Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
info@listarfish.it
www.listarfish.it

Manual

IDK®25-OH-Vitamin D ELISA

For the in vitro determination of 25-OH-vitamin D in dried blood

Valid from 2022-09-27

REF **K 2020DBS** Σ 96 +8°C +2°C IVD CE

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

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1. INTENDED USE

IDK® 25-OH-Vitamin D ELISA is an enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of 25-OH-vitamin D (D_2 and D_3) from dried blood. This value allows monitoring of the vitamin D status and detection of a possible insufficiency or excess. The assay is an *in vitro* diagnostic medical device and intended to be used by professional users in a laboratory environment. It is performed manually.

2. SUMMARY AND EXPLANATION OF THE TEST

Vitamin D_3 is photochemically synthetised from its precursor 7-dehydrocholesterol in the skin under the influence of ultraviolet light (UV-B). **Vitamin D_2** originates from plant food and is resorbed via the small intestine. Vitamin D_2 and D_3 are metabolised in the same way.

Vitamin D is bound to vitamin D binding protein (VDBP) in the bloodstream and metabolised to 25-OH vitamin D in the liver. This 25-hydroxylation is dependent on the substrate supply. 25-OH-vitamin D has a low biological activity, but is present in the circulation with the highest concentration of all D metabolites. Due to its high affinity to VDBP, it represents the storage form of vitamin D. The serum concentration of 25-OH vitamin D is therefore the best indicator of vitamin D supply.

In the kidneys, 25-hydroxyvitamin D is further metabolised to 1,25-dihydroxyvitamin D, which is the most active vitamin D metabolite and acts as a hormone (D hormone). It regulates calcium resorption from the intestine, bone mineralisation, osteoblast differenciation and bone matrix synthesis. Furthermore, the neuromuscular function is influenced by the D hormone.

Even mild vitamin D deficiency with a 25-OH vitamin D content of 20–29 ng/ml or 50–74 nmol/l leads to a secondary parathyroid hormone increase and increased bone resorption via reduced calcium absorption.

In the German general population aged > 50 years, vitamin D status is significantly correlated with bone density. Therefore, vitamin D deficiency is one of the most important risk factors for senile osteoporosis. The early detection of a vitamin D deficiency allows for an effective prevention of fractures by vitamin D supplementation. Severe vitamin D deficiency (< 20 ng/ml or < 50 nmol/l) results in rickets (in children) or osteomalacia (in adults), which are both characterised by a disturbed bone formation and defective matrix mineralisation. An excess of vitamin D (drug overdose) leads to a hypercalcemia syndrome.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 2020DBS	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 2020DBS	WASHBUF	Wash buffer concentrate, 50x	1 x 30 ml
K 2020DBS	SAMDIL	Sample diluent, ready-to-use	1 x 22 ml
K 2020DBS	STD	Standards, ready-to-use	6 x 200 µl
K 2020DBS	EXBUF	Extraction buffer, ready-to-use	1 x 10 ml
K 2020DBS	CTRL 1 CTRL 2	Controls, ready-to-use (see specification for range)	1 x 200 µl 1 x 200 µl
K 2020DBS	CONJ	Conjugate, peroxidase labelled, ready-to-use	1 x 22 ml
K 2020DBS	SUB	Substrate, ready-to-use	1 x 12 ml
K 2020DBS	STOP	Stop solution, ready-to-use	1 x 12 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- If applicable, dried blood carrier
- Calibrated precision pipettors and 10–1 000 µl single-use tips
- Multi-channel pipets or repeater pipets
- 1.5 ml reaction vials (disposable)
- Vortex
- Absorbent paper or paper towel
- Microplate reader (450 nm, reference wave length 620 or 690 nm)

*Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. PREPERATION AND STORAGE OF REAGENTS

- Bring all **reagents and samples to room temperature** (20–25 °C) and mix well.
- The test is **temperature-sensitive** and should only be carried out at a **room temperature of 20–25 °C**. Immundiagnostik AG cannot guarantee the accuracy of the measurement results at deviating room temperatures.

- To run assay more than once, ensure that reagents are stored at conditions stated on the label.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:50** before use (15 ml WASHBUF + 735 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. The crystals must be redissolved at room temperature or in a water bath at 37 °C before dilution of the buffer solution. The **WASHBUF** is stable at **2–8 °C** until the expiry date stated on the label.
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.
- **Note: Microtiter strips:** Once the vacuum-sealed aluminum bag has been opened, all unused strips must be put back into the aluminium bag together with the desiccant bag. Close the aluminium bag and store it at 2–8 °C.

6. PREPARATION OF THE ASSAY

Obtaining dried blood samples

Suitable sample material is **50 µl whole blood** dripped onto an Immundiagnostik AG dried blood carrier and completely dried (we recommend the special dry blood carrier for the determination of vitamin D: "D-Vital ID" - DZ9025ID). If other dried blood carriers are used, we recommend that laboratories validate these themselves.

Preparation of dried blood samples (= exctraction)

a) Remove the pre-punched dried blood spot from the dried blood carrier and place it rolled up in a 1.5 ml reaction vial.

b) Add 200 µl EXBUF and make sure that the dried blood spot is completely covered with EXBUF, if necessary vortex briefly.

c) Incubate samples for 30 min at room temperature (20–25 °C).

d) Thoroughly vortex the extracted dried blood sample again.

Sample storage

Dried blood samples (approximately 24 h after sampling) can be stored in a sealed bag with desiccant, protected from light at **-20 °C, room temperature and 37 °C** for **4 weeks** and for 1 week at up to 50 °C.

7. ASSAY PROCEDURE

Principle of the test

This **sandwich ELISA** is intended for the quantitative determination of 25-OH vitamin D (D_2 and D_3) from dried blood. The standards, controls and prepared samples are incubated with the first and second antibody in the microtitre plate for 20 minutes. The resulting vitamin D antibody-immune complex is detected with a third, peroxidase-labelled antibody by adding tetramethylbenzidine (TMB). The colour development is stopped by the addition of stop solution, and the resulted yellow colour is measured at 450 nm using a microtiter plate reader. The colour intensity is positively proportional to the concentration of Vitamin D in the sample.

A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated using the values obtained from the standard. 25-OH-vitamin D in the patient sample is determined directly from this curve.

Test procedure

Only reagents and samples that are at room temperature 20–25 °C may be used in the test. For this purpose, open the kit and remove the individual components required. Mix reagents and samples carefully before use and avoid foam formation. Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 °C.

Due to its temperature sensitivity, the test should only be performed at a room temperature of 20–25 °C. This is the only way to guarantee valid test results.

Furthermore, we recommend the determination of duplicate values.

Mark the positions of standards/controls/samples on a protocol sheet.

1.	Add **20 µl of extracted dried blood** and **10 µl of the standards/controls** into the respective wells.
2.	Add **200 µl** sample diluent (SAMDIL) into each well and mix gently for **30 s**.
3.	Incubate for **20 min at room temperature** (20–25 °C).
4.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the last washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
5.	Add **200 µl conjugate** (CONJ) into each well and mix gently for **30 s**.

6.	Incubate for **10 min at room temperature** (20–25 C).
7.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the last washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
8.	Add **100 µl substrate** (SUB) into each well and mix gently for **10 s**.
9.	**Incubate for 5–10 min at room temperature** (20–25 °C) **in the dark.**
10.	Add **100 µl stop solution** (STOP) into each well and mix gently until the blue colour completely changes to yellow.
11.	Determine **absorption** within **15 min** with an ELISA reader at **450 nm**. If the highest extinction of the standards is above the range of the photometer, absorption must be measured immediately at **405 nm** and the obtained results used for evaluation. If possible, the extinctions from each measurement should be compared with extinctions obtained at a reference wavelength, e. g. 595 nm, 620 nm, 630 nm, 650 nm and 690 nm can be used.

8. RESULTS

We recommend the 4-parameter algorithm for result calculation.

4-parameter algorithm

It is recommended a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.001).

The usage of the 4-parameter algorithm is strongly recommended. If it is not possible to use the 4-parameter algorithm for result calculation, it is possible to switch to a point-to-point calculation.

Point-to-point calculation

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

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 control of the paired values should be done manually.

9. LIMITATIONS

Measurement range

Samples with concentrations lower than the measurement range (= LoQ 1.25 ng/ml for dried blood) or above 150 ng/ml cannot be clearly quantified.

Dried blood samples cannot be diluted.

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Reference ranges for 25-OH-vitamin D_3

Deficiency (seriously deficient)	< 20 ng/ml or < 50 nmol/l
Insufficiency (deficient)	20–29 ng/ml or 50–74 nmol/l
Sufficiency (adequately supplied)	> 30 ng/ml or > 75 nmol/l

Literature references

The following literature for the vitamin D reference values can be found in the references: Grant et al., Soldin et al., Visser et al., Wicherts et al.

Conversion factor

1 ng/ml = 2.5 nmol/l

1 nmol/l = 0.4 ng/ml

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, VDBP) and serve only as orientation.

11. PERFORMANCE CHARACTERISTICS

Accuracy – Precision

Dried blood:

Repeatability (Intra-Assay); n = 20

Sample	Mean value [ng/ml]	S.D.	CV [%]
1	8.82	0.42	4.8
2	37.55	3.47	9.2

Reproducibility (Inter-Assay); n = 48

Sample	Mean value [ng/ml]	S.D.	CV [%]
1	9.68	1.18	12.2
2	44.3	2.66	6.0

Analytical sensitivity

Dried blood:

Limit of blank, LoB	0.31 ng/ml
Limit of detection, LoD	0.73 ng/ml
Limit of quantitation, LoQ	1.25 ng/ml
Detection range	1.52–150 ng/ml

Analytical specificity

100 % cross-reactivity to 25-OH Vitamin D_3 and 25-OH Vitamin D_2.

Accuracy – Trueness

24 DBS samples were tested with 25-OH vitamin D levels ranging from 17.52 ng/ml to 56.4 ng/ml. The test results were compared with the results of a commercially available 25(OH)-vitamin D ELISA kit. The comparison data showed a linear regression and demonstrated a good correlation between serum and DBS samples, with a correlation coefficient (R) of 0.921.

2. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- Control samples should be analysed with each run.
- Human materials used in kit components were 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.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide or ProClin are hazardous to health and the environment. Substrates for enzymatic colour reactions may also cause skin and/or respiratory irritation. Any contact with the substances must be avoided. Further safety information can be found in the safety data sheet, which is available from Immundiagnostik AG on request.
- The stop solution consists of diluted hydrochloric acid (HCl), 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 up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.
- As a precaution, it is recommended that the human material used is always considered potentially infectious.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch as wells from already opened microtiter plates are exposed to different conditions than sealed ones.
- Reagents should not be used beyond the expiration date stated on kit label.
- Substrate solution should remain colourless until use.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- Quality control guidelines 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 incorrect use.
- All serious incidents occurring in connection with the product must be reported to Immundiagnostik AG and (within the Union market) to the competent reporting authority of the respective member state.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

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Used symbols: