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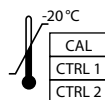
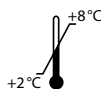
Manual

Vitamin B₆ HPLC Kit

*For the determination of vitamin B₆ in serum, plasma
and whole blood*

Valid from 2022-09-06

REF KC2150



IVD **CE**



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Table of Contents

1. INTENDED USE	15
2. SUMMARY AND EXPLANATION OF THE TEST	15
3. PRINCIPLE OF THE TEST	15
4. MATERIAL SUPPLIED	16
5. MATERIAL REQUIRED BUT NOT SUPPLIED	17
6. STORAGE AND PREPARATION OF REAGENTS	17
7. SPECIMEN COLLECTION AND PREPARATION	17
8. ASSAY PROCEDURE	18
<i>Test procedure</i>	18
<i>Chromatographic conditions</i>	18
9. TREATMENT OF THE COLUMN	19
10. RESULTS	19
<i>Calculation</i>	19
<i>Typical chromatogram</i>	19
11. QUALITY CONTROL	20
<i>Reference ranges</i>	20
<i>Controls</i>	20
12. PERFORMANCE CHARACTERISTICS	20
<i>Precision and reproducibility</i>	20
13. DISPOSAL	21
14. TROUBLESHOOTING	21
15. PRECAUTIONS	22
16. GENERAL NOTES ON THE TEST	23
17. REFERENCES	23

1. INTENDED USE

This HPLC application is intended for the quantitative determination of vitamin B₆ in plasma, serum and EDTA whole blood. For *in vitro* diagnostic use only.

2. SUMMARY AND EXPLANATION OF THE TEST

Vitamin B₆ is the term for pyridoxin, pyridoxal and pyridoxamin and the appropriate phosphate products. All forms can be transformed into the active form pyridoxal-5-phosphate (here referred to as vitamin B₆).

Vitamin B₆ functions as a coenzyme and is essential for more than 50 reactions in the protein metabolism thereby synthesizing, transforming or degrading amino acids. Vitamin B₆ supports the resorption of amino acids and their transport into the cells. Furtheron vitamin B₆ contributes to the synthesis of neuro transmitters and amine products (histamin).

Due to the fact that vitamin B₆ contributes to a variety of different reactions lack of vitamin B₆ results in various clinical pictures.

Applications:

- determination of vitamin B₆ status
- homocysteinaemia
- skin diseases
- movement disorders
- Anaemia, depression

3. PRINCIPLE OF THE TEST

The first step in the determination of vitamin B₆ includes the sample preparation with additional derivatisation. During the precipitation, higher molecular substances are removed. After centrifugation the supernatant is used for derivatisation (20 min at 60 °C), thereby transforming the vitamin B₆ 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 10 minutes. The quantification is performed with the delivered plasma calibrator; the concentration is calculated via integration of the peak area/heights.

Summary

This HPLC application for the determination of vitamin B₆ 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 columns.

As for many other parameters, the advantage of HPLC analytics is 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.	Label	Kit components	Quantity
K 0005.15	RECSOL	Reconstitution solution	1 x 15 ml
KC2150	PREC	Precipitation reagent (serum, plasma, EDTA-whole blood)	1 x 30 ml
KC2100	MOPHA	Mobile phase (important: do not recirculate)	1 x 1 000 ml
	CAL	Calibrator; lyophilised (see specification data sheet for concentration)	4 x
	DER	Derivatisation solution (contain KCN)	1 x 25,5 ml
	CTRL1	Control1; lyophilised	4 x
	CTRL2	Control2; lyophilised	4 x

For reorders of single components, use the catalogue number followed by the label as product number.

The HPLC column (KC2100RP), can be ordered separately from Immundiagnostik. To extend the lifetime of your HPLC column, pre-columns (KC2100VS) are highly recommended. These and also the pre-column holders (KC2100VH) 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 C₁₈ column
- Thermoshaker

6. STORAGE AND PREPARATION OF REAGENTS

- **The lyophilised calibrator (CAL)** is stable at **-20 °C** until the expiry date stated on the label. Before use, the CAL has to be reconstituted with **500 µl reconstitution solution (RECSOL)**. The concentration of vitamin B₆ 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 reconstitution solution (RECSOL)**. The concentration of vitamin B₆ slightly changes from lot to lot, the exact concentration is stated on the specification data sheet.
- 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

Plasma, serum and EDTA-whole blood are suitable for use in the assay.

Vitamin B₆ is light- and temperature-sensitive; therefore, samples have to be protected from light and cooled and centrifuged immediately.

The samples are stable in the dark at 2–8 °C for one week. For longer storage, samples should be frozen at -20 °C.

