



QUICK REFERENCE

ClinRep[®] Complete Kit "Vitamin B1 in Whole Blood"

HPLC conditions:

Pump (mobile phase):	1.0 ml/min
Autosampler:	Injection interval: 6.0 min
Column heater:	35 °C
Fluorescence detector:	Excitation: 376 nm Emission: 435 nm
Pump (reagent N):	0.1 ml/min

For the mixing of the mobile phase with reagent N a commercially available T-connector (order no.: FK5121) has to be installed between the analytical column and the fluorescence detector.

Please note:

- For using the mobile phases A and B (order nos.: 27008 and 27009) of the ClinRep[®] chromatography kit for the determination of the vitamins B1, B2 and B6 (combikit), please consult the according quick reference (order no.: 27053) for appropriate HPLC conditions.
- A combined determination of vitamins B1 and B6 from whole blood may be performed (if necessary) with the HPLC-conditions for vitamin B6. Column heater: 25 °C; fluorescence detector: 370 nm (excitation), 470 nm (emission); pump (reagent N): 0.2 ml/min.

Reconstitution of the lyophilized whole blood calibrator:

Add exactly 2.0 ml HPLC-water to the vial. To dissolve the material completely, we recommend a so called roller-mixer. A treatment of 30 to 60 min with such an instrument is sufficient. The concentrations of the analytes are given on the data sheet.

Preparation of the enzyme solution:

The working solution of reagent B has to be prepared freshly. For this purpose, take 10 mg of reagent B and add 1 ml of reagent S. The volume of the working solution is chosen according to the number of samples you have in your series of analyses. The freshly prepared working solution is stable for 1 week when stored at 4 °C.

Sample preparation:

Extraction:

500 µl reagent A	300 µl whole blood (calibrator, control, patient)
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↓ mix for 30 sec (vortex-mixer)

centrifugate for 5 min ↓ at 10000 x g

Enzymatic treatment

400 µl supernatant

<i>with enzymatic treatment</i> ^(*)		<i>without enzymatic treatment</i> ^(*)	
add 50 µl	↓ reagent B	add 50 µl	↓ reagent S
mix shortly	↓ (vortex-mixer)	mix shortly	↓ (vortex-mixer)
incubation at 20 °C	↓ (min. 3 h., max. 16 h.)		

Oxidation:

450 µl sample	50 µl reagent C	100 µl reagent D
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↓ mix for 30 sec (vortex-mixer)

↓ add 100 µl reagent E

↓ mix shortly (vortex-mixer)

HPLC analysis:

inject 50 µl of the supernatant

(*): For the determination of vitamin B1 as TPP follow the path without enzymatic treatment. Otherwise vitamin B1 may be determined as "total thiamine". In this case perform the sample preparation including enzymatic treatment.

Test run/calibration run:

Test run: inject 50 µl of the test solution (testing of separation performance).

Calibration run: inject 50 µl of the prepared calibrator (calibration).

Calculation:

Calculation of unknown samples has to be done using the external standard method via peak areas.

The whole blood calibrator supplied with the kit is used for calibration of the HPLC-system and has to be prepared like a patient sample.

The test solution supplied with the kit is intended for qualitative check of the HPLC-system (integration parameters, retention times, peak separation and -shape, etc.). This test solution has to be injected directly (after reconstitution) into your HPLC-system without sample preparation, because it is matrix-free, but only dissolved in a stabilizing medium.

