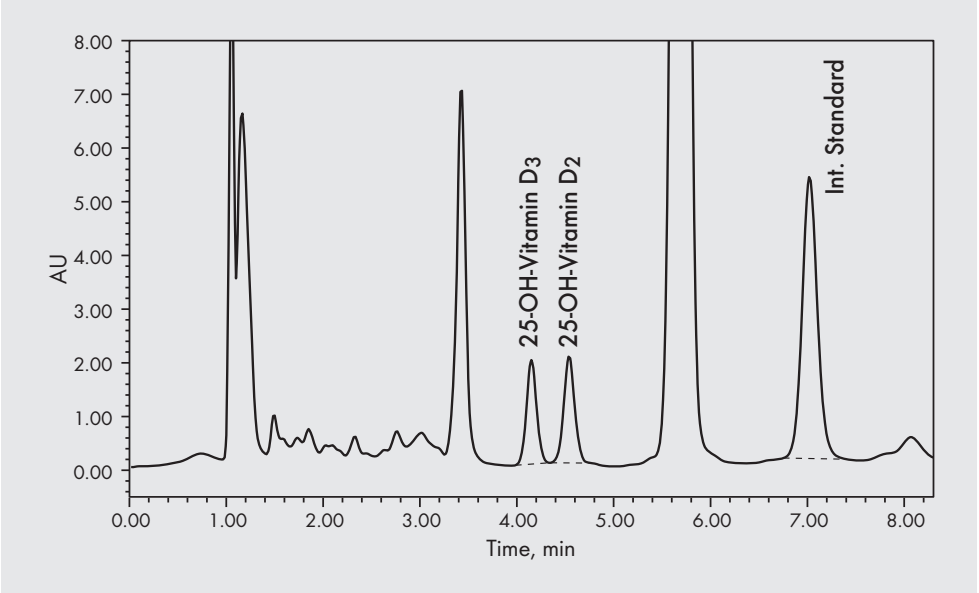
25-OH-Vitamin D_3/D_2 in Serum/Plasma

Reagent Kit for HPLC Analysis

> Vitamins D_3 and D_2 with one analysis
> Improved separation
> Quality controls and calibrator



For the diagnosis of a bone mineralisation malfunction 25-OH-vitamin D_3 is a recognized clinical determinant. 25-OH-vitamin D_2 is measured for monitoring the therapy of vitamin D deficiency using vitamin D_2.

This reagent kit allows the simultaneous and safe chromatographic determination of 25-OH-vitamin D_3 and 25-OH-vitamin D_2 on a simple isocratic HPLC system with UV detection. By means of efficient protein precipitation and selective solid phase extraction interfering components are removed. The analytes are quantified by the inclusion of a stable internal standard. This kit combines fast sample throughput with cost efficiency.

Specifications

Linearity: 2–250 µl

Limit of quantification: 1.1–1.4 µl/g

Recovery: 86 % (25-OH-vitamin D_3)
87 % (25-OH-vitamin D_2)

Intraassay: CV = 0.9–3.0 % (25-OH-vitamin D_3)
CV = 0.8–1.9 % (25-OH-vitamin D_2)

Interassay: CV = 2.3–3.3 % (25-OH-vitamin D_3)
CV = 1.9–4.6 % (25-OH-vitamin D_2)

Analysis time: 12 minutes

Pre-Analytic Treatment

Serum or plasma is used for analysis. Samples are stable up to 1 week at +2 to +8 °C. For longer storage (maximum 1 year), deep-freeze samples below -18 °C.

Sample Preparation

→ Mix 500 µl serum/plasma with 50 µl Internal Standard in a light protected reaction vial.
→ Add 500 µl Precipitation Reagent, vortex-mix for 20 s.
→ Cool down 10 min to 4 °C.
→ Centrifuge 5 min at 15000 x g.
→ Apply complete supernatant to a labelled Sample Clean Up Column and draw through by centrifugation (1 min at 400 x g) or suction, discard effluent.
→ Draw 2 x 1 ml Wash Buffer 1 through column by centrifugation (1 min at 400 x g) or suction, discard effluents.
→ Draw 75 µl Wash Buffer 2 through column by centrifugation (400 x g, about 1 min) or suction, discard effluent.
→ Change collection vial, apply 200 µl Elution Buffer to the column, draw through completely by centrifugation (1 min at 400 x g) or suction. The eluates must be collected into glass vials!
Dilute the eluate with 20 µl ultrapure water (HPLC grade) and mix.
→ Inject 25 µl eluate into the HPLC system.

HPLC Parameters

For the HPLC analysis of 25-OH-vitamin D_3/D_2 an isocratic HPLC system with UV detection is required.

Injection volume:	25 µl
Flow rate:	0.7 ml/min
Wavelengths:	265 nm
Column temperature:	ambient (~ 25 °C)

Ordering Information

These products are not available in all countries

Order no.	*Product*
38038	Reagent kit for the analysis of 25-OH-Vitamin D_3/D_2 in Serum/Plasma For 100 analyses
	Components available separately:
38031	Mobile Phase, 1000 ml
38032	Mobile Phase, 10 x 1000 ml
38033	25-OH-Vitamin D_3/D_2 Serum Calibration Standard, 5 x 2 ml (lyoph.)
38004	Internal Standard, 5 ml
38005	Precipitation Reagent, 50 ml
38006	Wash Buffer 1, 200 ml
38007	Wash Buffer 2, 7.5 ml
38009	Elution Buffer, 20 ml
38008	Sample Clean Up Columns, 50 pcs.
33005	Reaction vials, amber coloured (light protection), 100 pcs.
	Startup Accessories:
38130	HPLC column, equilibrated, with test chromatogram, 1 pc.
15010	PEEK prefilter housing, 1 pc.
15011	PEEK-encased prefilter, 2 µm, 5 pcs.
18001	Precolumn cartridge holder 4/10, 1 pc.
18038	Precolumn cartridge 4/10, 1 pc.
	Chromsystems Calibrators and Controls for 25-OH-Vitamin D_3/D_2 in Serum/Plasma (lyoph.):
62028	3PLUS1® Multilevel Serum Calibrator Set 25-OH-Vitamin D_3/D_2, 4 x 1 ml
38033	25-OH-Vitamin D_3/D_2 Serum Calibration Standard, 5 x 2 ml
0028	25-OH-Vitamin D_3/D_2 Serum Control, Bi-Level (I + II), 2 x 5 x 2 ml
0029	25-OH-Vitamin D_3/D_2 Serum Control, Level I, 5 x 2 ml
0030	25-OH-Vitamin D_3/D_2 Serum Control, Level II, 5 x 2 ml