Diagnostics Biochem Canada Inc.
Manufacturing Innovative IVD for the World
Since 1973

25-Hydroxyvitamin D [25(OH)D] ELISA

REF CAN-VD-510 | CE | **IVD**

Effective Date: May 25, 2026 | Version: CE-8.0

1. INTENDED PURPOSE & USE

For the quantitative measurement of 25-Hydroxyvitamin D [25(OH)D] in human serum and plasma by an ELISA (Enzyme-Linked Immunosorbent Assay).

This kit is intended for professional use only and is for laboratory use only. For *in vitro* diagnostic use only. Intended to be used manually but may be adaptable to open automated analyzers. The user is responsible for validating the performance of this kit with any automated analyzers.

2. LIMITATIONS RELATED TO INTENDED PURPOSE & USE

1. This test is not intended to be used for screening purposes.
2. This test is not intended for home testing or self-testing.
3. The kit is calibrated for the determination of 25(OH)D in human serum and plasma. The kit is not calibrated for the determination of 25(OH)D in other specimens of human or animal origin.
4. The interpretation of the results should recognise the conditions that can affect vitamin D levels, such as medications, food supplements or extreme exposure to sun light or UV rays.
5. The results obtained with this kit shall never be used as the sole basis for a clinical diagnosis and for therapeutic decisions.
6. Although common interfering substances have been evaluated with this test, other substances that have not been evaluated such as drugs and the occurrence of heterophilic antibodies in individuals regularly exposed to animals or animal products have the potential of causing interferences.

3. SUPPLEMENTAL INFORMATION

Vitamin D concentration in blood should be measured regularly to ensure that satisfactory physiological levels are maintained year round (see references). Vitamin D is assimilated from food sources (both vitamin D2 and vitamin D3) or produced in the skin by sun exposure (vitamin D3). The body stores both vitamin D2 and vitamin D3 mainly in the form of 25-hydroxyvitamin D2 or 25-hydroxyvitamin D3 respectively. Therefore, the best approach to assess the physiological levels of vitamin D is to analyze the total concentration of both hydroxylated forms [25(OH)D].

4. PRINCIPLE OF THE TEST

The 25(OH)D ELISA is a two-step competitive immunoassay.
In the first incubation step, 25(OH)D present in calibrators, controls and specimen samples is dissociated from binding proteins (e.g. vitamin D binding protein). The dissociated 25(OH)D is bound by anti-25(OH)D antibodies that are immobilized on the microplate wells.
Excess and unbound materials are removed by a washing step.
In the second incubation step, a 25(OH)D-biotin:streptavidin-HRP conjugate is added which competes with the antibody-bound 25(OH)D for a limited number of anti-25(OH)D antibody binding sites.
After another washing step that removes unbound materials, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue-coloured product that is inversely proportional to the amount of 25(OH)D present. Following an incubation, the enzymatic reaction is terminated by the addition of the stopping solution, converting the colour from blue to yellow. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of 25(OH)D in specimen samples and controls can be directly read.

5. PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory *in vitro* use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
   - Do not pipette by mouth.
   - Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
   - Wear protective clothing and disposable gloves.
   - Wash hands thoroughly after performing the test.
   - Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Do not use the kit beyond the expiry date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibrator curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or serum/plasma pools which should be included in every run at a high and low level for assessing the reliability of results.
12. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper procedural techniques or pipetting, incomplete washing, or improper reagent storage.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
14. The TMB Substrate is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
15. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum and plasma samples.
16. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
17. Samples values above the measuring range of the kit are reported as >160 ng/mL. Do not further dilute samples as this may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
20. To prevent the contamination of reagents, do not pour reagents back into the original containers.
21. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
22. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
23. This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
24. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
25. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
26. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shaker and/or speed is used, the user is responsible for validating the performance of the kit.
27. Do not reuse the microplate wells, they are for SINGLE USE only.
28. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
29. Avoid exposing kit reagents, serum and plasma specimens to intense light.
30. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
31. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the European Member State in which the user and/or the patient is established.

6. SAFETY CAUTIONS AND WARNINGS

6.1 BIOHAZARDS

The reagents should be considered a potential biohazard and handled with the same precautions applied to blood specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

The calibrators and controls provided with the kit contain processed human serum/plasma that has been tested by approved methods and found to be negative for the presence of HIV 1/2, HCV, HIV-1 NAT, HCV NAT and RPR. However, no test method can offer complete assurance that any viable pathogens are absent. Therefore, these components should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen, following good laboratory practices.

6.2 CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

7. SPECIMEN COLLECTION, STORAGE AND PRE-TREATMENT

7.1 Specimen Collection & Storage

Serum

Approximately 0.05 mL of serum is required per duplicate determination. Collect 4–5 mL of venous blood into an appropriately labelled tube and allow it to clot. Centrifuge at room temperature and carefully transfer the serum into a new storage tube or container. Serum samples may be stored at 2-8°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later time.
Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

Plasma

Approximately 0.05 mL of plasma is required per duplicate determination. Collect 4–5 mL of venous blood into an appropriately labelled EDTA plasma tube and allow it to clot. Centrifuge at room temperature and carefully transfer the plasma into a new storage tube or container. Plasma samples may be stored at 2-8°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later time.
Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

7.2 Specimen Pre-Treatment

Specimen pre-treatment is not required.

8. REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Calibrated single-channel pipette to dispense 25 µL.
2. Calibrated multi-channel pipettes to dispense 50 µL and 150 µL.
3. Calibrated multi-channel pipettes to dispense 300 µL (if washing manually).
4. Automatic microplate washer (recommended).
5. Disposable pipette tips.
6. Distilled or deionized water.
7. Calibrated absorbance microplate reader with a 450 nm filter and an upper OD limit of 3.0 or greater.
8. Disposable glass test tubes or glass bottles.
9. Vortex mixer.

9. REAGENTS PROVIDED

1. MPL — Microplate

Contents:	One anti-25(OH)D monoclonal antibody-coated 96-well (12x8) microplate in a resealable pouch with desiccant.
Format:	Ready to Use
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

2. BIOT | CONJ | CONC — Biotin Conjugate Concentrate

Contents:	One bottle containing 25-Hydroxyvitamin D-biotin conjugate in a protein-based buffer with a non-mercury preservative.
Format:	Concentrated; Requires Preparation
Volume:	1.0 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.
Preparation of Conjugate Working Solution:	**X101** **Dilute 1:101 Before Use** See section *10. Preparation of Conjugate Working Solution.*

3. STA | HRP | CONJ | CONC — Streptavidin HRP Conjugate Concentrate

Contents:	One bottle containing Streptavidin-Horse Radish Peroxidase (HRP) conjugate in a protein-based buffer with a non-mercury preservative.
Format:	Concentrated; Requires Preparation
Volume:	0.3 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.
Preparation of Conjugate Working Solution:	**X101** **Dilute 1:101 Before Use** See section *10. Preparation of Conjugate Working Solution.*

4. CAL | A – F Calibrator A – F

Contents:	Six bottles of calibrator containing specified 25-hydroxyvitamin D concentrations. Human plasma-based matrix with a non-mercury preservative. Prepared by spiking matrix with defined quantities of 25-hydroxyvitamin D. Calibrators are traceable to NIST SRM 972 and to concentrations determined by UV spectrophotometric analysis using a molar extinction coefficient of 18,300 M-1cm-1 at 264 nm. Listed below are approximate concentrations, please refer to vial labels for exact concentrations. Concentrations: 0, 10, 20, 40, 80, 160 ng/mL.
Format:	Ready to Use
Volume:	1.0 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

5. CONTROL | 1 – 2 Control 1 – 2

Contents:	Two bottles of control containing different 25-hydroxyvitamin D concentrations. Human plasma-based matrix with a non-mercury preservative. Prepared by spiking matrix with defined quantities of 25-hydroxyvitamin D. Refer to the QC certificate for the target values and acceptable ranges.
Format:	Ready to Use
Volume:	1.0 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

6. INC | BUFF Incubation Buffer

Contents:	One bottle containing a buffer with a non-mercury preservative.
Format:	Ready to Use
Volume:	20 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

7. ASY | BUFF Assay Buffer

Contents:	One bottle containing a protein-based buffer with a non-mercury preservative.
Format:	Ready to Use
Volume:	20 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

8. TMB | SUB TMB Substrate

Contents:	One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Format:	Ready to Use
Volume:	16 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

9. STOP Stopping Solution

Contents:	One bottle containing 1M sulfuric acid.
Format:	Ready to Use
Volume:	6 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.
Safety:	Refer to product SDS.  **Warning**

10. WASH | BUFF | CONC Wash Buffer Concentrate

Contents:	One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Format:	Concentrated; Requires Preparation
Volume:	50 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks. Following Preparation: The wash buffer working solution is stable for 2 weeks following preparation, assuming Good Laboratory Practices are adhered to. To prevent microbial growth, prepare the wash buffer working solution in a clean container and store under refrigerated conditions (2-8°C) when not in use.
Preparation of Wash Buffer Working Solution:	**X10 Dilute 1:10 Before Use** Dilute 1:10 in distilled or deionized water before use. If the whole microplate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of distilled or deionized water.

10. PREPARATION OF CONJUGATE WORKING SOLUTION

The conjugate working solution consists of a 1:101 dilution of the biotin Conjugate Concentrate and a 1:101 dilution of the Streptavidin-HRP Conjugate Concentrate into the same aliquot of Assay Buffer. The resulting conjugate working solution is a biotin:streptavidin-HRP complex.

Preparation

1. To a disposable glass test tube or glass bottle, first add the required volume of Assay Buffer.

   Only **glass** may be used for preparing the conjugate working solution. The use of other types of materials, including plastic, may lead to low OD values.

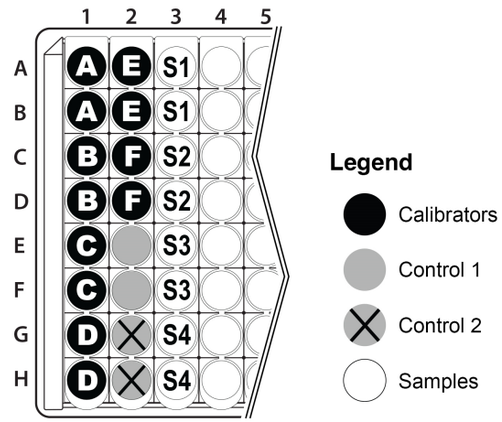
2. To the glass tube or bottle containing the assay buffer from step 1, add a 1:101 volume of Biotin Conjugate Concentrate and a 1:101 volume of Streptavidin HRP Conjugate Concentrate.

   For example, if the whole plate is to be used, add 16 mL of Assay Buffer to a glass bottle. add 0.16 mL of the Biotin Conjugate Concentrate and 0.16 mL of the Streptavidin-HRP Conjugate Concentrate.

   It is very important to add the assay buffer to the glass tube or bottle **first** and then add the conjugate concentrates to the assay buffer. Failure to prepare the working conjugate solution in this order may lead to low OD values.

3. Mix the working conjugate thoroughly using a vortex mixer and store in a dark place until it is used in the assay procedure. Discard any that is left over; do not store for future use.

11. RECOMMENDED ASSAY LAYOUT



12. ASSAY PROCEDURE

Specimen Pre-Treatment: None

All kit components, controls and specimen samples must reach room temperature prior to use. Calibrators, controls, and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. After all kit components have reached room temperature, **mix** gently by inversion.
2. **Prepare** the Conjugate Working Solution (see section *10.* Preparation of Conjugate Working Solution) and Wash Buffer Working Solution (See section *9. Reagents Provided* section, *10. Wash Buffer Concentrate*).
3. **Plan** the microplate wells to be used for calibrators, controls, and samples. See section *11. Recommended Assay Layout.* Remove the strips from the microplate frame that will not be used and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator.
4. **Pipette 25 µL** of each calibrator, control, and specimen sample into assigned wells.
5. **Pipette 150 µL** of the Incubation Buffer into each well (the use of a multi-channel pipette is recommended).
6. Gently tap the microplate frame for 10 seconds to mix the contents of the wells and **incubate** the microplate at room temperature in a dark place (no shaking) for **60 minutes**.
7. **Wash** the microplate wells with an automatic microplate washer (preferred) or manually as stated below.

   Automatic: Using an automatic microplate washer, perform a **3-cycle** wash using **300 µL/well** of Wash Buffer Working Solution (3 x 300 µL). One cycle consists of aspirating all wells then filling each well with 300 µL of Wash Buffer Working Solution. After the final wash cycle, aspirate all wells and then tap the microplate firmly against absorbent paper to remove any residual liquid.

   Manually: For manual washing, perform a **3-cycle** wash using **300 µL/well** of Wash Buffer Working Solution (3 x 300 µL). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waste container, then pipetting 300 µL of Wash Buffer Working Solution into each well using a multi-channel pipette. After the final wash cycle, aspirate all wells by briskly emptying the contents over a waste container and then tap the microplate firmly against absorbent paper to remove any residual liquid.
8. **Pipette 150 µL** of the Conjugate Working Solution into each well (the use of a multi-channel pipette is recommended).
9. Gently tap the microplate frame for 10 seconds to mix the contents of the wells and **incubate** the microplate at room temperature in a dark place (no shaking) for **30 minutes**.
10. **Wash** the microplate wells again as stated in step 7.
11. **Pipette 150 µL** of TMB Substrate into each well (the use of a multi-channel pipette is recommended).
12. Gently tap the microplate frame for 10 seconds to mix the contents of the wells and **incubate** the microplate at room temperature in a dark place (no shaking) for **10-15 minutes**.
13. **Pipette 50 µL** of Stopping Solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed as was used for addition of the TMB Substrate. Gently tap the microplate frame to mix the contents of the wells.
14. **Measure** the optical density (absorbance) in the microplate wells using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the Stopping Solution.

13. CALCULATIONS

1. Calculate the mean optical density for each calibrator, control and specimen sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a calibrator curve.
3. The immunoassay software will calculate the concentrations of the controls and specimen samples using the mean optical density values and the calibrator curve.
4. If a sample value is greater than 160 ng/mL then report as >160 ng/mL; do not further dilute and retest.

14. QUALITY CONTROL

When assessing the validity of the test results, the following criteria should be evaluated:

1. The calibrator A mean optical density meets the acceptable range as stated in the QC Certificate.
2. The calibrator with the highest concentration meets the % binding acceptable range as stated in the QC Certificate. % Binding = (OD of calibrator/OD of calibrator A) x 100.
3. The values obtained for the kit controls are within the acceptable ranges as stated in the QC certificate.
4. The results of any external controls that were used meet the acceptable ranges.

15. TYPICAL DATA

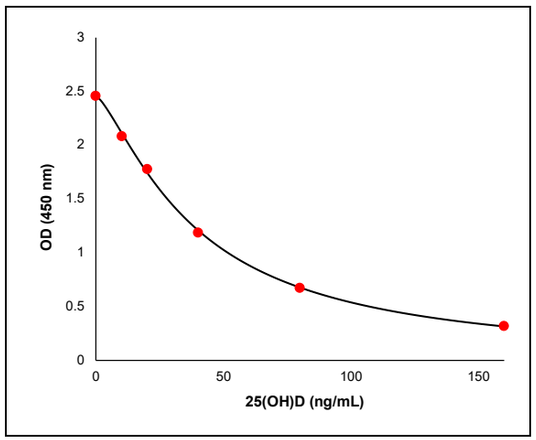
15.1 TYPICAL TABULATED DATA

Sample data only. **Do not** use to calculate results.

Calibrator	Mean OD (450 nm)	% Binding	Value (ng/mL)
A	2.461	100	0
B	2.084	85	10
C	1.780	72	20
D	1.191	48	40
E	0.676	27	80
F	0.318	13	160
Unknown	1.702	-	21.2

15.2 TYPICAL CALIBRATOR CURVE

Sample curve only. **Do not** use to calculate results.



16. PERFORMANCE CHARACTERISTICS

16.1 SENSITIVITY

The analytical sensitivity study was performed according to the CLSI EP17-A guideline. The limit of detection (LoD) was determined from the analysis of 64 samples of the blank and a low value sample and it was calculated as follows:

$LoD = \mu_B + 1.645\sigma_B + 1.645\sigma_S$,

where σ_B and σ_S are the standard deviation of the blank and low value sample and μB is the mean value of the blank.

The Limit of Detection (LoD) was determined to be 5.5 ng/mL.

16.2 SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with 25(OH)D3 cross-reacting at 100%.

Compound	% Cross-Reactivity
25 (OH)D3	100
25 (OH)D2	100
1,25 (OH)2D3	8.3
3-epi-25 (OH)D3	66
Vitamin D2	< 1.0
Vitamin D3	< 1.0

16.3 INTERFERENCES

An interference study was performed according to the CLSI EP07-A2 guideline. No significant interference was observed for concentrations of up to 7.5 mg/mL haemoglobin, 200 µg/mL Bilirubin (conjugated and unconjugated), 5.5 mg/mL triglycerides, 2.6 mg/mL cholesterol, 10 mg/mL ascorbic acid, 40 µg/mL Biotin and 10 µg/mL caffeine.

16.4 PRECISION

The precision study was performed according to the CLSI EP05-A3 guideline.

The experimental protocol used a nested components-of-variance design with 21 testing days, two runs per testing day, and two replicate measurements per run (a 21 x 2 x 2 design) for each sample. Data was analyzed with a two-way nested ANOVA and summarized in the table below.

Sample	Mean (ng/mL)	Repeatability		Within Location	
		SD (ng/mL)	CV%	SD (ng/mL)	CV%
1	21.87	1.09	5.0	1.77	8.1
2	36.57	1.01	2.8	3.17	8.7
3	45.01	1.07	2.4	4.45	9.9
4	60.25	2.82	4.7	6.21	10.3

16.5 COMPARATIVE STUDIES

The DBC 25(OH)D ELISA kit (y) was compared to a higher level test (LC-MS/MS) (x). The comparison of 40 serum samples yielded the following linear regression results:

y = 0.93x – 4.68, r = 0.96

17. REFERENCE RANGES

Data presented here are from samples collected in Florida (USA) from putatively healthy Black, White and Hispanic individuals of both genders and between 20 and 60 years old. Population reference ranges for 25(OH)D vary widely depending on age, ethnic background, geographic location and season. Population-based ranges correlate poorly with serum 25(OH)D concentrations that are associated with biologically and clinically relevant vitamin D effects and are therefore of limited clinical value.

Each laboratory shall establish their own reference ranges.

N	Mean (ng/mL)	Median (ng/mL)	95% Reference Interval (ng/mL)
120	24.6	23.5	12.6 – 42.3

Additional Information

Results from the NHANES III study[1] yielded a mean of 30 ng/mL among 15,390 individuals.

The Institute of Medicine at Washington DC (2) concluded that the levels of vitamin D can be associated with health conditions as in the following table:

25(OH)D (ng/mL)	Health Status
< 12	Vitamin D deficiency leading to rickets in infants and children and osteomalacia in adults.
12–20	Generally considered inadequate for bone and overall health in healthy individuals.
≥ 20	Generally considered adequate for bone and overall health in healthy individuals.
> 60	Emerging evidence links potential adverse effects to such high levels.

Another source reports the following threshold levels:

25(OH)D (ng/mL)	Health Status
< 10	Severe deficiency. Could be associated with osteomalacia or rickets.
10–19	Mild to moderate deficiency. May be associated with increased risk of osteoporosis or secondary hyperparathyroidism.
20–50	Optimum levels in the healthy population; patients with bone disease may benefit from higher levels within this range.
51–80	Increased risk of hypercalciuria. Sustained levels > 50 ng/mL 25OH-VitD along with prolonged calcium supplementation may lead to hyper-calciuria and decreased renal function.
> 80	Toxicity possible. 80 ng/mL is the lowest reported level associated with toxicity in patients without primary hyperparathyroidism who have normal renal function. Most patients with toxicity have levels > 150 ng/mL. Patients with renal failure can have very high 25(OH)D levels without any signs of toxicity, as renal conversion to the active hormone 1,25(OH)D is impaired or absent.

These reference ranges represent clinical decision values that apply to males and females of all ages, rather than population-based reference values.

18. LITERATURE

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19. SYMBOLS GLOSSARY

Symbol	Definition	Symbol	Definition
REF	Catalogue number		Manufacturer
LOT	Batch code		Date of manufacture
IVD	In vitro diagnostic medical device		Biological risks
UDI	Unique Device Identifier		Consult instructions for use
X #	Dilute 1:# Before Use	Rx ONLY	Prescription only: Device restricted to use by or on the order of a physician
QTY	Quantity		Keep away from sunlight
	Use-by date	EC REP	Authorized representative in the European Community/ European Union
	Do not re-use		Temperature limit
	Caution	Σ	Contains sufficient for <n> tests
LYO	Lyophilized	RUO	For Research Use Only. Not for use in diagnostic procedures.

The definitions of symbols used for kit component names are described in the *Reagents Provided* section.

20. CHANGE HISTORY

Previous Version:	7.2 (Combined)	New Version:	CE-8.0
Changes:	New IFU format with numbered headings. **HEADING** Removal of country-specific regulatory information. **1. INTENDED PURPOSE & USE** Addition: This kit is intended for professional use only and is for laboratory use only. For in vitro diagnostic use only. Intended to be used manually but may be adaptable to open automated analyzers. The user is responsible for validating the performance of this kit with any automated analyzers. **2. LIMITATIONS RELATED TO INTENDED PURPOSE & USE** 1 and 2 added. **4. PRINCIPLE OF THE TEST** Content same, wording changed to match standardized text. **5. PROCEDURAL CAUTIONS AND WARNINGS** Additional cautions and warnings added. Some previous limitations added to this section. **8. REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED** Addition of vortex mixer. **9. REAGENTS PROVIDED** Addition of symbols for all components and safety information if applicable. In-use stability statement added for all components. Control low and high now called control 1 and 2, respectively. 6. Incubation Buffer **Change** **Blue dye** was removed from the contents description. **10. PREPARATION OF CONJUGATE WORKING SOLUTION** Warning symbols added. Changed from 2 steps to 3 steps. Step 3 added use of vortex mixer. **11. RECOMMENDED ASSAY LAYOUT** New section added. **12. ASSAY PROCEDURE** Component names revised to match symbol definitions. **13. CALCULATIONS** Addition of more explanatory text. **14. QUALITY CONTROL** New section added. **15.1 TYPICAL TABULATED DATA** Table data updated. **17. REFERENCE RANGES** Column heading changed from: Range (2.5th–97.5th percentile) (ng/mL) To: 95% Reference Interval (ng/mL)		
	19. SYMBOLS GLOSSARY Addition of symbols and definitions. **20. CHANGE HISTORY** New section added. **21. GENERAL INFORMATION** Addition of product complaints, warranty and limitation of liability sections. EC REP changed from Emergo Europe to MedEnvoy Global B.V. Build: *v1.3D* BASE: *v9.0*		

21. GENERAL INFORMATION

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Product Complaints

In the case of product complaints, the user shall submit in writing to the distributor or manufacturer a description of the complaint and provide accompanying data and/or information.

Warranty

DBC guarantees that the product is free of defects and will perform within the product specifications when the product is used prior to the expiration date, according to the intended purpose and use, and according to the instructions for use provided with the product. Any deviations from the intended purpose and use, instructions for use, modifications to kit components or use beyond the expiration date will invalidate any warranty claims.

Limitation of Liability

DBC liability in all circumstances whether in tort (including negligence) or at common law, and for any damage or loss, including but not limited to loss of profit and loss of sales, suffered whether direct, indirect, consequential, incidental or special is limited to the purchase price of the product(s) in question.