Instructions for Use

25-OH-Vitamin D ELISA

Enzyme immunoassay for the quantitative measurement of total 25-OH-Vitamin D (Vitamin D2 and Vitamin D3) in human serum.

REF **RE53041**

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EU: IVD CE

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1. INTENDED USE

Enzyme immunoassay for the quantitative measurement of total 25-OH-Vitamin D (Vitamin D2 and D3) in human serum.

2. SUMMARY AND EXPLANATION

Vitamin D refers to a group of fat-soluble secosteroids being responsible for intestinal absorption of calcium, iron, magnesium, phosphate and zinc. The most important vitamin Ds in humans are vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol). Vitamin D3 is synthesized in the skin from the cholesterol precursor 7-dehydrocholesterol and is the major source of vitamin D in humans. In the liver vitamin D is further metabolized via hydroxylation to 25-hydroxyvitamin D (25-OH-vitamin D). 25-OH-vitamin D is the major circulating metabolite of vitamin D. 25-OH-vitamin D is the precursor for other vitamin D metabolites, but shows also limited activity by itself. The most active metabolite is 1,25-dihydroxyvitamin D, which is produced in the kidney by 1-hydroxylation of 25-OH-vitamin D. Yet, it is widely accepted that determination of circulating 25-OH-vitamin D (25-OH-vitamin D3 + 25-OH-vitamin D2) provides currently the best information on a patient's vitamin D status.

3. TEST PRINCIPLE

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the competition principle. An unknown amount of antigen present in the sample and a fixed amount of enzyme labelled antigen compete for the binding sites of the antibodies coated onto the wells. After incubation, the wells are washed to stop the competition reaction. After the substrate reaction, the intensity of the developed colour is inversely proportional to the amount of the antigen in the sample. Results of samples can be determined directly using the standard curve.

4. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
9. Avoid contact with Stop solution. It may cause skin irritations and burns.
10. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely. For this reason reagents should be treated as potential biohazards in use and for disposal.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sunlight. The storage and stability of specimens and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the indicated expiry after the kit is opened. Make sure that the opened bag is tightly closed when stored at 2-8 °C.

6. SPECIMEN COLLECTION AND STORAGE

Serum

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8°C	≤ -20°C (Aliquots)	Keep away from heat or direct sunlight.
Stability:	3 days	> 3 days	Avoid repeated freeze-thaw cycles.

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Coated with antibodies against 25-OH-Vitamin D2 and D3 (monoclonal).
1 x 1 mL	CAL A	**Standard A** Ready to use. Contains: biological matrix with stabilizers and ≤ 1.0 % ProClin.
1 x 5 x 1 mL	CAL B-F	**Standard B-F** Ready to use. Exact concentrations see vial labels or QC certificate. Contains: 25-OH-Vitamin D, biological matrix with stabilizers and ≤ 1.0 % ProClin.
2 x 1 mL	CONTROL 1 + 2	**Control 1 + 2** Ready to use. Concentrations / acceptable ranges see QC certificate. Contains: 25-OH-Vitamin D, biological matrix with stabilizers and ≤ 1.0 % ProClin.
1 x 12 mL	INCBUF	**Incubation Buffer** Ready to use. Contains: casein and < 2.0 % ProClin
1 x 0.15 mL	BIOTIN CONC	**25-OH-Vitamin D Biotin** Concentrate (101x) Contains: Biotin in Buffer with stabilizers.
1 x 15 mL	ENZCONJ	**Enzyme Conjugate** Ready to use. Contains: streptavidin conjugated to HRP.
1 x 100 mL	WASHBUF CONC	**Wash Buffer** Concentrate (10x) Contains: phosphate buffer, Tween.
1 x 15 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains: TMB, Buffer, stabilizers.
1 x 15 mL	TMB STOP	**TMB Stop Solution** Ready to use. Contains: 1 M H_2SO_4.

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 25; 75; 100; 1000 µL
2. Vortex mixer
3. 8-Channel Micropipettor with reagent reservoirs
4. Wash bottle, automated or semi-automated microtiter plate washing system
5. Orbital shaker (500 rpm)
6. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
7. Bidistilled or deionised water
8. Paper towels, pipette tips and timer

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
7. Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
8. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

Preparation of concentrated components

Dilute / dissolve	Component	with	Diluent	Relation	Remarks	Storage	Stability
140 µL	**BIOTIN CONC**	14 mL	**ENZCONJ**	1:101	Prepare at least 90 min before use. Mix without foaming.	RT (18-25°C)	8 hours Prepare freshly and use only once.
100 mL	**WASHBUF CONC**	900 mL	bidist. water	1:10	Mix vigorously.	2-8°C	4 weeks
⚠	**INCBUF**	Mix carefully. Mix without foaming.					

11. TEST PROCEDURE

1.	Pipette **25 µL** of each **Standard, Control and sample** into the respective wells of the Microtiter Plate.
2.	Pipett**e 75 µL** of **Incubation Buffer** into all wells.
3.	**Incubate** microtiter plate for **90 min at RT (18-25 °C)** on an orbital shaker (500 rpm).
4.	Discard incubation solution. **Wash** plate **4x with 350 µL** of **diluted Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel. Automatic microtiter plate washers should be adjusted to overflow mode.
5.	Pipette **100 µL** of **diluted Biotin** in each well.
6.	**Incubate** microtiter plate for **30 min at RT (18-25 °C)** on an orbital shaker (500 rpm).
7.	Discard incubation solution. **Wash** plate **4x with 350 µL** of **diluted Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel. Automatic microtiter plate washers should be adjusted to overflow mode.
8.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
9.	**Incubate** microtiter plate for **15 min at RT (18-25 °C)** on an orbital shaker (500 rpm).
10.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
11.	Measure optical density with a photometer at **450 nm** within **15 min** after pipetting the Stop Solution (Reference-wavelength: 600-650 nm).

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or comparable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

13. CALCULATION OF RESULTS

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logistics or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentration of the samples can be read directly from the standard curve.

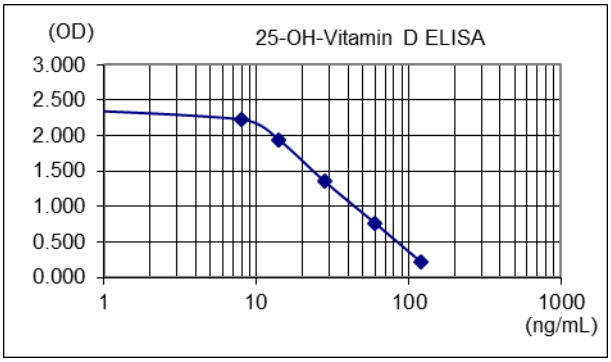
Conversion:

25-OH-Vitamin D (ng/mL) x 2.5 = nmol/L

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	25-OH-Vitamin D (ng/mL)	OD_{Mean}	OD/OD_{max} (%)
A	0.0	2.439	100
B	8	2.230	91
C	14	1.942	80
D	28	1.356	56
E	60	0.766	31
F	120	0.220	9



14. EXPECTED VALUES

Recent literature has suggested the following ranges for the classification of 25-OH-Vitamin D status:

ng/mL	level
<10	Deficient
10 - 29	Insufficient
30 - 100	Sufficient
>100	Potential Toxicity

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.
It is recommended that each laboratory establishes its own range of normal values.

In an in-house study, apparently healthy subjects showed the following results:

n	Range (ng/mL)	Mean (ng/mL)	Median (ng/mL)	5th Percentile - 95th Percentile (ng/mL)
120	7.8 - 58.3	23.5	22.3	10.8 - 41.9

15. LIMITATIONS OF THE PROCEDURE

The following blood components do not have a significant effect (+/-20% of expected) on the test results up to the below stated concentrations:

Hemoglobin	1.25 mg/mL
Bilirubin	5.00 mg/mL
Triglyceride	22.8 mg/mL

16. PERFORMANCE

16.1. Analytical Specificity (Cross Reactivity)

Substance	Cross Reactivity (%)
25-OH-Vitamin D3	110%
25-OH-Vitamin D2	84%
Vitamin D3	5.4%
Vitamin D2	1.8%
24,25-$(OH)_2$-Vitamin D3	>100 %

Substance	Cross Reactivity (%)
25,26-$(OH)_2$-Vitamin D3	>100 %
3-epi-25-OH-Vitamin D3	0.1%
1,25-$(OH)_2$-Vitamin D3	8.8%
1,25-$(OH)_2$-Vitamin D2	0.1%

16.2. Evaluation of Detection Capability according to CLSI-EP17-A2.

Limit of Blank (LoB)

The LoB study was conducted during three days of testing by two operators. The runs were performed with high and low kit controls and with five different blank samples. For each sample four replicates were tested using two reagent lots.
Limit of Blank = 6.2 ng/mL

Limit of Detection (LoD)

The LoD study was conducted during three days of testing by two operators. The runs were performed with high and low kit controls and with five different low concentrated human serum samples. For each sample four replicates were tested using two reagent lots.

Limit of Detection = 11.6 ng/mL

Limit of Quantitation (LoQ)

The LoQ study was conducted during three days of testing by two operators. The runs were performed with high and low kit controls and seven different low to mid concentrated human serum samples. For each sample four replicates were tested using two reagent lots.
Limit of Quantitation = 8.2 ng/mL

16.3. Linearity

The linearity study was conducted during one day of testing by one operator. The run was performed with high and low kit controls and with three different human serum samples spiked with 25-OH-Vitamin D and diluted with analyte free serum. Each sample was tested in duplicate using one reagent lot.

Range: 11.8 – 98.9 ng/mL (94 – 126 %)
Serial dilution up to1:8

16.4. Method Comparison versus LC-MS

The Method comparison study was conducted during one day of testing by one operator. The run was performed with high and low kit controls and with 40 different human serum samples.

IBL-Assay = 1.007 x ID-LC-MS/MS – 3.964
r = 0.944
n = 40

16.5. Precision

Performance parameters were measured according to Clinical & Laboratory Standards Institute (CLSI) guidelines.

The Intra-Assay and Inter-Assay study was conducted during 20 days using one reagent lot. Two runs were performed per day with high and low kit controls and with a panel of five human serum samples. Each sample was run in duplicate. The results were calculated according CLSI-EP05-A3.

Sample	Mean conc. (ng/mL)	within run (Intra-Assay)		total Precision (Inter-Assay)	
		SD (ng/mL)	CV	SD (ng/mL)	CV
1	11.9	1.4	11.9%	1.9	15.6%
2	21.0	1.1	5.3%	1.7	8.0%
3	29.2	1.7	5.8%	2.0	6.9%
4	44.3	1.4	3.2%	2.3	5.3%
5	63.8	2.2	3.4%	3.5	5.5%

The intra-assay precision showed a mean CV from 5.9% and a range of 3.2% - 11.9% for the five different human serum samples.

The inter-assay precision showed a mean CV from 8.3% and a range of 5.3% - 15.6% for the five different human serum samples.

The between lot variation study was conducted during 5 days of testing. Each run were performed per day with high and low kit controls and with a panel of five human serum samples. Each sample was tested in five replicate per run with 3 different reagent lots. The results were calculated according CLSI-EP05-A3.

Sample	Mean conc. (ng/mL)	total Precision (Inter-Lot)	
		SD (ng/mL)	CV
1	14.3	2.9	20.1%
2	22.9	2.7	11.6%
3	31.6	2.8	8.8%
4	46.1	3.3	7.1%
5	80.1	4.9	6.1%

The between lot variation showed a mean CV from 10.7% and a range of 6.1%– 20.1% for the five different human serum samples.

17. PRODUCT LITERATURE REFERENCES

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Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.


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