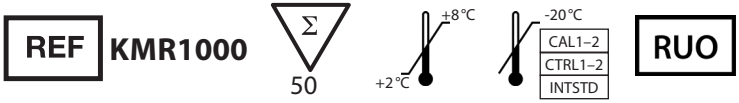
Manual

1,25-$(OH)_2$-Vitamin D_3/D_2 ImmuTube® LC-MS/MS kit

For the determination of 1,25-$(OH)_2$-vitamin D_3/D_2 in plasma and serum

Valid from 2021-10-15



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1. INTENDED USE

1,25-$(OH)_2$-vitamin D_3/D_2 ImmuTube® LC-MS/MS kit is an assay for the quantification of 1,25-$(OH)_2$-vitamin D_3 and 1,25-$(OH)_2$-vitamin D_3 in serum and plasma after immunoaffinity enrichment by LC-MS/MS. The assay is a research tool for manual use by professional laboratory staff. For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

Vitamin D is either produced in the skin (under the influence of UV light) or taken up from nourishment. The storage type of vitamin D, namely 25-hydroxy vitamin D, is formed in the liver. The hormone 1,25-dihydroxy vitamin D (D hormone) is formed in a second hydroxylation step in the kidney. The responsible enzyme, the kidney 1α-hydroxylase, is subjected to a rigid control through hormones (especially parathyroid hormone) and its activity is influenced by the serum concentrations of calcium and phosphate.

The serum concentration of 1,25-dihydroxy vitamin D normally re-adjusts itself to the demands of metabolism. The reason for a deficiency of 1,25-dihydroxy vitamin D can be found in metabolic disturbances, caused either by genetic defects of the enzyme 1α-hydroxylase (rare) or kidney malfunctions (more common). Even a slightly impaired kidney function can lead to a decrease of the 1,25-dihydroxy vitamin D concentration.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KMR0001	ACTSOL	Activation solution	1.5 ml
KMR0002	RECSOL	Reconstitution solution	15 ml
KMR1000	CAL1-2	Calibrators 1 and 2; lyophilised (for concentration, see product specification)	5 vials (à 600 µl) per level
	CTRL1-2	Controls 1 and 2; lyophilised (for concentration, see product specification)	5 vials (à 600 µl) per level
	INTSTD	Internal standard	600 µl
	MOPHAA	Mobile phase A	500 ml
	MOPHAB	Mobile phase B	500 ml
	SOLA	Solution A	25 ml
KMR1100		Extraction kit	see point 4.

For reorders of single components, please use the catalogue number followed by the label without space as product number.

The following accessories for the ImmuTube® LC-MS/MS application can be ordered seperately at Immundiagnostik AG:

- tuning solution for 1,25-$(OH)_2$-vitamin D_3/D_2 (KMR1000TU)
- tuning solution for the internal standard (KMR1000TS)
- UPLC column (KMR1000SP)
- in-line filter (KMR1000IF)
- in-line filter holder (KMR1000IH)

Please ask for our single component price list.

4. CONTENT OF THE EXTRACTION KIT

Cat. No.	Label	Kit components	Quantity
KMR0003	WASHSOL	Wash solution	80 ml
KMR1100	COLUMNS	ImmuTube®-Columns for extraction of 1,25-$(OH)_2$-vitamin D_3/D_2	50 pieces
	ELUSOL	Elution solution	20 ml

For reorders of single components, please use the catalogue number followed by the label without space as product number.

The extraction kit can be ordered separately from Immundiagnostik AG under catalog number KMR1100.

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Glass tubes (inner diameter 10 mm)
- 500 ml graduated cylinder, LC-MS/MS suitable
- Precision pipettors and disposable tips to deliver 10–1 000 µl
- Repeating dispenser
- Centrifuge capable of 10 000 *g* for 1.5 ml Eppendorf reaction tubes and 550 *g* for glass tubes, respectively
- Vortex mixer
- Vacuum centrifuge or nitrogen distributor
- Standard laboratory disposable plastic reagent vials (inner diameter 10 mm)
- Overhead rotator
- LC-MS/MS system
- LC-MS vials

6. PREPARATION AND STORAGE OF REAGENTS

Storage

The test reagents should be stored protected from light, dry and their specified storage temperature (CAL1-2, CTRL1-2, INTSTD: -20 °C; all others 2–8 °C). The test reagents stored in this way are usable until the indicated expiry date.

Note: After preparation of the test reagents for the test procedure other stabilities might apply (see respective preparation step).

Preaparation of the mobile phases and test reagents

Before use, the mobile phases (MOPHAA and MOPHAB) and solution A (SOLA) must be activated by adding activation solution (ACTSOL) according to the following chart:

Components			ACTSOL [µl]
Name	[ml]		
Mobile phases (MOPHAA and MOPHAB)	500	+	500
Solution A (SOLA)	25		25

Prior use mobile phases should be degassed.

Note: After activation with activation solution (ACTSOL), the components mobile phase A (MOPHAA), mobile phase B (MOPHAB) and solution A (SOLA) can be stored up to 2 weeks. It is therefore recommended to prepare only as much as is needed for the test approach.

Attention: The activation solution (ACTSOL) must be added under the fume cupboard. All vessels to be used must be absolutely clean, free of detergents and preferably made of LC-MS/MS suitable glass.

Preaparation of the calibrators and controls

Dissolve calibrators (CAL1–2) and controls (CTRL1–2) in 600 µl of reconstitution solution (RECSOL) each while 30 s vortexing.

7. SAMPLE PREPARATION

Serum and plasma samples are suited for the assay.

The samples must be centrifuged before use (minimum 5 min at 10 000 *g*).

Control samples should be analysed with each run.

Prior to use in the assay, allow all samples and reagents to come to room temperature (18–26 °C).

Mix samples and reagents well before use.

1.	Vortex ImmuTubes® carefully and centrifuge (30 s at 500–1 000 rpm) that no suspension remains in the lid.
2.	Label the lids of ImmuTubes®, open ImmuTubes®, add quickly 500 µl of calibrator (CAL), control (CTRL) or sample. Add 10 µl of internal standard (INTSTD) in each ImmuTube®, close ImmuTubes® and mix gently.
3.	Incubation for 1 h at room temperature in an overhead rotator (15–20 rpm).
4.	Insert closed ImmuTubes® in plastic reagent vials, centrifuge for 1 min at 550 *g*.
5.	Open the outlet of the ImmuTubes®, then the lid and centrifuge for 2 min at 550 *g* to dryness. Discard flow-through.
6.	Add 500 µl of wash solution (WASHSOL) and centrifuge for 2 min at 550 *g* to dryness; discard the flow-through. Carry out this wash step three times in total.
7.	Label new glass tubes, place ImmuTubes® in the labelled glass tubes.
8.	Add 250 µl of-elution solution (ELUSOL), centrifuge for 2 min at 550 *g* and collect the eluate with the 1,25-$(OH)_2$ vitamin D_3/D_2 in the glass tubes.
9.	Evaporate the eluate under a nitrogen stream at 37 °C or in a vacuum centrifuge.
10.	Vortex the residue for 1 min in 165 µl of activated solution A (activated SOLA).
11.	Injection into the LC-MS/MS system (see application note).

8. LC-MS/MS METHOD

Please refer to the application note or contact lcms@immundiagnostik.com for the parameters for setting the LC-MS/MS method.

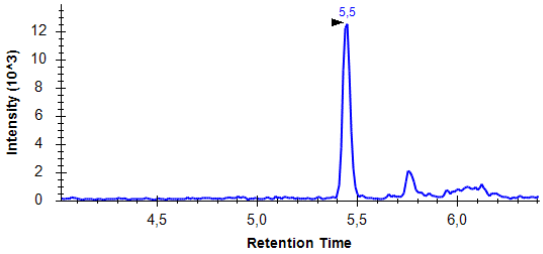
9. CALCULATION

The linear regression is used as model for evaluation of the results. The two calibrator concentration points are connected by a strait line. The samples can be calculated using the obtained line.

