



QUICK REFERENCE

ClinRep[®] Complete Kit "Vitamin B6 in Plasma / Whole Blood"

HPLC-conditions:

Pump (Mobile Phase):	1.0 ml/min
Autosampler:	Injection interval: 10.0 min
Column Heater:	25 °C
Fluorescence-Detector:	Excitation: 370 nm Emission: 470 nm
Pump (Reagent N):	0.2 ml/min

For merging the mobile phase and reagent N a commercially available T-union (order no.: FK5121) is installed in the capillary between the analytical column and the fluorescence detector.

Please note:

- If you use the mobile phases A and B for determination of the vitamins B1, B2 and B6 (order nos.: 27008 and 27009), please use the quick reference (order no.: 27053) for the ClinRep[®] chromatography kit for the vitamins B1, B2 and B6 to adjust the correct settings at the HPLC instruments.
- The determination of vitamin B6 *from plasma* may be alternatively performed at 35 °C. The injection interval may be decreased to 8.0 min.

Reconstitution of the lyophilized matrix-calibrators (serum, whole blood):

Serum calibrator (order no.: 26013): Add exactly 3.0 ml HPLC-water to the vial and mix for 15 min. When all material is dissolved, the solution is ready to use.

Whole blood calibrator (order no.: 24513): 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 minutes with such an instrument is sufficient.

The concentrations of the analytes are given on the data sheet.

Sample preparation:

Extraction:

100 µl plasma / whole blood (calibrator, control, patient)	100 µl precipitant P
---	-------------------------

↓ mix 30 sec

↓ centr. for 5min at 10000xg

HPLC-analysis:

inject 50 µl of the supernatant

Test run/Calibration run:

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

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 matrix calibrators are used for calibration of the HPLC-system. They have to be prepared like the patient samples.

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

In case of any problems it may be helpful to calibrate with the standard solution. This is possible, but then you have to change the concentrations and calculation factors according to the table below (right column):

Designation	Calibration with the serum calibrator	Calibration with the standard solution
Concentration analyte	see data sheet	see data sheet
Sample amount	1.0	1.0
Scale factor	1.0	2.0

For explanation of the designations see also manual (order no.: 26052) section 5.6.2.

The exact designation of the various parameters differs according to the calculation unit. In addition, it may be necessary to enter further parameters. Thus, prior to carrying out determinations using the external standard method, please read the operation instructions of your integrator/computer carefully.

For manual calculation of the concentrations of the analytes see also manual section 5.6.3.

