EAGLE BIOSCIENCES

Vitamin B_6 HPLC Assay

Catalog Number: VB631-H100
100 Tests
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

EAGLE BIOSCIENCES, INC.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 866-419-2019 Fax: 617-419-1110
www.EagleBio.com

1. Intended purpose

The Eagle Biosciences Vitamin B_6 HPLC Assay is intended for the quantitative determination of vitamin B_6 in serum and plasma. The Vitamin B_6 HPLC Assay kit is for research use only and not to be used in diagnostic procedures.

2. Introduction

The vitamins pyridoxin, pyridoxal and pyridoxamin and the appropriate phosphate products are summarized as vitamin B_6. All forms can be transformed into the active form pyridoxal-5-phosphate. Vitamin B_6 functions as a coenzyme and is essential for more than 50 reactions in the protein, carbohydrate and fatty acid metabolism thereby synthesizing, transforming or degrading amino acids. In protein metabolism vitamin B_6 supports the resorption of amino acids and their transport into the cells. Furthermore vitamin B_6 contributes to the synthesis of neurotransmitters and amine products (histamine).

Due to the fact that vitamin B_6 contributes to a variety of different reactions lack of vitamin B6 results in various clinical pictures as muscle dystrophia, skin diseases, or disturbances of the nervous system. High risk groups for reduced vitamin B_6 concentrations in serum are lactating women, women taking oral contraceptives with high amount of estrogen and chronic drinkers.

The Eagle Biosciences Vitamin B_6 HPLC Assay makes it possible 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 (IC2100rp) and the controls (IC2100ko). Both can be supplied by Eagle Biosciences. Beside the complete test kits it is possible to order all components separately. Please request our single component pricelist.

3. Warnings and precautions

- All reagents of the Vitamin B_6 HPLC Assay kit are strictly intended for research use only and are not to be used for diagnostic procedures.
- Test kit and column are concerted. Using alternative columns might cause in insufficient separation, resulting in false high results. The given test characteristics might not be fulfilled.
- Do not interchange Vitamin B_6 HPLC Assay kit components from different lots.
- Calibrator and controls contain human blood. It was tested and found negative for HBsAg, anti-HIV-1/2, and anti-HCV. No test can guarantee the absence of HBsAg or HIV, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- The precipitating reagent contains acid and has to be handled carefully. It is corrosive and causes burns. It should be handled with gloves, eye protection, and appropriate

protective clothing in a hood. Any spill should be wiped out immediately with copious quantities of water. Do not breathe vapor and avoid inhalation. In case of an accident or indisposition contact immediately a physician.

- Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
- Do not pipette by mouth.
- Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
- Reagents should not be used beyond the expiration date shown on kit label.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

4. Materials Provided

Article no.	Component	Designation	Amount
IC2100lm	ELU	Mobile phase	1000 ml
IC2100ka	CAL	Calibrator, (lyoph. 4 ml)	1 vial
IC2100fr	PREC	Precipitation reagent	5 ml
IC2100rl	RECON	Reconstitution solution	10 ml
IC2100dl	DERIVAT	Derivatisation solution (contains KCN)	3 x 8.5 ml

5. Additional special equipment

- 1.5 ml reaction tubes (Eppendorf)
- Centrifuge
- Various pipettes
- HPLC with Fluorescence-detector
- HPLC column Vitamin B6 (IC2100rp)
- Heatable shaker or water bath
- Vortex mixer

6. Reagent preparation

- Reconstitute the **calibrator (CAL)** in **4 ml** reconstitution solution (RECON), divide the calibrator in several portions and store them at -20 °C. Avoid repeated freeze-thaw circles. The concentration of vitamin B6 might have minor changes from lot to lot.
- All other test reagents are stable at 2-8 °C, up to the date of expiry stated on the label.

7. Specimen

- EDTA-plasma and serum could be used in this test system. For the determination of EDTA-whole blood a whole blood calibrator and a special adapted precipitation reagent is available.
- 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 1 week. For longer storage samples should be frozen at -20 °C.

8. Procedure

Principle of the Method

For the determination of vitamin B_6 a precipitation step to remove high molecular substances is performed first. After centrifugation the supernatant is mixed with a derivatisation solution and incubated for 20 min at 60°C. The fluorescent probe is then cooled (2-8°C), centrifuged and injected into the HPLC system. The isocratic separation via HPLC at 30°C lasts 10 minutes. The chromatograms are recorded by a fluorescence detector. The quantification is performed with the delivered plasma calibrator; the concentration is calculated via integration of the peak heights respectively areas.

Sample preparation

1. Pipette into 1.5 ml reaction tubes:

   200 µl sample, CAL or CTRL

   +

   50 µl PREC

2. Mix well. Leave the tubes for **10 minutes at 2-8°C** and centrifuge afterwards at 10.000g for 2 minutes.

3. Mix

   100 µl supernatant

   +

   250 µl DERIVAT

4. Incubate for **20 minutes at 60°C** on a shaker or in a water bath; cool to 2-8°C and centrifuge at 10.000g for 5 minutes

5. Inject **20 µl** of the supernatant for chromatography into the HPLC-system. The supernatant is stable in the dark for 5 days at 2-8°C.

