

# Vitamin B<sub>1</sub> in Whole Blood and Vitamin B<sub>6</sub> in Whole Blood/Plasma

**With Pre-mixed Neutralisation Tubes**

## 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
- > Simplified sample prep with Pre-mixed Tubes

### 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 s (vortex).
- Centrifuge for 5 min with at least 9000 x g.
- Add 100 µl of Derivatisation Mix and 250 µl of supernatant to a labeled Pre-mixed Neutralisation Tube, mix briefly.
- Incubate for 25 min at 60 °C (water bath).
- 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

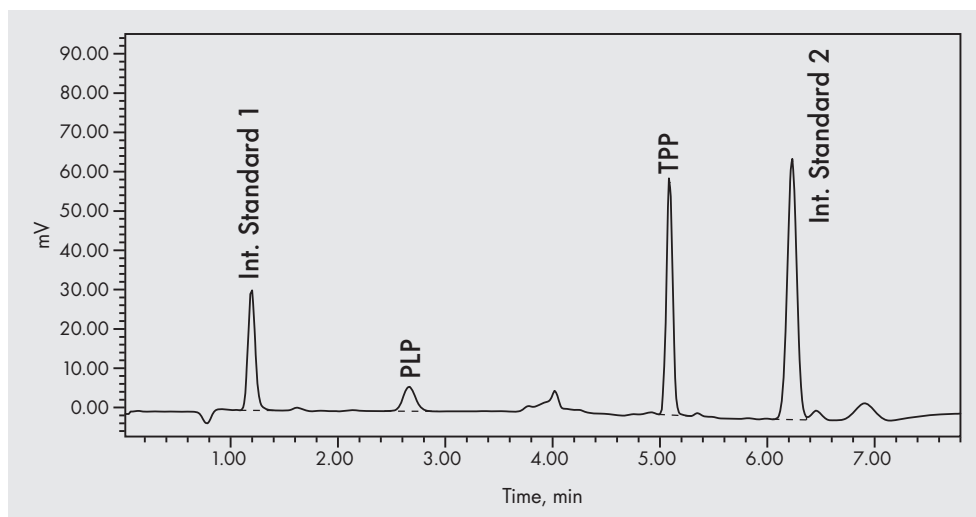
For the combined Chromsystems HPLC analysis of vitamins B<sub>1</sub> and B<sub>6</sub> in whole blood or plasma every common HPLC system with binary pump and programmable fluorescence detector is suitable.

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 B<sub>1</sub> (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 metabolised due to vitamin B<sub>1</sub> deficiency destroy cells in the myocard and central nervous system. As the active form, TPP concentrations in whole blood are more conclusive than the concentration of total thiamine. Vitamin B<sub>6</sub> 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 B<sub>1</sub> in whole blood and vitamin B<sub>6</sub> in whole blood and plasma. The efficient sample preparation with Pre-mixed Tubes 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/Premix	HPLC reagent kit Vitamin B <sub>1</sub> in whole blood and Vitamin B <sub>6</sub> in whole blood/plasma with Pre-mixed Neutralisation Tubes For 100 analyses
<b>Components available separately:</b>	
52001	Mobile Phase A, 1000 ml
52022	Mobile Phase B, 1000 ml
52003	Vitamins B <sub>1</sub> /B <sub>6</sub> 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.
<b>Accessories:</b>	
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.
<b>Chromsystems calibrator and controls for vitamin B<sub>1</sub> and vitamin B<sub>6</sub> in whole blood (lyoph.):</b>	
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
<b>Chromsystems calibrator and controls for vitamin B<sub>6</sub> in plasma (lyoph.):</b>	
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