8. ASSAY PROCEDURE

Test procedure

Pipet into 1.5 ml reaction tubes (e. g. Eppendorf) 200 µl calibrator, patient sample or control 1 or 2.
Add 300 µl precipitation reagent (PREC) and vortex for at least 30 s
Incubate for 10 min at 2–8 °C
Centrifuge for 2 min at 10 000 g
Add to each 250 µl supernatant 250 µl derivatisation solution (DER) and mix
Incubate for 20 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 . It is stable for 5 days at 2–8 °C in the dark.
Inject 50 µl of the supernatant into the HPLC

Chromatographic conditions

Column material: Bischoff Prontosil Eurobond, 5 µm
Merck Lichrospher 5 µm
MZ-Inertsil ODS-2; 5 µm

Column dimension: 125 × 4 mm

Flow rate: 1–1.5 ml/min

Please refer to the quality certificate of the column

Fluorescence detection: Excitation: 320 nm
Emission: 415 nm

Temperature: 30 °C

Injection volume: 20 µl

Running time: 7 min (dialysis patients: 15 min)

9. TREATMENT OF THE COLUMN

It is recommended to use a guard column 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 0.5 ml/min). Before use, the system should be equilibrated with ~ 30 ml mobile phase (MOPHA).

10. RESULTS

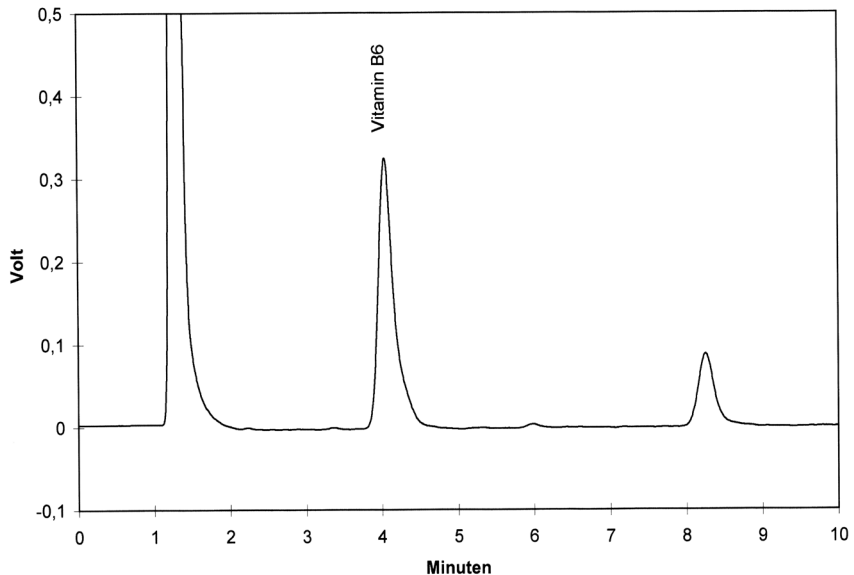
Calculation

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

* see label

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

Typical chromatogram



11. QUALITY CONTROL

Reference ranges

Serum and plasma

- Based on Immundiagnostik AG studies of samples of apparently healthy persons (n = 90), a mean value of 4.3–17.9 ng/ml was estimated.
- Published reference range: 3.6–18 ng/ml (14.6–72.8 pmol/ml); source: Lehrbuch für klinische Chemie und Pathobiochemie, Schattauer Stuttgart / New York, 1987

Whole blood

8.7–27.2 ng/ml*

*Source: den Ottolander, Diagnostisch Kompas, 1997 Ziekenfondsraad, Amstelveen, Holland

We recommend each laboratory to establish its own reference range. The above mentioned values are only for orientation and may 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.

12. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay CV: 2.3 % (28.4 ng/ml) [n = 12]

Inter-Assay CV: 5.4 % (12.9 ng/ml) [n = 12]

Linearity: up to 250 ng/ml

Detection limit: 1.04 ng/ml

Recovery: 84.1 % (whole blood)
97.0 % (plasma)

13. 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.

14. 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
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

Problem	Possible reason	Solution
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

15. 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.

















16. 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 sent to Immundiagnostik AG along with a written complaint.
- The assay should always be performed according to the enclosed manual.

17. REFERENCES

1. Ambrosch, A. et al., 2001. Relation between homocysteinaemia and diabetic neuropathy in patients with Type 2 diabetes mellitus. *Diabetic medicine : a journal of the British Diabetic Association*, **18**(3), pp.185–92.
2. Dierkes, J., Domröse, U., et al., 2001. Homocysteine lowering effect of different multivitamin preparations in patients with end-stage renal disease. *Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation*, **11**(2), pp.67–72.
3. Dierkes, J., Westphal, S., et al., 2001. Vitamin supplementation can markedly reduce the homocysteine elevation induced by fenofibrate. *Atherosclerosis*, **158**(1), pp.161–4.

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
	Contains plasma derivatives or human blood		Consult instructions for use
	Consult specification data sheet		Do not re-use
	Unique Device Identification		Contains material of animal origin
	Medicinal substance		Contains material of human origin