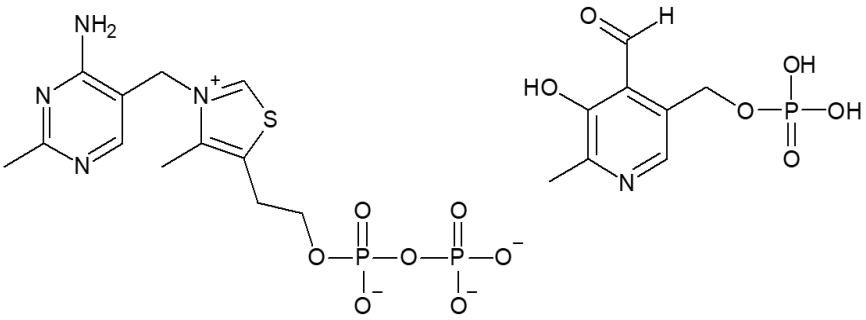

Fig 1: Thiamine pyrophosphate (vitamin B_1, left) and pyridoxal-5'-phosphate (vitamin B_6, right)

3. MATERIAL SUPPLIED

Cat. No.	Identifier	Kit components	Quantity
KM5100	AUTOWASH	Autosampler wash solution	1 x 1 000 ml
	PREC	Precipitation solution	3 x 46 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 250 ml

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

4. MATERIAL REQUIRED BUT NOT SUPPLIED

The following accessories are required for the *IDK*® Vitamin B_1 & B_6 LC-MS/MS application (not included in the kit):

- Precision pipettors and disposable tips to deliver 10–1 000 µl
- Centrifuge, 10 000 *g* (for reagent tubes or 96-well plate, depending on test procedure)
- Vortex mixer
- Standard laboratory disposable plastic reagent tubes (e.g. 1.5 ml) and shaker for the reagent tubes (for test procedure in reagent tubes)
- 2 ml 96-well plate and shaker for the plate (for test procedure in 96-well plate)
- LC-MS/MS system and suitable LC-MS vials / 96-well plate for the autosampler
- Ultrapure water*

  *Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696/LC-MS grade), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

The following accessories for the *IDK*® Vitamin B_1 & B_6 LC-MS/MS application can be ordered seperately at Immundiagnostik AG:

- HPLC column (KM5100SP)
- all single components

Please ask for our single component price list. Please 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	48 h
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	48 h
After reconstitution:	-20 °C	1 week

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 2 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 2 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.

6. STORAGE, STABILITY AND PREPARATION OF SAMPLE

Storage and Stability

Use whole blood (EDTA- and Heparin-tubes).

Avoid freeze-thaw cycles.

Samples can be stored: 1 month (-20 °C)

Sample preparation

#	96-well plate	1.5 ml reaction tube
1.	Add 50 µl INTSTD in one well respectively reaction tube.	
2.	Add 50 µl sample, CAL or CTRL to the INTSTD presented. Mix thoroughly.	
3.	Add 400 µl PREC.	
4.	Seal plate and shake for at least 30 min.	Seal tubes and vortex for 30 s.

5.	-	Shake the tubes for at least 30 min.
6.	Centrifuge for 5 min at 10 000 *g*.	
7.	Use directly the plate for the autosampler or transfer 200 µl of the supernatant (from step 6.) in a suitable microtiter plate for the autosampler.	Transfer 200 µl of the supernatant (from step 6.) in a suitable vial or microtiter plate for the autosampler.
8.	Injection into the LC-MS system (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

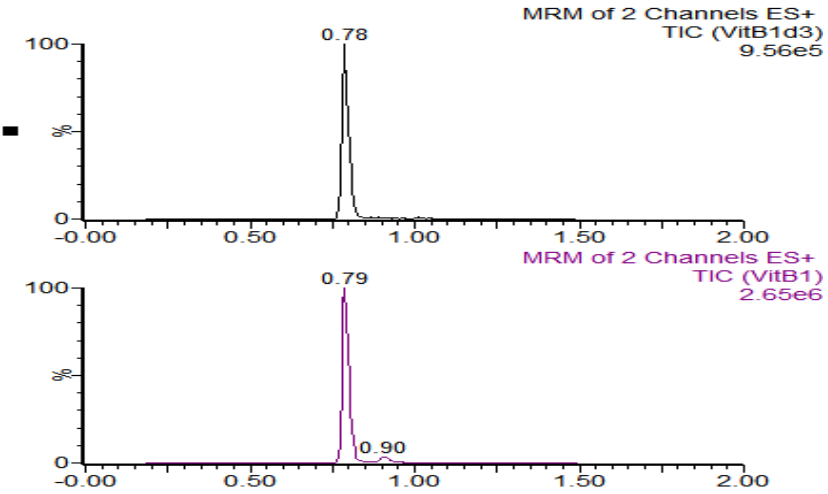

Fig. 2: Chromatogram (recorded with Waters TQS LC-MS/MS) for the internal standard (top) and analyte (bottom) of vitamin B_1

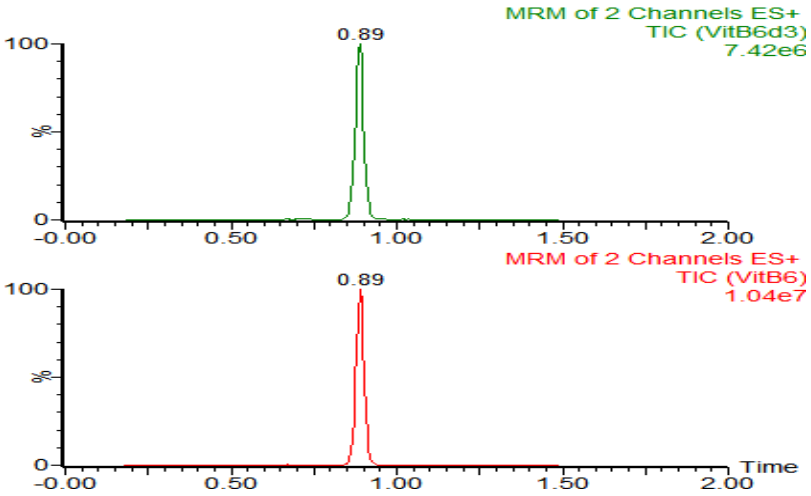

Fig. 3: Chromatogram (recorded with Waters TQS LC-MS/MS) for the internal standard (top) and analyte (bottom) of vitamin B_6

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

VitaVitamin B_1:	60–120 nmol/l	(25.5–50.9 ng/ml)	[4]
Vitamin B_6:	35–110 nmol/l	(8.6–27.2 ng/ml)	[5]

We recommend each laboratory to establish its own reference range.

10. TESTCHARACTERISTICS

Measuring range with limit of quantification (LLOQ)

Analyte	nmol/l (ng/ml)
Vitamin B_1	9.4–8 000 (4.0–3 394)
Vitamin B_6	8.0–3 000 (2.0–741)

Repeatability

Vitamin B_1

Level	Measured value [nmol/l (ng/ml)]	Standard Deviation [nmol/l (ng/ml)]	CV (%)	N
Level I	41.1 (17.4)	1.0 (0.4)	2.4	20
Level II	212.1 (90.0)	4.0 (1.7)	1.9	20
Patient sample	147.5 (62.6)	2.9 (1.2)	1.9	20

Vitamin B_6

Level	Measured value [nmol/l (ng/ml)]	Standard Deviation [nmol/l (ng/ml)]	CV (%)	N
Level I	22.5 (5.6)	0.5 (0.1)	2.4	20
Level II	122.7 (30.3)	2.5 (0.6)	2.1	20
Patient sample	90.9 (22.5)	2.0 (0.5)	2.2	20

11. PRECAUTIONS

- Human material used in the kit components was tested and found to be negative for HBsAg, anti-HIV 1/2 and anti-HCV. 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.
- The test components contain organic solvents. Avoid contact with skin or mucous membranes.

12. DISPOSAL

Autosampler wash solution (AUTOWASH), precipitation solution (PREC), mobile phase A (MOPHAA) and mobile phase B (MOPHAB) must be disposed as nonhalogenated solvents.

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. Stanley, N. N. "Cardiac Beriberi: Two Modes of Presentation." BMJ: 567-569.
2. Huang, et al. "Vitamin B6 Supplementation Improves Pro-inflammatory Responses in Patients with Rheumatoid Arthritis." European Journal of Clinical Nutrition (2010): 1007-013.
3. Huang, et al. "Prediction of All-cause Mortality by B Group Vitamin Status in the Elderly." Clinical Nutrition (2011): 191-98.
4. Hooijkaas, et al. "Handboek medische laboratoriumdiagnostiek" (2013): 820 - 821.
5. Hooijkaas, et al. "Handboek medische laboratoriumdiagnostiek" (2013): 825.

Used symbols: