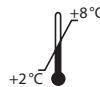


1,25-(OH)₂-Vitamin D₃/D₂ ImmuTube[®] LC-MS/MS extraction kit

*For the extraction of 1,25-(OH)₂-vitamin D₃/D₂
from plasma and serum*

Valid from 2021-10-15

REF KMR1100



RUO



Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany

Tel.: +49 6251 70190-0

Fax: + 49 6251 70190-363

e.mail: info@immundiagnostik.com

www.immundiagnostik.com

Table of Contents

1. INTENDED USE	2
2. INTRODUCTION	2
3. CONTENT OF THE EXTRACTION KIT	3
4. MATERIAL REQUIRED BUT NOT SUPPLIED	4
5. PREPARATION AND STORAGE OF REAGENTS	4
6. SAMPLE PREPARATION	4
7. PRECAUTIONS	5
8. DISPOSAL	5
9. TECHNICAL HINTS	5
10. GENERAL NOTES ON THE TEST	5
11. REFERENCES	6

1. INTENDED USE

The 1,25-(OH)₂-Vitamin D₃/D₂ ImmuTube® LC-MS/MS extraction kit is intended for the extraction of 1,25-dihydroxyvitamin D₃/D₂ from serum and plasma via immuno-affinity enrichment and can be used for sample preparation for LC-MS/MS applications. It is 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 1 α -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. A better information is obtained by measuring the precursor metabolite 25-hydroxy vitamin D.

The reason for a deficiency of 1,25-dihydroxy-vitamin D can be caused by metabolic disorders, e.g. genetic defects of the enzyme 1 α -hydroxylase (rare) or by kidney dysfunction (more common). Even slightly impaired kidney function can lead to a drop in 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.

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 hyper-calcaemia. Also in idiopathic hypercalcaemia a relatively high level of 1,25-dihydroxy vitamin D is found. Increased concentrations of 1,25-dihydroxyvitamin 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 D₃). 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 D₃/D₂ as a measure of 1,25-dihydroxy vitamin D status provides an objective, quantitative measure of the biological response to vitamin D administration.

Indications of possible research areas

- 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
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 following accessories for the ImmuTube® LC-MS/MS extraction kit can be ordered separately at Immundiagnostik AG:

- 1,25-(OH)₂-Vitamin D₃/D₂ ImmuTube® LC-MS/MS kit (KMR1000)
- tuning solution for 1,25-(OH)₂-vitamin D₃/D₂ (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. 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 <i>g</i> .
5.	Open the outlet of the ImmuTubes®, then the lid and centrifuge for 2 min at 550 <i>g</i> 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 Immunodiagnostik 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 solvent.

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.

10. GENERAL NOTES ON THE TEST

- ImmuTube® is a brand of Immunodiagnostik 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 sent 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-Dihydroxy Vitamin D₃-26,23-lactone interferes in determination of 1,25-dihydroxy Vitamin 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-dihydroxy Vitamin 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.

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		

Immundiagnostik AG

Stubenwald-Allee 8a
64625 Bensheim, Germany

Tel.: +49 6251 70190-0

Fax: +49 6251 70190-363

info@immundiagnostik.com

www.immundiagnostik.com

