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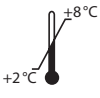
**Manual**

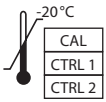
# Vitamin $B_6$ HPLC Kit

***For the determination of vitamin $B_6$ in serum and plasma***

Valid from 2022-09-05

REF **KC2100**


IVD CE

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## 1. INTENDED USE

This HPLC application is intended for the quantitative determination of vitamin $B_6$ in EDTA-plasma and serum. For *in vitro* diagnostic use only.

## 2. SUMMARY AND EXPLANATION OF THE TEST

Vitamin $B_6$ 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 $B_6$).

Vitamin $B_6$ functions as a coenzyme and is essential for more than 50 reactions in the protein metabolism thereby synthesizing, transforming or degrading amino acids. Vitamin $B_6$ supports the resorption of amino acids and their transport into the cells. Furtheron vitamin $B_6$ contributes to the synthesis of neuro transmitters and amine products (histamin).

Due to the fact that vitamin $B_6$ contributes to a variety of different reactions lack of vitamin $B_6$ results in various clinical pictures.

**Applications:**

- determination of vitamin $B_6$ status
- homocysteinaemia
- skin diseases
- movement disorders
- Anaemia, depression

## 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 (20 min at 60 °C), thereby transforming the vitamin $B_6$ 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**

The application of vitamin $B_6$ for HPLC makes it possible to determine the vitamin in an easy, fast and precise method. The kits 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 |
| KC2100 | MOPHA | Mobile phase (important: do not recirculate) | 1 x 1 000 ml |
| | CAL | Calibrator; lyophilised (see specification data sheet for concentration) | 4 x |
| | PREC | Precipitation reagent (serum, plasma) | 1 x 5 ml |
| | 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 $C_{18}$ 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 $B_6$ 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 $B_6$ 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

EDTA-plasma and serum are suitable for use in the assay.

Vitamin $B_6$ 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)<br>**200 µl calibrator**, **patient sample** or **control 1 or 2.** |
| Add **50 µl precipitation reagent** (PREC) and mix |
| Incubate for **10 min** at **2–8 °C** |
| Centrifuge for **2 min** at **10 000 *g*** |
| Add to each **100 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 20 µl** of the supernatant into the HPLC |

### *Chromatographic conditions*

| | | |
|---|---|---|
| Column material: | Bischoff Prontosil Eurobond, 5 µm<br>Merck Lichrospher 5 µm<br>MZ- Inertsil ODS-2; 5 µm | |
| Column dimension: | 125 × 4 mm | |
| Flow rate: | 1–1.5 ml/min<br>Please refer to the quality certificate of the column | |
| Fluorescence detection: | Excitation:<br>Emission: | 320 nm<br>415 nm |
| Temperature: | 30 °C | |
| Injection volume: | 20 µl | |
| Running time: | 7 min (dialysis patients: 15 min) | |

It is recommended to use a guard column to extend column life.

## 9. TREATMENT OF THE COLUMN

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

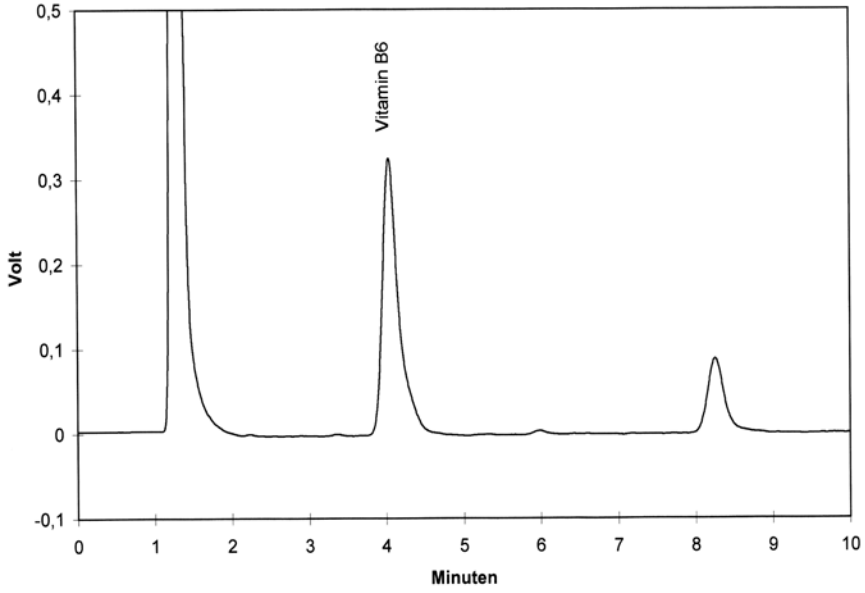
### *Calculation*

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

* see label

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

### *Typical chromatogram*



## 11. QUALITY CONTROL

### *Reference ranges*

- 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

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**: | 8.3 % (6.6 ng/ml) | [n = 6] |
| | 6.0 % (15.1 ng/ml) | [n = 6] |
| **Inter-Assay CV**: | 5.9 % (10.5 ng/ml) | [n = 13] |
| | 5.9 % (27.2 ng/ml) | [n = 13] |

### *Linearity*

up to 250 ng/ml

### *Detection limit*

0.8 ng/ml

### *Recovery*

98.3 %

## 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 send 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

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