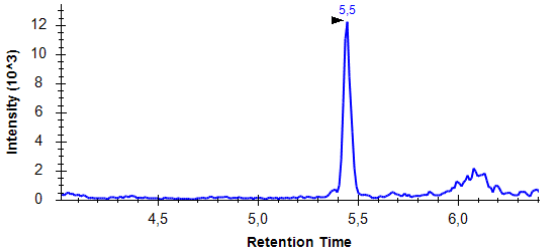
10. EXAMPLES OF CHROMATOGRAMS

1,25-$(OH)_2$-vitamin D_3

Quantifier (*m/z* = 399.3 > 151):

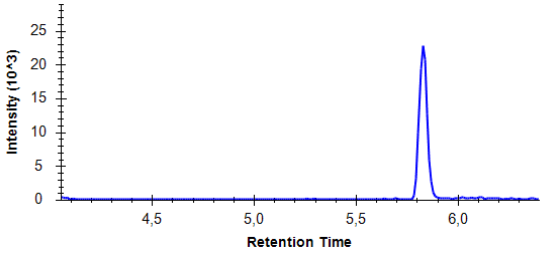


Qualifier (*m/z* = 399.3 > 135.1):

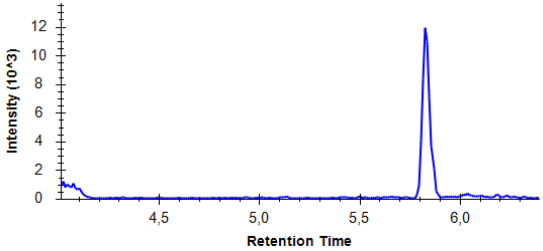


1,25-$(OH)_2$-vitamin D_2

Quantifier (*m/z* = 411.1 > 151):

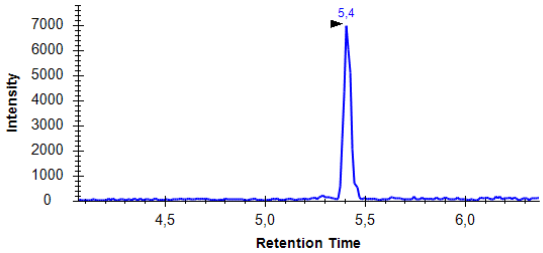


Qualifier (*m/z* = 411.1 > 135.1):

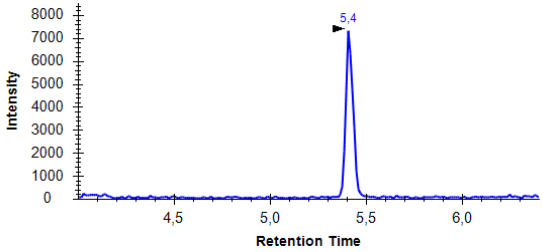


Internal standard

Quantifier (*m/z* = 405.1 > 151):



Qualifier (*m/z* = 405.1 > 135.1):



11. QUALITY CONTROL

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results may not be valid, if one or more values of the quality control sample are outside the acceptable range (see product specification).

We recommend each laboratory to establish its own norm concentration range.

12. PERFORMANCE CHARACTERISTICS

Precision

Repeatability (intra-day); n=22

1,25-$(OH)_2$-vitamin D_3		1,25-$(OH)_2$-vitamin D_2	
[pg/ml]	**CV [%]**	**[pg/ml]**	**CV [%]**
113.0	7.2	115.0	5.3
338.9	4.0	367.1	2.7

Reproducibility (Inter-day); n=14

1,25-$(OH)_2$-vitamin D_3		1,25-$(OH)_2$-vitamin D_2	
[pg/ml]	**CV [%]**	**[pg/ml]**	**CV [%]**
118.8	12.2	119.1	6.7
345.0	9.7	340.4	8.4

Analytical sensitivity

The detection limit (LLOD) designates the lowest concentration of the analyte that can still be detected.

