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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 2022-09-28

REF **KM1000**

Σ 50

+8 °C / +2 °C

-20 °C

CAL1–6
CTRL1–3
INTSTD

IVD

CE

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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 an *in vitro* diagnostic tool for manual use by professional laboratory staff. It is used for the determination of the vitamin D metabolite composition in patients with suspected impaired vitamin D metabolism. It can thus be used as a support in the differentiated assessment of the vitamin D status, also in the context of a therapy of vitamin D deficiency by supplementation of vitamin D.

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. Deviations from the normal range of 1,25-dihydroxy vitamin D must therefore always be interpreted in the context of the remaining parameters of the calcium metabolism. The serum concentration of 1,25-dihydroxy vitamin D decreases only in seldom cases of vitamin D deficiency. For the diagnosis of vitamin D deficiency the precursor metabolite, 25-hydroxyvitamin D, should be measured.

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.

Since 1,25-dihydroxy vitamin D has important functions in calcium metabolism as well as supplementing secretion of parathyroid hormone from the parathyroid glands, increasing kidney malfunctioning leads to development of renal osteopathy, which is characterised by osteomalacia and osteitis fibrosa.

Treatment of renal osteopathy consists of the administration of 1,25-dihydroxy vitamin D (calcitriol) or the prohormone 1α-hydroxy vitamin D. In renal tubules malfunctions decreased or relatively low levels of 1,25-dihydroxy vitamin D (e.g. diabetes insipidus, Fanconi syndrome) are found. A non-physiological over-production of 1,25-dihydroxy vitamin D arises in granulomatosis (e.g. sarcoidosis), where extra-

renal synthesis of 1,25-dihydroxy vitamin D occurs. This can lead to hypercalcaemia. Also in idiopathic hypercalciuria a relatively high level of 1,25-dihydroxy vitamin D is found. Increased concentrations of 1,25-dihydroxy vitamin D can be seen in case of non-functional vitamin D receptors (rare), during calcium deficient nutrition, as well as a result from overproduction of parathyroid hormone (primary hyperthyroidism).

Supplemental vitamin D is available in two distinct forms: ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3). Pharmacopoeias have officially regarded these two forms as equivalent and interchangeable, based on studies of rickets prevention in infants. The determination of 1,25 dihydroxy vitamin D_3/D_2 as a measure of 1,25 dihydroxy vitamin D status provides an objective, quantitative measure of the biological response to vitamin D administration.

Indications

- Defect of kidney functions
  Chronic kidney failure
  Haemodialysis following kidney transplantation
- Renal osteopathy
- Osteomalacia from various types of vitamin D metabolism disturbances
- Kidney tubules function disturbances (diabetes insipidus, Fanconi-Syndrom)
- Monitoring of therapy with active vitamin D metabolites
- Ideopathic hypercalciuria
- Hypercalcaemia

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KM0001	ACTSOL	Activation solution	1 x 1.5 ml
KM0002	RECSOL	Reconstitution solution	1 x 15 ml
KM1000	CAL1–6	Calibrators 1–6; lyophilised (for concentration, see product specification)	2 vials (à 600 µl) per level
	CTRL1–3	Controls 1–3; lyophilised (for concentration, see product specification)	3 vials (à 600 µl) per level
	INTSTD	Internal standard	1 x 600 µl
	MOPHAA	Mobile phase A	1 x 500 ml
	MOPHAB	Mobile phase B	1 x 500 ml
	SOLA	Solution A	1 x 25 ml
KM1100		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 (KM1000TU)
- tuning solution for the internal standard (KM1000TS)
- UPLC column (KM1000SP)
- in-line filter (KM1000IF)
- in-line filter holder (KM1000IH)

Please ask for our single component price list.

Immundiagnostik AG offers further options for derivatization and sample preparation for the 1,25-$(OH)_2$-vitamin D_3/D_2 LC-MS/MS application. Please contact us for your individual solution.

4. CONTENT OF THE EXTRACTION KIT

Cat. No.	Label	Kit components	Quantity
KM0003	WASHSOL	Wash solution	1 x 80 ml
KM1100	COLUMNS	ImmuTube®-Columns for extraction of 1,25-$(OH)_2$-vitamin D_3/D_2	1 x 50 pieces
	ELUSOL	Elution solution	1 x 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 KM1100.

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 (CAL1–6, CTRL1–3, 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 (CAL1–6) and controls (CTRL1–3) 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.

