



EAGLE
BIOSCIENCES

Vitamin B₁ (Thiamine Pyrophosphate) HPLC Assay

Catalog Number: VB131-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: 617-419-2019 Fax: 617-419-1110

www.EagleBio.com



1. Intended purpose

The Eagle Biosciences Vitamin B₁ (Thiamine Pyrophosphate) HPLC Assay is intended for the quantitative determination of vitamin B₁ (thiamine diphosphate or thiamine pyrophosphate) in EDTA-blood. The Vitamin B₁ (Thiamine Pyrophosphate) HPLC Assay kit is for research use only and not to be used in diagnostic procedures.

2. Introduction

Vitamin B₁ is a water soluble vitamin. It consists out of a pyrimidine and a thiazol ring, which are synthesized independently and linked afterwards. Vitamin B₁ is de novo produced by many plants and microorganisms. Humans and animals have to take it up by nutrition. They are only able to do the phosphorylation reaction of the thiamin molecule.

Thiamin is found free and as mono-, di- and triphosphate. The different forms can be transferred into each other. The metabolic active form is thiamin diphosphate in the body and thiamin triphosphate in the brain. Thiamin diphosphate (TDP) works as thermostable coenzyme in many enzymes of the carbohydrate and amino acid metabolism. In most reactions TDP catalyzes the cleavage of C-C bonds. In the nervous system vitamin B₁ is needed for the stimulation of nerve cells.

The classical disease of patients with a lack of vitamin B₁ is Beriberi, which is known from regions where people eating predominantly white rice. The symptoms are paralysis, muscle dystrophia and heart failure. Other diseases are the Wernicke encephalopathy, the Korsakow syndrome and some forms of the Landry`s paralysis. In industrialized countries alcoholism seems to be the most important reason for a lack of vitamin B₁. In alcoholics the thiamin resorption is disturbed. Due to a damage of the liver the storage capacity of vitamin B₁ is reduced.

The Eagle Biosciences Vitamin B₁ (Thiamin) 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 (IC2201rp) and the controls (IC2201ko). Both can be supplied by The Eagle Biosciences. Beside the complete test kit it is possible to order all components separately. Please request our single component price list.

3. Warnings and precautions

- All reagents of the Vitamin B₁ (Thiamine Pyrophosphate) HPLC Assay kit are strictly intended for Research Use Only.



- 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 the Eagle Biosciences Vitamin B₁ (Thiamine Pyrophosphate) HPLC Assay components from different lots.
- The hydrolysis 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 immediately contact 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. Material delivered in the test package

Article no.	Component	Designation	Amount
IC2201lm	ELU	Mobile phase	1000 ml
IC2201ka	CAL	Calibrator, (lyoph. 1 ml)	1 vial
IC2201fr	PREC	Precipitation reagent	5 ml
IC2201rb	REAC	Reaction buffer	5 ml
IC2201vl	DIL	Dilution solution	20 ml
IC2201lc	SOLC	Solution C	5.5 ml
IC2201dl	DERIVAT	Derivatisation solution (lyoph.)	1 vial



5. Additional special equipment

- 1.5 ml reaction tubes (Eppendorf)
- Centrifuge
- Various pipettes
- HPLC with fluorescence-detector
- HPLC column Vitamin B1 (IC2201rp)
- Heatable shaker or water bath
- Vortex mixer

6. Reagent preparation

- Reconstitute the **calibrator (CAL)** in 1 **ml dilution solution (DIL)**, divide the calibrator in several portions and store them at -20 °C. Avoid repeated freeze-thaw circles. The concentration of vitamin B1 might have minor changes from lot to lot.
- Reconstitute the **derivatisation solution (DERIVAT)** in **5.5 ml solution C (SOLC)**. The dissolved derivatisation solution is stable for 6 month at 2-8°C.
- All other test reagents of the Vitamin B₁ (Thiamine Pyrophosphate) HPLC Assay are stable at the temperature given on the label, up to the date of expiry.

7. Specimen

- EDTA blood can be used in the Vitamin B₁ (Thiamine Pyrophosphate) HPLC Assay.
- Vitamin B1 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 day. For longer storage samples should be frozen at -20 °C. Avoid freezing the samples a second time.



8. Procedure

Principle of the method

- For the determination of vitamin B1 a precipitation step to remove high molecular substances is performed first. After centrifugation the supernatant is mixed with a derivatisation solution and incubated for 10 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 5 minutes. The chromatograms are recorded by a fluorescence detector. The quantification is performed with the delivered EDTA-blood calibrator; the concentration is calculated via integration of the peak heights respectively areas.

Sample and standard preparation

1. Pipette into 1.5 ml reaction tubes:

50 µl sample, CAL or CTRL

+

150 µl DIL

+

50 µl PREC

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

Mix **150 µl** supernatant

+

50 µl REAC

+

50 µl DERIVAT

3. Incubate for **10 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.



- Inject **40-50 µl** of the supernatant for chromatography into the HPLC-system (To achieve the best separation, the exact volume is given in the specification of the Vitamin B1 HPLC Column (sold separately))

The supernatant is stable in the dark for 3 days at 2-8°C.

Chromatographic conditions

Column material:	Vitamin B1 column (IC2201rp)
Column dimension:	125 mm x 4 mm
Flow rate:	0.8-1.2 ml/min
Fluorescence detection:	Excitation 365 nm Emission 440 nm
Running time:	5 min
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 85% acetonitrile/deionized water. Before use, the system should be equilibrated with approx. 30 ml eluent.

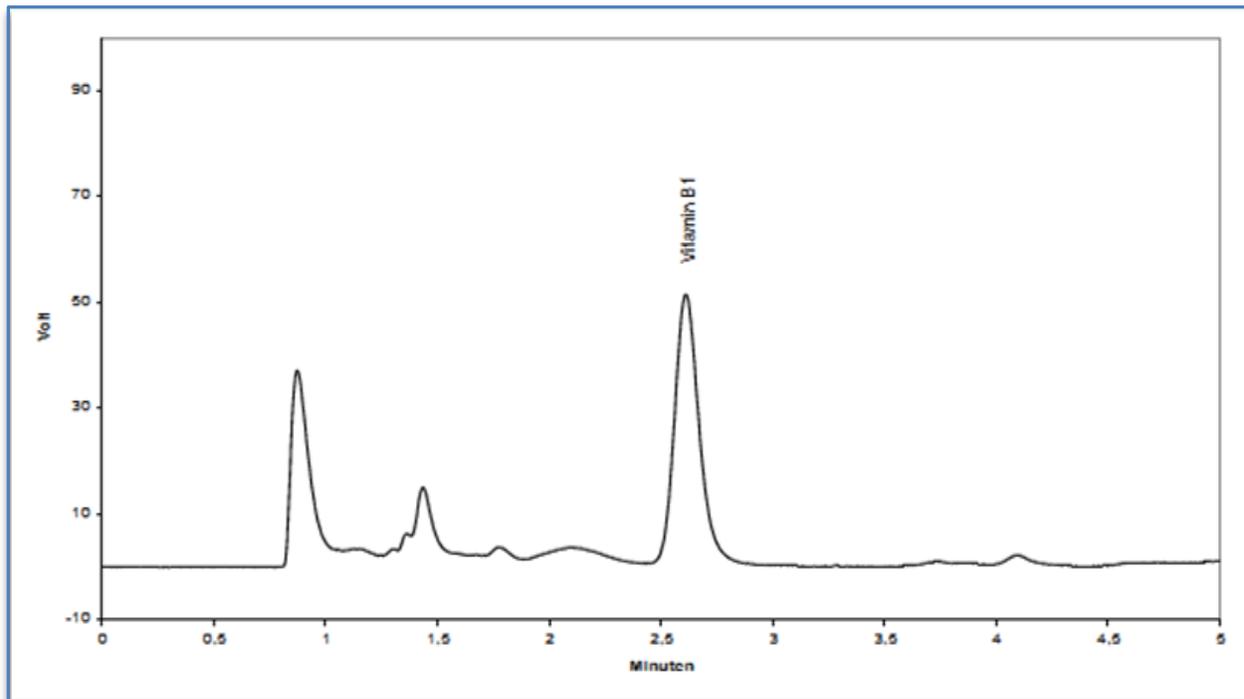
9. Calculation of analytical results

Calculation

$$\text{Conc. sample} = \frac{\text{peak area patient} * \text{conc. calibrator}}{\text{peak area calibrator}}$$



Typical chromatogram



10. Internal quality control

Expected values

35 – 99 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.

11. Validation data

Precision and reproducibility

Intra-Assay CV:	0.9 % (36.6 ng/ml)	[n = 6]
	1.5 % (85.6 ng/ml)	[n = 6]
Inter-Assay CV:	3.4 % (37.1 ng/ml)	[n = 6]
	3.8 % (85.0 ng/ml)	[n = 6]



Linearity	up to 500 ng/ml
Detection limit	0.2 ng/ml
Recovery	99.6 %

12. Limitations of the method

The measurement of serum and plasma samples is possible with the Vitamin B₁ (Thiamin) HPLC Assay but not recommended, because the concentration are most below the detection limit. Lipemic samples should not be measured.

13. Disposal

The mobile phase (ELU) and derivatisation solution (DERIVAT) must be disposed as non-halogenated solvent. 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 connections
	Lamp of detector is altered	Renew lamp
No peaks	Injector is congested	Check injector
Double peaks	Dead volume in fittings and/or at the head of column	Renew fittings and/or column



Problem	Possible reason	Solution
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 the mobile phase and pump head
	Autosampler vials contaminated	Use new vials or clean them with methanol
Broad peaks, tailing	Precolumn / column exhausted	Renew 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
	Detector lamp is too old	Renew lamp

15. Literature references

- 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.
- Herbeth B., et al. (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.

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