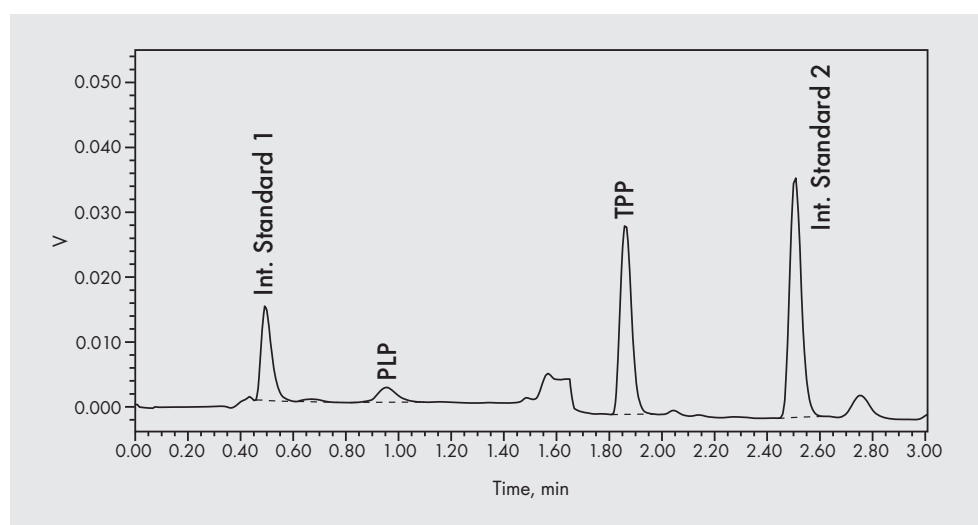


High Throughput UHPLC Combined Vitamin B₁/B₆ Analysis Reagent Kit for UHPLC Analysis

- > UHPLC separation in less than 3 min
- > Each parameter safeguarded by a specific internal standard



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.

The new Chromsystems UHPLC Kit for the combined analysis of vitamin B₁ in whole blood and B₆ in whole blood or plasma combines the features of the proven HPLC method with the distinct advantages of UHPLC technology. The efficient sample preparation with Pre-mixed Tubes involves only one precipitation step and subsequent derivatisation. Analyte separation takes approximately 3 minutes. The two specifically developed internal standards for both parameters and matching quality controls ensure precise and accurate results.

**With Pre-mixed
Neutralisation Tubes**

Specifications

Limit of quantification:
PLP 5 µg/l (whole blood)
TPP 5 µg/l (whole blood)
PLP 1.4 µg/l (plasma)

Intraassay: CV < 4 %
Interassay: CV < 6 %

Linearity: Vit. B₆ (PLP) 500 µg/l
Vit. B₁ (TPP) 750 µg/l

Analysis time: approx. 3 min

Pre-Analytic Treatment

Specimens: whole blood or plasma.
Stability of samples: Store samples light protected. Samples are stable at +2 to +8 °C 1 day, below -18° C up to 2 weeks.

Sample Preparation

- > Add in a light protected vial to 200 µl whole blood/plasma, 100 µl Internal Standard and 300 µl Precipitation Reagent, mix 30 s (vortex).
- > Centrifuge 5 min with at least 9000 x g.
- > Add 100 µl Derivatisation Mix in a labelled Pre-mixed Neutralisation Tube and add 250 µl of the supernatant from step 1, mix briefly.
Do not centrifuge the precipitate!
- > Incubate 25 min at 60° C (water bath).
- > Cool down for 10 min at +2 to +8 °C and centrifuge for 2 min with at least 9000 x g.
- > Transfer the supernatant into a light protected autosampler vial, inject 2.5–10 µl into the UHPLC system.

UHPLC Parameters

For the combined Chromsystems UHPLC analysis of vitamins B₁ and B₆ in whole blood and plasma, any major UHPLC system with binary pump and programmable fluorescence detector is suitable.

Pressure: < 600 bar
Injection volume: 2.5–10 µl
Flow rate: 0.7 ml/min
Detection wavelengths:
Start: EX 320 nm, EM 415 nm
After approx. 1.6 min switch to EX 367 nm, EM 435 nm
Column temperature: ambient (~ 25°C)

Ordering Information

Order no.	Product
52952/UHPLC	UHPLC reagent kit Vitamin B ₁ in whole blood and Vitamin B ₆ in whole blood/plasma

Content for 1000 analyses:
 Mobile Phase A, 2 x 1000 ml
 Mobile Phase B, 1000 ml
 Whole Blood Calibration Standard (lyoph.), 5 x 5 x 1 ml
 Internal Standard, 10 x 10 ml
 Precipitation Reagent, 10 x 30 ml
 Pre-mixed Neutralisation Tubes, 10 x 100 pcs.
 Derivatisation Reagent 1 (lyoph.), 10 x 2 x 0.3 ml
 Derivatisation Reagent 2, 10 x 15 ml
 Reaction vials, amber coloured (light protection), 10 x 100 pcs.
 UHPLC column, 1 pc.

Components available separately:

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
52906	Pre-mixed Neutralisation Tubes, 100 pcs.
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:

15060	UHPLC prefilter housing, 1 pc.
15061	UHPLC prefilter, 0.5 µm, 5 pcs.

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

