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Manual

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

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

Valid from 2022-05-25

REF **KM1100**

Σ 50

+2°C ±8°C

IVD

CE

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Table of Contents

1. INTENDED USE

The 1,25-$(OH)_2$-Vitamin D_3/D_2 ImmuTube® LC-MS/MS extraction kit is intended for the sample preparation of 1,25-$(OH)_2$-vitamin D_3/D_2 for the 1,25-$(OH)_2$-vitamin D_3/D_2 LC-MS/MS application by means of extraction from human serum and plasma via immunoaffinity enrichment (ImmuTube®). It is for manual use by laboratory professionals. For *in vitro* diagnostic use only.

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 characterized 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 D2) 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. 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	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 following accessories for the ImmuTube® LC-MS/MS extraction kit can be ordered seperately at Immundiagnostik AG:

- 1,25-$(OH)_2$-vitamin D_3/D_2 ImmuTube® LC-MS/MS kit (KM1000)
- 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. MATERIAL REQUIRED BUT NOT SUPPLIED

- Glass tubes (inner diameter 10 mm)
- 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

5. PREPARATION AND STORAGE OF REAGENTS

The test reagents should be stored protected from light, dry and their specified storage temperature (2–8 °C). The test reagents stored in this way are usable until the indicated expiry date.

6. 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 carried along 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 sample, 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 the 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 fresh glass tubes, place ImmuTubes® in the labeled 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 a solvent which is adjusted to the mobile phase.

7. PRECAUTIONS

- 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.

8. DISPOSAL

Elution solution (ELUSOL) must be disposed as non-halogenated solvents.

9. 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.

10. GENERAL NOTES ON THE TEST

- This product has been produced and placed on the market in accordance with the regulation (EU) 2017/746 (IVDR).
- ImmuTube® is a brand of Immundiagnostik AG.
- All reagents in the kit package are for *in vitro* diagnostic use only.
- This instruction for use replaces the version dated 2022-05-24.
- 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.
- Serious incidents are to be reported to Immundiagnostik AG and the national regulatory authorities.
- 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.

11. 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.
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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.

Used symbols:


Temperature limitation

REF Catalogue number

IVD In Vitro Diagnostic Medical Device

→REF To be used with


Manufacturer


Contains sufficient for <n> tests

LOT Lot number


Use by


Attention


Consult instructions for use


Consult specification data sheet


Do not re-use

UDI Unique Device Identification