

Vitamin B₁ in Whole Blood and Vitamin B₆ in Whole Blood/Plasma

Reagent Kit for HPLC Analysis

- > Each parameter safeguarded by a specific internal standard
- > No post-column derivatisation required
- > One sample preparation and one run for both parameters

Specifications

Limit of quantification:
 TPP 2.0 µg/l (whole blood)
 PLP 4.5 µg/l (whole blood), 0.5 µg/l (plasma)
 Intraassay: CV < 3 %
 Interassay: CV < 6 %
 Linearity: PLP 500 µg/l
 TPP 750 µg/l
 Analysis time: < 7 min plasma
 < 9 min whole blood

Pre-Analytic Treatment

Specimens: whole blood or plasma
 Stability of samples: protect sample from light, at +2 to +8 °C stable for 1 day, for longer storage deepfreeze below -18 °C (maximum 2 weeks).

Sample Preparation

- In a light protected vial mix 200 µl whole blood or plasma with 100 µl Internal Standard and 300 µl Precipitation Reagent, mix for at least 30 sec (vortex).
- Centrifuge for 5 min with at least 9000 x g.
- In a new light protected vial mix 250 µl Neutralisation Reagent and 100 µl Derivatisation Mix with 250 µl supernatant obtained above, mix briefly.
- Incubate for 25 min at 60 °C (waterbath).
- Cool sample for 10 min at +2 to +8 °C, then centrifuge for 2 min with at least 9000 x g.
- Transfer supernatant into a light protected autosampler vial, inject 25–50 µl into the HPLC system.

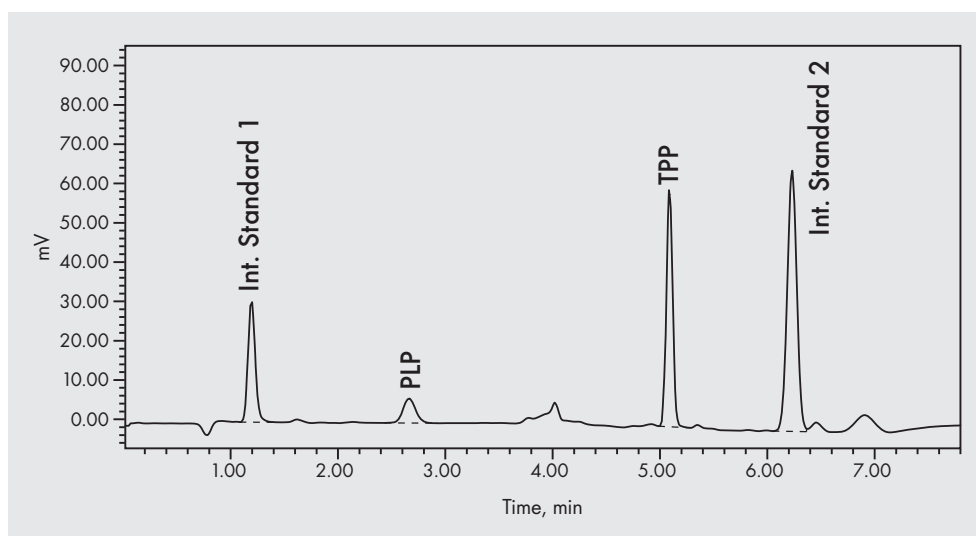
HPLC-Parameter

For the combined Chromsystems HPLC analysis of vitamins B₁ and B₆ in whole blood or plasma every common HPLC system with binary pump and programmable fluorescence detector is suitable.

Injection volume: 25–50 µl
 Flow rate: 1.5–2.3 ml/min
 Gradient profile: specific binary gradients for whole blood and plasma

Detection wavelengths:
 Start EX 320 nm, EM 415 nm, after approx. 3.8 min switch to EX 367 nm, EM 435 nm

Column temperature: ambient (~25 °C)



Vitamin B₁ (thiamine) is ingested with food; it is water-soluble and heat sensitive. The active form (thiamine pyrophosphate, TPP) acts as a co-enzyme for several enzymatic reactions in carbohydrate metabolism, e.g. for pyruvate decarboxylation. Thiamine is also important for oxidative glucose metabolism and deficiency negatively impacts brain and nerve cells, as these cells depend on the energy generated by glucose. Metabolic products that cannot be further metabolized due to vitamin B₁ deficiency destroy cells in the myocardium and central nervous system. As the active form, TPP concentrations in whole blood are more conclusive than the concentration of total thiamine. Vitamin B₆ is comprised of the pyridoxine-group pyridoxine, pyridoxamine and pyridoxal. It is ingested with food and transferred via several enzymatic conversions into its active form, pyridoxal-5'-phosphate (PLP). PLP acts as a co-factor, e.g. in amino acid metabolism, in the formation of haemoglobin or neurotransmitters in the brain. If PLP cannot be synthesised due to enzyme deficiency, strong convulsions, especially in newborns, are induced.

This Chromsystems reagent kit facilitates the combined analysis of vitamin B₁ in whole blood and vitamin B₆ in whole blood and plasma. The sample preparation processes different matrices, thus whole blood samples can easily be measured in the same sequence with plasma samples. Vitamin molecules are derivatised during sample preparation which renders the common post-column derivatisation unnecessary. The separation takes place with a binary gradient. The two specifically developed internal standards for both parameters and matching quality controls ensure precise and accurate results.

Ordering Information

Order no.	Product
52052	HPLC reagent kit Vitamin B ₁ in whole blood and Vitamin B ₆ in whole blood/plasma For 100 analyses

Components available separately:

52001	Mobile Phase A, 1000 ml
52022	Mobile Phase B, 1000 ml
52003	Vitamins B ₁ /B ₆ Whole Blood Calibration Standard (lyoph.), 5 x 1 ml
52044	Internal Standard, 10 ml
52005	Precipitation Reagent, 30 ml
52006	Neutralisation Reagent, 25 ml
52007	Derivatisation Reagent 1 (lyoph.), 2 x 0.3 ml
52008	Derivatisation Reagent 2, 15 ml
33005	Reaction vials, amber coloured (light protection), 100 pcs.

Accessories:

52100	HPLC column, equilibrated, with test chromatogram, 1 pc.
15010	PEEK prefilter housing, 1 pc.
15011	PEEK-encased prefilters, 2 µm, 5 pcs.
18001	Precolumn cartridge holder 4/10, 1 pc.
18052	Precolumn cartridge 4/10, 1 pc.

Chromsystems calibrator and controls for vitamin B₁ and vitamin B₆ in whole blood (lyoph.):

52003	Whole Blood Calibration Standard, 5 x 1.0 ml
0164	Whole Blood Control Bi-Level (I + II), 2 x 5 x 2.0 ml
0165	Whole Blood Control Level I, 5 x 2.0 ml
0167	Whole Blood Control Level II, 5 x 2.0 ml

Chromsystems calibrator and controls for vitamin B₆ in plasma (lyoph.):

36005	Plasma Calibration Standard, 5 x 1.0 ml
0031	Plasma Control Bi-Level (I + II), 2 x 5 x 2.0 ml
0038	Plasma Control Level I, 5 x 2.0 ml
0039	Plasma Control Level II, 5 x 2.0 ml