Detection limit of 1,25-$(OH)_2$-vitamin D_3: 5.68 pg/ml

Detection limit of 1,25-$(OH)_2$-vitamin D_2: 12.01 pg/ml

It should be noted that the detection limit depends not only on the application method but also on the instrument.

13. PRECAUTIONS

- The quality control guidelines should be followed.
- Human material used in the kit components was tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- The GHS symbols indicated on the individual components and specifications of the material safety data sheets (available on request from Immundiagnostik AG) must be noted. When working with these reagents, the legal protective precautions must be adhered to.

14. DISPOSAL

Mobile phases (MOPHAA, MOPHAB), solution A (SOLA), activation solution (ACTSOL) and elution solution (ELUSOL) must be disposed as non-halogenated solvents. The calibrators (CAL1-2) and controls (CTRL1-2) should be disposed due to their treatment as potentially infectious material in accordance with local regulations.

15. TECHNICAL HINTS

- Do not mix different lot numbers of any kit component.
- Reagents should not be used beyond the expiration date shown on the kit label.
- The assay should always be performed according the enclosed manual.
- Plugs and caps of different reagents should not be swapped.

16. GENERAL NOTES ON THE TEST

- ImmuTube® is a brand of Immundiagnostik AG.
- All reagents in the kit package are for research use only.
- The guidelines for laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from wrong use.

- Please contact Immundiagnostik AG if one or more components of the kit are damaged, missing (see material supplied) or precipitates are visible in the ready-to-use solutions.
- Warranty claims and complaints in respect of deficiencies must be lodged within 14 days after receipt of the product. The product shall be send to Immundiagnostik AG together with a written complaint.

17. REFERENCES

1. Armbruster, F. et al., 1990. Extraktion und chromatographische Trennung von 1,25-(OH)2-Vitamin D aus Serum oder Plasma ohne Hochleistungs-Flüssigkeitschromatographie (HPLC). *Das Ärztliche Laboratorium*, **36**, pp.75–80.
2. Durham, B. et al., 1995. Comparison of the IDS Gamma-B 1,25 dihydroxy Vitamin D assay system with the Nichols Institute radioreceptor assay system. In *Proceedings of the ACB National Meeting*. Glasgow, UK: The Association of Clinical Biochemists.
3. Hollis, B.W., 1995. 1,25-DihydroxyVitamin D3-26,23-lactone interferes in determination of 1,25-dihydroxyVitamin D by RIA after immunoextraction. *Clinical chemistry*, **41**(9), pp.1313–4.
4. Hollis, B.W., 1996. Assessment of Vitamin D nutritional and hormonal status: what to measure and how to do it. *Calcified tissue international*, **58**(1), pp.4–5.
5. Iqbal, S.J. et al., 1996. Possible interference with calcipotriol on new IDS RIA for 1,25-dihydroxyVitamin D. *Clinical chemistry*, **42**(1), pp.112–3.
6. Schilling, M., Armbruster, F.P. & Schmidt-Gayk, H., 1987. Rapid, selective separation of 1 alpha, 25-dihydroxyvitamin D3 from serum with Extrelut-1 columns. *Clinical chemistry*, **33**(1), p.187.
7. Wildermuth, S. et al., 1993. Scintillation proximity assay for calcitriol in serum without high pressure liquid chromatography. *Clinica chimica acta; international journal of clinical chemistry*, **220**(1), pp.61–70.
8. Withold, W. et al., 1995. Evaluation of a radioimmunoassay for determination of calcitriol in human sera employing a 125I-labelled tracer. *European journal of clinical chemistry and clinical biochemistry : journal of the Forum of European Clinical Chemistry Societies*, **33**(12), pp.959–63.
9. Yuan, C. et al., 2011. Sensitive measurement of serum 1α,25-dihydroxyVitamin D by liquid chromatography/tandem mass spectrometry after removing interference with immunoaffinity extraction. *Rapid communications in mass spectrometry : RCM*, **25**(9), pp.1241–9.

Used symbols:


Temperature limitation

Catalogue number

For research use only

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet