Version 25-04/24

User's Manual

25-OH Vitamin D (total) ELISA Kit

REF **DEIA2219**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

Enzyme immunoassay for the quantitative measurement of total 25-OH-Vitamin D (Vitamin D2 and D3) in human serum. For research use only. Not for use in diagnostic procedures.

General Description

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.

Principles of Testing

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.

Reagents And Materials Provided

Microtiter Plate Break apart strips. (1 x 12 x 8)

Coated with antibodies against 25-OH-Vitamin D2 and D3 (monoclonal)

Standard A Ready to use. (1 x 1 mL)

Contains: biological matrix with stabilizers and ≤ 1.0 % ProClin.

Standard B-F Ready to use. (1 x 5 x 1 mL)

Exact concentrations see vial labels or QC certificate.

Contains: 25-OH-Vitamin D, biological matrix with stabilizers and ≤ 1.0 % ProClin.

Control 1 + 2 Ready to use. (2 x 1 mL)

Concentrations / acceptable ranges see QC certificate.

Contains: 25-OH-Vitamin D, biological matrix with stabilizers and ≤ 1.0 % ProClin.

Incubation Buffer Ready to use. (1 x 12 mL)

Contains: casein and < 2.0 % ProClin

25-OH-Vitamin D Biotin Concentrate (101x) (1 x 0.15 mL)

Contains: Biotin in Buffer with stabilizers.

Enzyme Conjugate Ready to use. (1 x 15 mL)

Contains: streptavidin conjugated to HRP.

Wash Buffer Concentrate (10x) (1 x 100 mL)

Contains: phosphate buffer, Tween.

TMB Substrate Solution Ready to use. (1 x 15 mL)

Contains: TMB, Buffer, stabilizers.

TMB Stop Solution Ready to use. 1 x 15 mL

Contains: 1 M H_2SO_4.

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

Storage

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.

Specimen Collection And Preparation

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. The serum samples can be stored refrigerated (2-8°C) for up to 3days, for a longer storage they should be kept at -20 °C. The samples should not be frozen and thawed repeatedly.

Reagent Preparation

25-OH-Vitamin D Biotin Concentrate (101x)

Dilute 140 µL of 25-OH-Vitamin D Biotin Concentrate with 14 mL of Enzyme Conjugate. Prepare at least 90 min before use. Mix without foaming. The dissoved solution is stable for 8 hours at RT (18-25°C). Prepare freshly and use only once.

Wash Buffer Concentrate (10x)

Dilute 100 mL of Wash Buffer Concentrate with 900 mL of bidist. water. Mix vigorously. The DILUTED wash buffer is stable for 4 weeks at 4-8°C.

Assay Procedure

Before Assay

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.

Assay Steps

1. Pipette 25 µL of each Standard, Control and sample into the respective wells of the Microtiter Plate.
2. Pipette 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).

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. 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.

Calculation

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 Standard 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

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.

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.

Detection Limit

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

Specificity

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%

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 to 1:8

Precautions

1. For research use only. Not for use in diagnostic procedures.
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 Creative Diagnostics 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 Creative Diagnostics website or upon request directly from CD.
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.

Limitations

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