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Manual
Preliminary

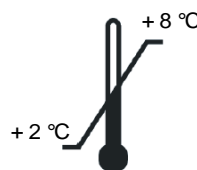
1,25-(OH)₂-Vitamin D₃/D₂ ImmunoTube[®] Extraction Kit

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

Valid from 30.06.2011



IMM-KM1100



1. INTENDED USE

The described ImmunoTube® Extraction kit is intended for the extraction of 1,25-dihydroxy vitamin D₃/D₂ from serum and plasma and can be used for sample preparation for LC-MS/MS applications. 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 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. For the diagnosis of vitamin D deficiency the precursor metabolite, 25-hydroxyvitamin D, should be measured.

The reason for a non-physiological deficiency of 1,25-dihydroxy vitamin D can be found in metabolic disturbances, caused either by genetic defects of the enzyme 1 α -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 1 α -hydroxy vitamin D. In renal tubules malfunctions decreased or relatively low levels of 1,25-dihydroxy vitamin D (e.g. diabetes insipidus, Fanconi-syndrom) 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 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

- 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
KM1100SI	COLUMNS	ImmunoTube [®] -Columns for isolation of 1,25-(OH) ₂ -Vitamin D ₃ from the sample	50 Columns
KM1100ER	ELUREAG	Elution reagent for ImmunoTube [®] , ready to use	20 ml
KM1100WL	WASHSOL	Wash solution for ImmunoTube [®]	80 ml

In addition, the following products can be purchased from Immundiagnostik AG:
 1,25-Dihydroxy-Vitamin D₃/D₂ ImmunoTube[®]LC-MS/MS Kit (KM1000)
 1,25-Dihydroxy-Vitamin D₃/D₂ LC-MS/MS Tuning Kit (KM1001)
 UPLC separation column (KM1000RP)
 HPLC separation column (KM1001RP).

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Glass tubes; LC-MS/MS-suitable
- Precision pipettors and disposable tips to deliver 10-1000 µl
- A repeating dispenser
- Centrifuge capable of 10000 x g for 1.5 ml Eppendorf reaction tubes and 550 x g for glass tubes, respectively
- Vortex-Mixer
- Vacuum centrifuge or nitrogen distributor
- Standard laboratory disposable plastic reagent vials
- Upside-down-shaker

5. PREPARATION AND STORAGE OF REAGENTS

The test reagents are stable until the expiry date when stored at 2-8°C.

6. SAMPLE PREPARATION WITH IMMUNOTUBE®-EXTRACTION FOR 50 SAMPLES

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 analyzed with each run.

1. Prior to use in the assay, allow all samples and reagents to come to room temperature (18-26°C). Mix well samples and reagents before use
2. Vortex carefully ImmunoTubes®, place in a suitable rack and make sure that no suspension remained on the ImmunoTubes® cover
3. Label the covers of ImmunoTubes®, open ImmunoTubes®, add quickly 500 µl of CAL/SAMPLE/CTRL (Calibrator/Sample/Control), close ImmunoTubes® and mix gently
4. "Mix-rotate" (end-over-end rotation) intensively for 1 h at RT. Let the remaining separation material on the inner side of the cover flow down
5. Insert closed ImmunoTubes® in plastic reagent vials, centrifuge for 1 min at 550 x g
6. Open the cover and then the outlet the ImmunoTubes® and centrifuge for 2 min at 550 x g to dryness. Discard flow-through
7. Add 500 µl of WASHSOL and centrifuge for 2 min at 550 x g to dryness. Discard flow-through Repeat this step twice
8. Label fresh glass tubes, place ImmunoTubes® in the labeled glass tubes
9. Add 250 µl of ELUREAG (Elution Reagent for ImmunoTubes®), centrifuge for 2 min at 550 x g and collect the 1,25-(OH) ₂ -Vitamin D ₃ /D ₂ eluates in the glass tubes
10. Evaporate the eluate under a nitrogen stream at 37°C or in a vacuum centrifuge
11. Vortex the remainder 1 min in 165 µl of activated Solution A; inject 50 µl in the UPLC-System, respectively 100 µl in the HPLC-System

7. 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 sample preparation should always be performed according to the enclosed manual.

8. DISPOSAL

The elution reagent for ImmunoTube® (ELUREAG) must be disposed as non-halogenated solvents.

9. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- 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.
- 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.

10. REFERENCES

1. Wildermuth S. et al., 1993; Clinica chimica Acta, 220, 61
2. Schilling M. et al., 1987; Clinical Chemistry, 33, 187
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Used symbols:



Temperature limitation



Catalogue Number



In Vitro Diagnostic Medical Device



Contains sufficient for <n> tests



Manufacturer



Use by



Lot number