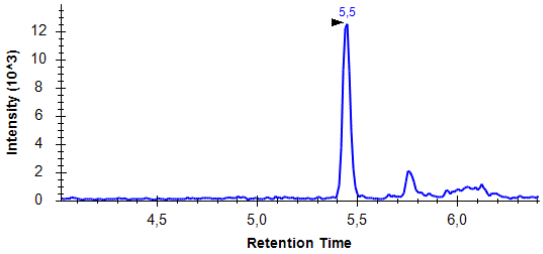
9. CALCULATION

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

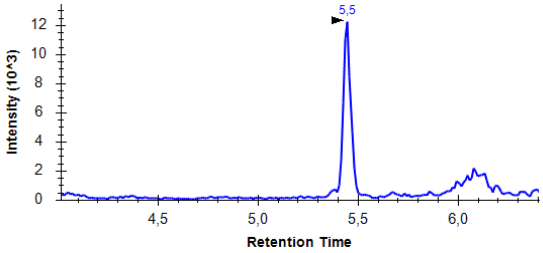
10. EXAMPLES OF CHROMATOGRAMS

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

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

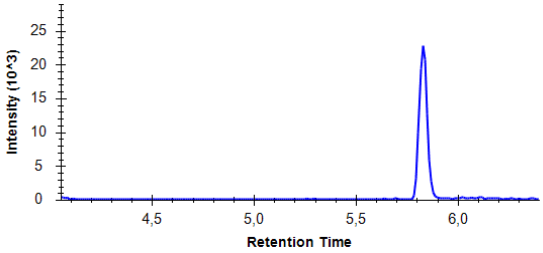


Qualifier (*m/z* = 399.3 > 135.1):

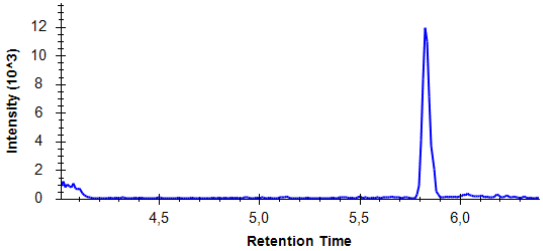


1,25-$(OH)_2$-vitamin D_2

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

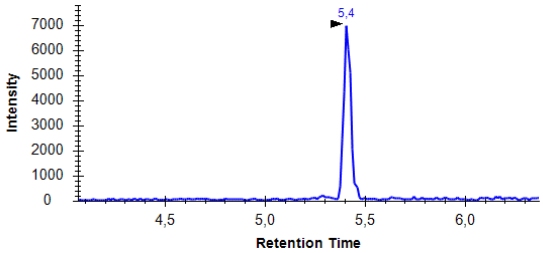


Qualifier (*m/z* = 411.1 > 135.1):

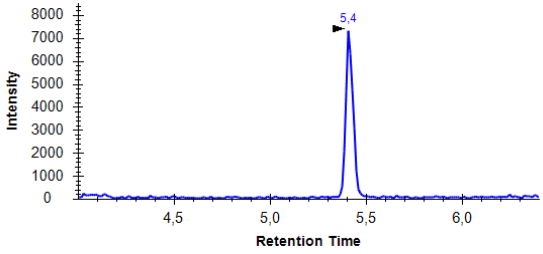


Internal standard

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).

Normal range (plasma or serum)

Healthy adults (age 20–50) : 17–53 pg/ml
Children up to 12: ca. 40 % higher values
Pregnant women (week 8–42): ca. 60 % higher values
Persons older than 70: ca. 40 % lower values

The normal range is independent of the season.

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

Linearity

Sample [pg/ml]	1,25-$(OH)_2$-vitamin D_3	1,25-$(OH)_2$-vitamin D_2
	Linearity [%]	
25	100.6	100.0
125	102.6	99.0
250	97.3	103.1
500	98.1	96.9
1 000	101.3	100.9

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.
- As a precaution, it is recommended that the human material used is always considered potentially infectious.

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 (CAL1–6) and controls (CTRL1–3) 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.
- The individual components of the kit are designed for a maximum of the specified number of test runs. Any part of the components that has already been used must not be reused, but must be disposed of properly in accordance with local regulations.

16. GENERAL NOTES ON THE TEST

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- ImmuTube® is a brand of Immundiagnostik AG.
- All reagents in the kit package are for *in vitro* diagnostic use only.
- The guidelines for medical 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.
- All serious incidents occurring in connection with the product must be reported to Immundiagnostik AG and (within the Union market) to the competent reporting authority of the respective member state.

- 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.
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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.
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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

In Vitro Diagnostic Medical Device

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet