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Manual

Vitamin B_1 HPLC Kit

For the determination of Vitamin B_1 (thiamin pyrophosphate) in EDTA-whole-blood

Gültig ab / Valid from 2022-09-12

REF **KC2201**

Σ 100

+8 °C
+2 °C

-20 °C
CAL
CTRL 1
CTRL 2

IVD

CE

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1. INTENDED USE

The Immundiagnostik Assay is intended for the quantitative determination of Vitamin B_1 in EDTA-blood. For *in vitro* diagnostic use only.

2. SUMMARY AND EXPLANATION OF THE TEST

Vitamin B_1 (thiamin) is a water-soluble Vitamin, which consists of a pyrimidine- and thiazolring linked via a methylene bridge. It is sensitive against alkaline solution, oxidation and reduction.

Vitamin B_1 is produced by plants and microorganisms. It is found free, peptide bound and as phosphoesters (mono-, di- and triphospho-esters). In animals and also in humans it is necessary to be supplemented by food. The intake of thiamin in the gut is maintained by active transport and passive diffusion. The different phosphoesters are synthesized by phosphorylation and dephosphorylation. The active form in metabolism is thiamin pyrophosphate and thiamin triphosphate in brain. After dephosphorylation thiamin is secreted by the kidney.

Thiamin pyrophosphate plays an important role as a co-enzyme in carbohydrate- and amino acid metabolism. An important reaction is the oxidative carboxylation. Thiamin itself is required for stimulating nerve cells. Beside this, it stimulates the fatty acid- and cholesterol-synthesis in nervous tissues.

A classical disease for the lack of Vitamin B_1 is Beri Beri, which is known from Asians eating predominantly white rice. The symptomes are paralysis, drop in muscle mass and heart failure. Other diseases are the Wernicke-encephalopathy, the Korsakow-syndrome and several forms of the Landry`s paralysis. Also many alcoholics have a deficient thiamin status.

Applications:

- determination of vitamin B_1 status
- disturbance in amino acid metabolism
- malabsorption caused by alcoholism
- suspicion for neuritis

3. PRINCIPLE OF THE TEST

The first step in the determination of vitamin B_6 includes the sample preparation with additional derivatisation. During the precipitation, higher molecular substances are removed. After centrifugation the supernatant is used for derivatisation (10 min at 60 °C), thereby transforming the vitamin B_1 into a fluorescent product. The sample is cooled, centrifuged and injected into the HPLC system.

The separation via HPLC follows an isocratic method at 30 °C using a reversed phase column; one run lasts about 12 minutes. The quantification is performed with the delivered EDTA-whole blood calibrator; the concentration is calculated via integration of the peak area/heights.

Summary

This HPLC application for the quantitative determination of vitamin B_1 allows to determine the vitamin in an easy, fast and precise method. The kit includes all reagents in ready to use form for preparation and separation of the samples with exception of the column.

Besides many other parameters, the advantage of HPLC method lies in the simultaneous handling of many analytes in a single test. The HPLC complete system enables even laboratories without experience in high performance liquid chromatography to use this technique for clinical chemical routines quickly and precisely. Mostly, a one-point calibration is sufficient for calibrating the test system – unlike immunoassays with up to 6 calibrators per test. It is possible to automate the sample application and calculation of the results so that even higher number of samples can be handled nearly without control. With short test series, the one-point calibration is much more economic than 6-point calibration for immunoassays.

4. MATERIAL SUPPLIED

Cat. No	Content	Kit Components	Quantity
KC2201	DIL	Dilution solution	1 x 20 ml
	PREC	Precipitating reagent (Acid)	1 x 5 ml
	MOPHA	Mobile phase; ready to use (important: do not recirculate)	1 x 1 000 ml
	CAL	Calibrator, lyophilised (see specification data sheet for concentration)	4 x
	REABUF	Reaction buffer	1 x 5 ml
	SOLC	Solution C	1 x 5.5 ml
	DER	Derivatisierungslösung	1 x 5.5 ml
	CTRL1	Control 1; lyophilised	4 x
	CTRL2	Control 2; lyophilised	4 x

The HPLC column (KC2201RP), can be ordered separately from Immundiagnostik. To extend the lifetime of your HPLC column, pre-columns (KC2201VS) are highly recommended. These and also the pre-column holders (KC2201VH) can also be ordered from Immundiagnostik. In addition to the complete kits, all components can also be ordered separately. Please ask for our single component price list.

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- 1.5 ml reaction tubes (e. g. Eppendorf)
- Centrifuge
- Various pipettes
- Vortex
- HPLC with fluorescence detector
- Reversed phase C_{18} column
- Thermoshaker

6. STORAGE AND PREPARATION OF REAGENTS

- **The lyophilised calibrator** (**CAL**, EDTA-whole blood with a defined thiamin pyrophosphate concentration) is stable at **-20 °C** until the expiry date stated on the label. Before use, resuspend the **calibrator** with 1 ml **dilution solution (DIL)**, aliquote and store at -20 °C. The concentration of vitamin B_1 slightly changes from lot to lot, the exact concentration is stated on the label.
- **The lyophilised controls 1 and 2** (**CTRL 1 and CTRL 2**) are stable at **-20 °C** until the expiry date stated on the label. Before use, they have to be reconstituted with each **250 µl dilution solution (DIL)**.
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.

7. SPECIMEN COLLECTION AND PREPARATION

EDTA-whole-blood is used in this assay.

Vitamin B_1 is light- and temperature-sensitive; therefore, samples have to be protected from light and cooled immediately after collection.

The samples are stable in the dark at 2–8 °C for one day. For longer storage, samples should be frozen at -20 °C. Do not re-freeze the samples.

8. ASSAY PROCEDURE

Test procedure

Pipet into 1.5 ml reaction tubes (e. g. Eppendorf) **50 µl sample (EDTA-whole blood)**, **calibrator**, or **control 1 or 2**
Add **150 µl dilution solution** (**DIL**) and **50 µl precipitating reagent (PREC), vortex** for at least **30 s**
Incubate for **10 min** at **2–8 °C**
Centrifuge for **10 min** at **10 000 *g***
Add to each **150 µl supernatant 50 µl reaction buffer** (**REABUF**) **and 50 µl derivatisation solution** (**DER**), **vortex** for at least **30 s**
Incubate for **10 min** at **60 °C** on a thermoshaker
Cool the tubes down at **2–8 °C**
Centrifuge for **5 min** at **10 000** g
Take the **supernatant**. (The sample is stable for 3 days at 2–8 °C in the dark)
Inject 50 µl of the supernatant for chromatography into the HPLC

Chromatographic conditions

Column material:	Bischoff Eurobond, 5 µm Lichrospher RP18 5 µm Nucleodur Sphinx RP18; 5 µm	
Column dimension:	125 × 4 mm	
Flow rate:	0.8–1.2 ml/min Please refer to the quality certificate of the column	
Fluorescence detection:	Excitation: Emission:	365 nm 440 nm
Temperature:	30 °C	
Injection volume:	50 µl	
Running time:	12 min	

Cartridge holder (KC2201RK) is necessary for the use of Nucleodur Sphinx RP18 cartridges. The cartridge holder can be used repeatedly.

9. TREATMENT OF THE COLUMN

It is recommended to use a guard column (KC2201VS) to extend column life.

After analysis, the column should be flushed with 30 ml ultrapure water (1 ml/min) and stored in 50% methanol in water (~ 30 ml, flow 0.7 ml/min). Before use, the system should be equilibrated with ~ 30 ml mobile phase (MOPHA).

10. RESULTS

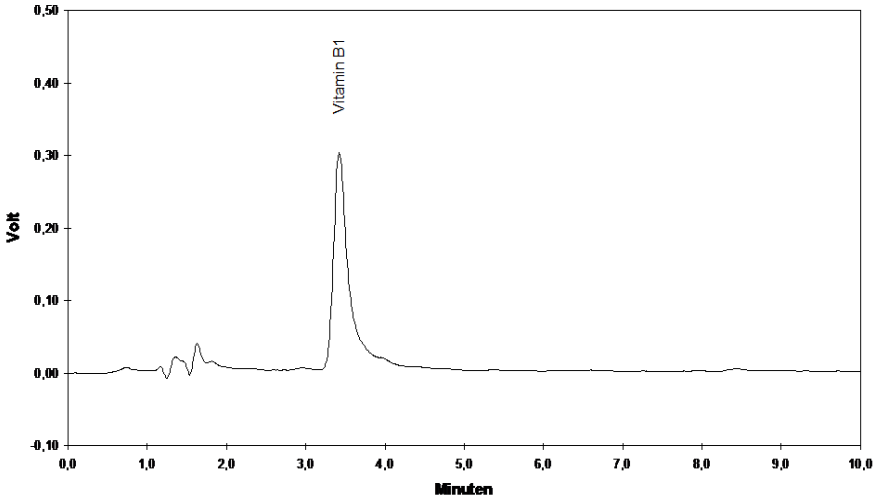
Calculation

$$\text{Sample concentration} = \frac{\text{Peak height sample} \times \text{calibrator concentration*}}{\text{Peak height calibrator}}$$

* see label

Tip: Alternatively, the peak area instead of the peak height can be used for quantification.

Typical chromatogram



11. LIMITATIONS

We recommend not to measure lipaemic patient samples. The measurement of serum and plasma samples is possible but not recommended, because the concentration is mostly below the detection limit.

12. QUALITY CONTROL

Normal rang

EDTA-whole blood

32–95 ng/ml (Mean value ± 2 SD)

We recommend each laboratory to establish its own reference range. The specification of the normal range for vitamin B_1 is for orientation purposes only, as the values depend strongly on the selection of the sample collective. The above information may therefore deviate from other published data.

Controls

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

13. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay CV:	3.3 % (31.2 ng/ml)	[n = 6]
	4.3 % (59.0 ng/ml)	[n = 6]
Inter-Assay CV:	3.2 % (33.0 ng/ml)	[n = 12]
	4.7 % (63.3 ng/ml)	[n = 12]

Linearity: up to 250 ng/ml

Detection limit: 0.5 ng/ml

Recovery: 84.1 % (whole blood)

14. DISPOSAL

The derivatisation solution (**DER**) must be oxidised with hydrogen peroxide, the pH value adjusted to 6–8, and disposed as aqueous salt solution. The mobile phase (**MOPHA**) and the precipitation reagent (**PREC**) must be neutralised with NaOH to neutral pH and disposed as salt solution.

Important: Reaction will produce heat, be careful!

Please refer to the appropriate national guidelines.

15. TROUBLESHOOTING

Problem	Possible reason	Solution
No signal	No or defect connection to evaluation system	Check signal cord and connection
	Detector lamp is altered	Change lamp
No peaks	Injector is congested	Check injector
Double peaks	Dead volume in fittings and / or column	Renew fittings and / or column
Contaminating peaks	Injector dirty	Clean injector
	Contamination at the head of the column	Change direction of the column and rinse for 30 min at low flow rate (0.2 ml/min) with mobile phase
	Air in the system	Degas pump
	Auto sampler vials contaminated	Use new vials or clean them with methanol

Problem	Possible reason	Solution
Broad peaks, tailing	Precolumn / column exhausted	Use new precolumn / column
Variable retention times	Drift in temperature	Use a column oven
	Pump delivers imprecise	Check pump, degas the system
	System is not in steady state yet	Rinse system mobile phase for 15 min
Baseline is drifting	Detector lamp did not reach working temperature yet	Wait
	Detector lamp is too old	Renew lamp
	System is not in steady state yet	Rinse system mobile phase for 15 min
	Pump delivers imprecise	Check pump, degas the system
Baseline is not smooth	Pump delivers imprecise	Check pump, degas the system
	Detector flow cell is dirty	Clean flow cell

16. PRECAUTIONS

- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- The precipitation reagent consists of an acid. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

- The test components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- Reagents should not be used beyond the expiration date stated on kit label.
- As a precaution, it is recommended that the human material used is always considered potentially infectious.
- As the derivatisation solution (DER) contains KCN, it should be pipetted under an fume hood.

17. GENERAL NOTES ON THE TEST

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- All reagents in the kit package are for *in vitro* diagnostic use only.
- Do not interchange different lot numbers of any kit component within the same assay.
- The guidelines for medical laboratories should be followed.
- Quality control guidelines should be observed.
- 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 incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.
- The assay should always be performed according to the enclosed manual.

18. REFERENCES

1. Tallaksen C.M.E., T. Bohmer, H. Bell (1991). Concomitant determination of thiamin and its phosphate esters in human blood and serum by high-performance liquid chromatography. J. Chromatogr, 564, 127-136.
2. Herbeth B., J. Zittoun, L. Miravet, M. Boureay-Causse, G. Carre-Guery, E. Delacoux, C. Le Devehat, A. Lemoine, J.P. Mareschi, J. Martin, G. Portier de Courcy and J. Sancho (1986). Reference intervals for vitamin B1, B2, E, D, retinol, and folate in blood: Usefulness of dietary selection criteria. Clin. Chem. 32/9, 1756-1759.

Used symbols: