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25-OH Vitamin D EIA Kit

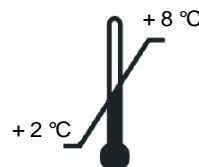
(EPBA)

For the determination of 25-OH Vitamin D in human serum and plasma

Valid from 29.10.2009



IMM-K 2110



EU Patent
Australisches Patent /Australien
patent

#EP1097132
763458

1. INTENDED USE

The *Immundiagnostik* Enzyme-Immuno-Assay (EIA) is intended for the quantitative determination of the 25-OH Vitamin D in plasma and serum. For in vitro diagnostic use only.

2. SUMMARY AND EXPLANATION OF THE TEST

Vitamin D is a steroid hormone involved in the intestinal absorption of calcium and the regulation of calcium homeostasis. There are two different forms of Vitamin D, named D₃ and D₂, which are very similar in structure. The D₂ is a synthetic product, which is predominantly absorbed by fortified food.

Physiological Vitamin D₃ levels result not only from dietary uptake but also by biosynthesis of 7-dehydrocholesterol and UV-light in skin because of sun exposure. In the liver, the vitamin is hydroxylated to 25-hydroxyvitamin D (25-OH Vitamin D), the major circulating metabolite of Vitamin D. Although 1,25-(OH)₂ Vitamin D portrays the biological active form of Vitamin D, which is synthesized in the kidney, it is widely accepted that the measurement of circulating 25-OH Vitamin D provides better information with respect to patients Vitamin D status and allows its use in diagnose hypovitaminosis (1,2).

The concentration of 25-OH Vitamin D decreases with age and a deficiency is common among elderly persons.

Clinical applications of 25-OH Vitamin D measurements are the diagnosis and therapy control of postmenopausal osteoporosis, rickets, osteomalacia, renal osteodystrophy, pregnancy, neonatal hypocalcemia and hyperparathyroidism. In addition, a prevalence of subclinical Vitamin D deficiency has been discussed lately in different European countries.

Vitamin D intoxication mostly occurs during a large intake of pharmaceutical preparations of Vitamin D and may lead to hypercalcemia, hypercalcuria and nephrocalcinosis in susceptible infants.

4. MATERIAL SUPPLIED

Cat. No	Content	Kit Components	Quantity
K 2110MTP	PLATE	one holder with precoated strips	12 x 8 wells
K 2110WP	WASBUF	ELISA wash concentrate 10x	1 x 100 ml
K 2110AP	ASYBUF	Assay buffer, ready to use	1 x 15 ml
K 2110BP	VDBP	Vitamin D binding protein (VDBP) (blue cap), lyophilized	1 vial
K 2110F	PREC	Precipitation Reagent (blue solution), ready to use	1 x 50 ml
K 2110AK	AB	Anti-VDBP antibody (yellow solution), ready to use	1 x 11 ml
K 2110ST	STD	Standards and NSB (0; 6.4; 16; 40; 100; 250 nmol/l) (white cap), ready to use	7 vials
K 2110KO	CTRL	Controls, ready for use (see specification for range)	2 vials
K 2110K	CONJ	Peroxidase labeled conjugate, ready to use	1 x 22 ml
K 2110TMB	SUB	TMB substrate (Tetramethylbenzidine)	2 x 15 ml
K 2110AC	STOP	ELISA stop solution, ready to use	1 x 15 ml

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Bidistilled water (aqua bidest.)
- Laboratory balance
- Deep freezer -20 °C
- Precision pipettors calibrated to deliver 10-1000 µl
- Horizontal microtiter plate shaker
- Multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc. (one time products)
- Microtiter plate reader 450 nm (reference wave length 620 or 690 nm)

5. PREPARATION AND STORAGE OF REAGENTS

- To run the assay more than one time, make sure that the reagents are carefully stored at conditions stated on the label. Prepare just the appropriate amount necessary for the assay. The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with the less than **100 µl volume** should be centrifuged before use to avoid loss of volume.
- The ELISA **WASHBUF** (wash buffer concentrate) should be diluted with aqua bidest. **1:10** before use (add 900 ml of a. bidest. to 100 ml concentrate), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals have to be redissolved at room temperature or at 37°C using a water bath **before dilution of the buffer solutions**. The buffer concentrates are stable at **2-8°C** until the expiry date stated on the label. Diluted **buffer solution** could be stored in a closed container at **2-8°C for 1 month**.
- Incubate the **ASYBUF** (Assay buffer) for **10 minutes at 37 °C** in a water bath before use.
- Dissolve **vitamin D binding protein (VDBP, blue cap)** in **11 ml** of assay buffer, mix well (no vortexer) and leave at room temperature for 30 min. Reconstituted VDBP is stable at -20°C until expiry date stated on the label. Avoid freezing/thawing cycles. We recommend to aliquot the VDBP (use glass containers). Frozen VDBP have to incubate for 10 minutes at 37 °C in a water bath before use. Avoid repeated freeze-thaw cycles!
- All other test reagents are ready for use. The test reagents are stable up to the date of expiry (see label of test package) when stored at **2-8 °C**.

6. SPECIMEN COLLECTION AND PREPARATION

1. Vitamin D is an inert substance. The samples can be stored at room temperature, however, Immundiagnostik AG recommends storage at 2-8°C after collection when the measurement will be performed within the first 24 h. Otherwise, the samples must be stored at -20°C. Avoid repeated freeze-thaw cycles.
2. **Hemolysis** does not disturb the results; whole blood is not suitable.
3. Untreated **lipemic** samples lead to incorrect results. Therefore we recommend:

a.) Centrifuge lipemic samples 10 min at 3.000xg.

The lipid phase floats up. Collect the aqueous phase.

b.) For highly lipemic samples we recommend using a special delipidation kit (Catalogue No. K 1003A). This kit was developed for lipemic samples in clinical laboratories.

4. Indicated incubation times and temperatures must be observed exactly.

5. Mix samples well before use.

7. ASSAY PROCEDURE

Principle of the test

This test kit is a competitive protein binding assay for the measurement of 25-OH Vit D. It is based on the competition of 25-OH Vit D present in the sample with 25-OH Vit D tracer, for the binding pocket of vitamin D binding protein (VDBP, Gc-globulin). Since all circulating 25-OH Vit D is bound to VDBP in vivo, samples have to be precipitated with precipitation reagent to extract the analyte. The supernatant can be used without further treatment within the test.

In the first incubation step, sample, calibrator, control, the vitamin D binding protein and the VDBP-Antibody, an antibody specific for this protein is added to the solid phase.

25-OH Vit D present in the sample then competes with the tracer, coated on the well for the specific binding site of the binding protein and the VDBP-Antibody is bound to the vitamin binding protein. Hence, with increasing concentrations of 25-OH Vit D in the sample, the amount of binding protein, immobilized to the well via the tracer, is reduced. After a washing step to remove unbound components, the quantitation of VDBP is achieved by incubation with a host specific peroxidase labeled antibody using TMB (tetramethylbenzidine) as enzyme substrate. An acidic stopping solution is then added to stop the reaction. The colour converts to yellow. The intensity of the yellow colour is indirectly proportional to the concentration of 25-OH Vitamin D in the sample. A dose response curve of the absorbance unit vs. concentration is generated using the results obtained from the calibrators. Concentrations of 25-OH Vitamin D, present in the patient samples, are determined directly from this curve.

Extraction procedure

Sample, Calibrator, NSB and Control preparation:

- Add **50 µl** calibrator, control, NSB (non specific binding control, assay buffer only) and samples into plastic V tubes (we recommend Eppendorf tubes with a cap).
- Add **400 µl** of precipitation reagent to calibrator, control, NSB and samples. Mix thoroughly on a Vortex mixer. Incubate for 30 min at -20 °C.
- Centrifuge for 10 min at 3000xg and at 4 °C. The supernatant contains the extracted 25-OH Vit D and must be assayed immediately. Please note: Keep samples during the transfer to the microtiter plate at 4 °C.

We recommend reconstitution of VDBP at room temperature for 30 min. This can be done during the incubation of the samples.

Test procedure

1. Prior to use in the assay allow all reagents and samples to come to room temperature (18 - 26 °C) and mix gentle, avoid foam formation
2. Mark the positions of STD (Standards)/SAMPLE/CTRL (Control) on a protocol sheet
3. Take microtiter strips out of the kit. Store unused strips covered at 2-8° C. Strips are stable until the expiry date stated on the label
4. Put the microtiterplate and the precipitated STD/PROBE/CTRL on a cool block or ice . The microtiterplate and the precipitated STD/PROBE/CTRL must be cooled during the complete pipetting step
5. Add 20 µl of STD/SAMPLE/CTRL (Standard/Sample/Control) in duplicate into respective well
6. Add 100 µl VDBP (binding protein) into each well, except the NSB

7. Add 100 µl ASYBUF (assay buffer) to the NSB wells
8. Add 100 µl AB (anti-VDBP antibody) into each well
9. Cover the plate tightly and incubate for 3 hours at 8 - 10 °C in the dark
10. Aspirate and wash the wells 5x with 250 µl of diluted wash buffer, remove remaining wash buffer by hitting the plate against paper towel after the last wash
11. Add 200 µl CONJ (Conjugate) into each well
12. Cover the plate tightly and incubate for 1 hour at 8 - 10 °C in the dark
13. Aspirate and wash the wells 5x with 250 µl of diluted wash buffer, remove remaining wash buffer by hitting the plate against paper towel after the last wash
14. Add 200 µl of SUB (Substrate) into each well
15. Incubate for 20 - 30 minutes at room temperature (18-26°C) in the dark
16. Add 50 µl of STOP (Stop solution) into each well
17. Determine the absorption with an ELISA reader at 450 nm against 620 nm (or 690 nm) as a reference. If no reference wave length is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620 nm (690 nm) as a reference

8. RESULTS

The following algorithms can be used alternatively to calculate the results. However, we recommend the use of option 1: 4-parameter-algorithm.

1. 4-parameter-algorithm

It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero calibrator has to be specified with a value smaller than 1 (e. g. 0.01).

2. Point-to-point-calculation

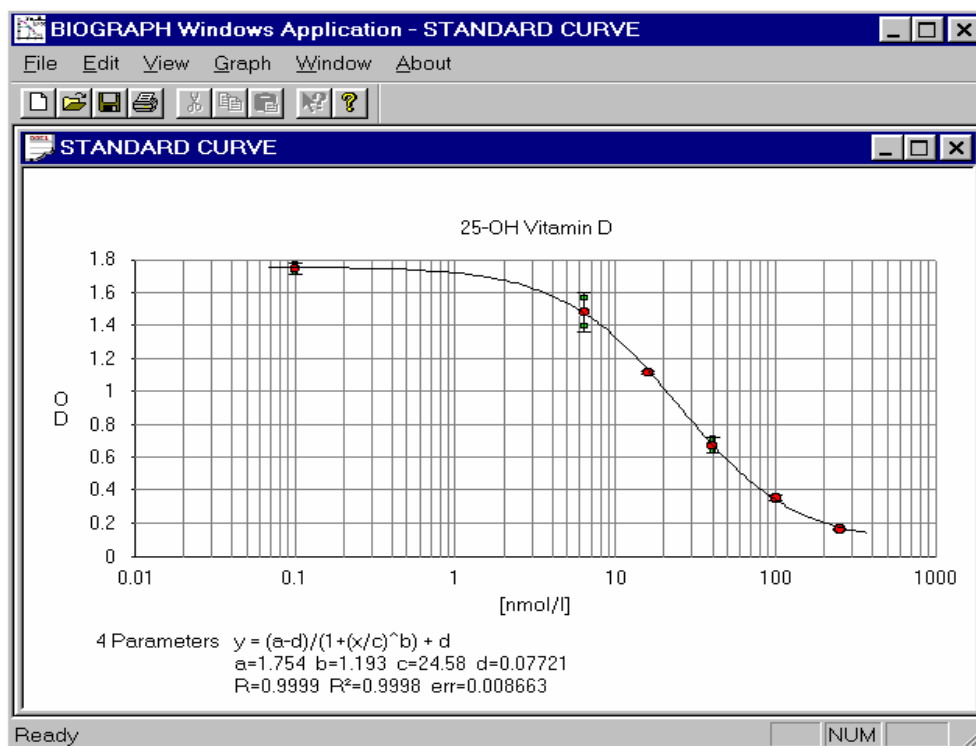
We recommend for the optical density a linear ordinate and for the concentration a linear abscissa.

3. Spline-algorithm

We recommend for the optical density a linear ordinate and for the concentration a logarithmic abscissa. When using a logarithmic abscissa, the zero calibrator has to be specified with a value smaller than 1 (e. g. 0.01).

The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a manual control of the paired values should be made.

Typical calibration curve



Calibrators	B/BO mean [%]	B/BO median [%]	SD B/BO [%] n = 17
NSB			
1	100	100	0
2	85	86	5.7
3	70	69	6.9
4	40	42	9.2
5	22	22	4.5
6	13	14	3.6

9. LIMITATIONS

Samples with a 25-OH Vitamin D level greater than the highest calibrator, should be diluted with pre-warmed ASYBUF (Assay buffer; 37 °C) and re-assayed.

Whole blood is not suitable. Untreated **lipemic** samples lead to wrong results.

10. QUALITY CONTROL

Control samples or serum pools should be analyzed with each run of calibrators and patient samples. Results generated from the analysis of the control samples should be evaluated for acceptability using appropriate statistical methods. In assays in which one or more of the quality control sample values lie outside the acceptable limits, the results for the patient sample may not be valid.

Expected values

Normal ranges for 25-OH-Vitamin D₃

1 ng/ml = 2.5 nmol/l

1 nmol/l = 0.4 ng/ml

Information from ASBMR 2006

Deficiency (seriously deficient)	< 12 ng/ml	resp. < 30 nmol/l
Insufficiency (deficient)	12 - 30 ng/ml	resp. 30 - 75 nmol/l
Sufficiency (adequately supplied)	> 30 ng/ml	resp. > 75 nmol/l

Society of Osteology SACHSEN E. V.

http://osteologie-sachsen.de/aktuelles_vitamin_d.html

Note

The vitamin D production in the skin is high variable and depends on the season- and daily time, degree of latitude, age, sun protection etc.