Chromatographic settings

Column material:	Bischoff Prontosil Eurobond, 5 µm	
Column dimension:	125 mm x 4 mm	
Flow rate:	1-1.5 ml/min	
Fluorescence detection:	Excitation	320 nm
	Emission	415 nm
Injection volume:	20 µl	
Running time:	7 min (Dialysis patients 15 minutes)	
Temperature:	30 °C	

Treatment of the HPLC column

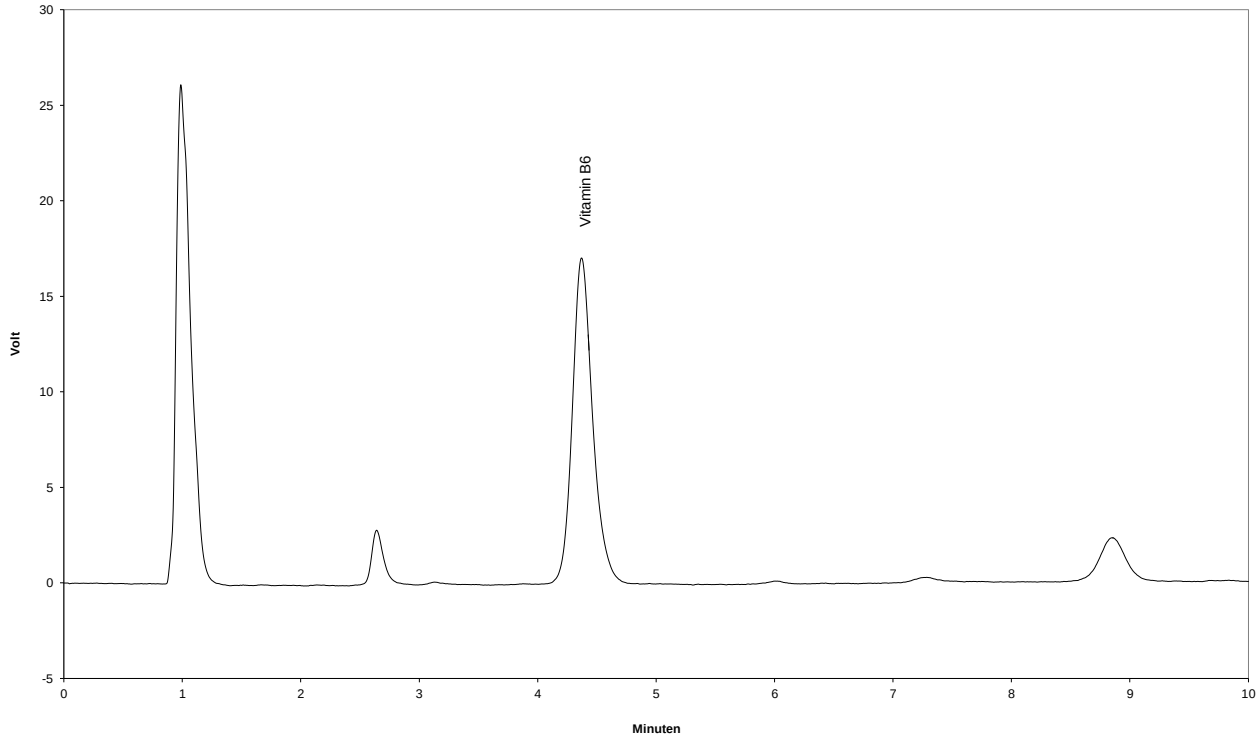
After the analysis the column should be flushed with 15 ml deionized water (1 ml/min) and stored in 50% methanol in deionized water (approx. 15 ml, flow 0.7 ml/min). Before use, the system should be equilibrated with approx. 30 ml ELU.

9. Calculation of analytical results

Calculation

$$\text{Conc. sample (ng/ml)} = \frac{\text{peak area patient} * \text{conc. calibrator (ng/ml)}}{\text{peak area calibrator}}$$

Typical chromatogram



10. Internal Quality Control

Reference intervals

4.1 – 43.7 ng/ml

We recommend that each laboratory should develop their own normal range. The values mentioned above are only for orientation and can deviate from other published data.

(Dierkes et al (2007). Plasma pyridoxal-5-phosphate and future risk of myocardial infarction in the European Prospective Investigation into Cancer and Nutrition Potsdam cohort. Am J Clin Nutr 86; 214-220)

11. Validation data

Precision and reproducibility

Intra-Assay CV:	2.5 % (5.9 ng/ml)	[n = 6]
	0.9 % (20.2 ng/ml)	[n = 6]
Inter-Assay CV:	2.9 % (6.1 ng/ml)	[n = 6]
	1.5 % (20.3 ng/ml)	[n = 6]

Linearity

up to 500 ng/ml

Detection limit

0.2 ng/ml

Recovery

97.1 %

12. Limitations of the method

To minimize interferences with whole blood, whole blood should be diluted 1:1 with deionized water. The calculated concentration must then be multiplied by 2.

13. Disposal

The derivatisation solution (DERIVAT) can be oxidized with hydrogen peroxide and after the pH value is adjusted in between 6-8, it can be disposed as aqueous salt solution. The mobile phase (ELU) and the precipitation solution (PREC) could be neutralized with NaOH and if the pH value is neutral it can be 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
	Autosampler 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
Baseline is drifting	Detector lamp did not reach working temperature yet	Wait
	Detector lamp is too old	Renew lamp
Continue baseline is drifting	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 flowcell is dirty	Clean flow cell

15. Literature references

- Ambrosch A. et al. (2000). Relation between homocysteinaemia and diabetic neuropathy in patients with Type 2 diabetes mellitus. Diabetic Med 18; 185-192.

- Dierkes J. et al. (2001). Vitamin supplementation can markedly reduce the homocysteine elevation induced by fenofibrate. Atherosclerosis 158; 161-164.

- Dierkes et al. (2001). Homocysteine lowering effect of different multivitamin preparations in patients with end-stage renal disease. J Renal Nut 11; 67-72.

- Dierkes et al (2007). Plasma pyridoxal-5-phosphate and future risk of myocardial infarction in the European Prospective Investigation into Cancer and Nutrition Potsdam cohort. Am J Clin Nutr 86; 214-220

For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences at info@eaglebio.com or at 866-411-8023.