The normal ranges depend on the method used (e. g. vitamin-D-release from the vitamin D binding protein, DBP) and serve only as orientation.

Literature references

Visser M, Deeg DJ, Puts MT, Seidell JC, Lips P. (2006) Low serum concentrations of 25-hydroxyvitamin D in older persons and the risk of nursing home admission. *Am J Clin Nutr.* Sep;84(3):616-22; quiz 671-2

Grant WB, Holick MF. Benefits and requirements of vitamin D for optimal health: a review. (2005) *Altern Med Rev.* Jun;10(2):94-111. Review

Wicherts IS, van Schoor NM, Boeke AJ, Visser M, Deeg DJ, Smit J, Knol DL, Lips P. (2007) Vitamin D status predicts physical performance and its decline in older persons. *J Clin Endocrinol Metab.* Jun;92(6):2058-65

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n=20)		
Probe	25-OH Vitamin D [nmol/l]	VC [%]
1	4,5	10,7

Inter-Assay (n=20)		
Probe	25-OH Vitamin D [nmol/l]	VC [%]
1	39,8	13,2
2	86,7	11,8

Cross reactivity

24,25-(OH) ₂ Vit D ₃	100 %
25-(OH)-Vit D ₃	100 %
25-OH Vit D ₂	100 %
1,25-(OH) ₂ Vit D ₃	< 1 %
Vit D ₂ & D ₃	< 1 %
Alphacalcidol	< 0.001 %

Recovery

10 samples were spiked with 156 nmol/l 25-OH Vitamin D calibrator and measured in the kit. The recovery was 94 per cent.

Spike [nmol/ml]	Vitamin D measured Mean [nmol/l]	Recovery [%]
156	146,7	94

Sensitivity

The sensitivity or minimum detection limit of this assay is defined as the smallest single value that can be distinguished from zero at the 2 x standard variation as confidence limit. ($B0 + 2 SD$)

Sensitivity n=20

Sample	Vitamin D mean value [OD]	Standard variation	Detection limit [nmol/l]
1	2.058	0.084	5.6

Sample dilution

Two patients' serum samples were diluted with pre-warmed ASYBUF (Assay buffer; 37 °C). The results in nmol/l are shown below:

Sample	Dilution	Observed [nmol/l]	Expected [nmol/l]
A	undiluted	135.8	135.8
	1:2	54.5	67.9
	1:4	30.8	33.9
	1:8	10.8	16.9
B	undiluted	127.8	127.8
	1:2	62.7	63.9
	1:4	33.4	31.9
	1:8	15.4	15.9

12. PRECAUTIONS

- For *in vitro* diagnostic use only.
- The quality control guidelines should be observed.
- 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.
- Reagents of the kit package contain sodium azide and thimerosal as bactericides. Sodium azide and thimerosal are toxic. The substrates for the enzymatic color reactions are described to be also toxic and carcinogenic. Contact with skin or mucous membranes has to be avoided.
- Stop solution consists of sulfuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped out immediately with copious quantities of water.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay.
- Reagents should not be used beyond the expiration date shown on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- The assay should always be performed according the enclosed manual.
- Incubation time, incubation temperature and the volumes of the different components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the test results. Immundiagnostik can therefore not be held responsible for any damage resulting from this.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and put on the market 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 observed.
- Incubation time, incubation temperature and volumes of the different 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 can therefore not be held responsible for any damage resulting from this.
- Warranty claims and complaints in respect of deficiencies must be lodged within 14 days of receipt of the product. The product shall be send to Immundiagnostik together with the complaint in writing.

15. REFERENCES

1. Scharla SH,et al. Exp Clin Endocrinol Diabetes 1996; 104:289-292
2. Scharla SH. et al Osteoporos Int 1998;8 (Supplement 2):S7-S12
3. Chapuy MC,et al. J Clin Endocrinol Metab 1996;81:1129-33
4. Oster P et al.. Akt Gerontol 1983;13:221-2
5. Scharla S. Schattauer Verlag, Stuttgart,1997; Seiten 217-242
6. Offermann G. Dtsch med Wschr. 1978;103:1387-1388

This Assay is developed by

Immundiagnostik, Bensheim and Biomedica, Wien.

Used symbols:



Temperature limitation



Catalogue Number



In Vitro Diagnostic Medical Device



Contains sufficient for <n> tests



Manufacturer



Use by



Lot number

Li StarFish distribuisce: