Medcaptain

CE

Instructions for Use

25-Hydroxyvitamin D (CLIA)

[Product Name]

25-Hydroxyvitamin D (CLIA)

[Packing Size]

24×1 Tests/Pkg (Calibrators included); 60×1 Tests/Pkg (Calibrators included); 60×1 Tests/ Pkg

[Intended Use]

25-Hydroxyvitamin D assay is a Chemiluminescent Immunoassay (CLIA) used for the quantitative determination of total 25-hydroxyvitamin D (25-OH-VD) in human serum or plasma, and it is mainly used for auxiliary diagnosis of vitamin D deficiency related diseases.

Vitamin D (VD) is a fat-soluble cyclopentane polyhydrophenanthrene compound. Currently known vitamin D includes vitamin D2~D7 [1-2]. The only active forms that really play a role in animal nutrition are VD_2 (ergocalciferol) and VD_3 (cholecalciferol). In humans, both VD_3 and VD_2 are bound to vitamin D-binding protein in the blood and transported to the liver [3]. Vitamin D itself is not active, and must be converted into a biologically active form after two consecutive hydroxylation reactions in the liver and kidney [3]. The first hydroxylation reaction occurs in the liver to produce 25-hydroxyvitamin D, and the second hydroxylation reaction occurs in the liver. Secondary hydroxylation occurs in the kidney, producing biologically active 1,25-dihydroxyvitamin D [3-6]. The quantity of 1,25-dihydroxyvitamin in the body is only 0.1% of that of 25-hydroxyvitamin D, and it is regulated by PTH, calcium and phosphorus [5]. 25-hydroxyvitamin D has strong stability in the body, therefore, the measurement of vitamin D in human blood circulation mainly detects the level of 25-OH-VD [7-8]. Vitamin D deficiency is closely related to the occurrence of many diseases, such as osteoporosis, diabetes, cardiovascular diseases, autoimmune diseases, and innate immune diseases [9-12]. Quantification of vitamin D in the body can help physician to identify the problem of VD deficiency, and give patients timely treatment with supplementation of vitamin D.

[Principle of the Assay]

This immunoassay adopts a competition format. The test principle is as follows:

(1) Sample, pretreatment reagent 1 (PT1), and pretreatment reagent 2 (PT2) are added to the reaction well, then mix and incubate.

(2) Anti-25-OH-VD antibody labeled with acridinium ester is added to the reaction well, after mixing and incubation, 25-OH-VD in the sample is bound by the anti-25-OH-VD antibody labeled with acridinium.

(3) Magnetic microparticle coated with a 25-OH-VD derivative is added to the reaction well, unreacted acridinium labeled anti-25-OH-VD antibody will bind to the magnetic microparticle coated with the 25-OH-VD derivative, while the 25-OH-VD bound antibody in the sample can no longer bind to the magnetic microparticle coated with the 25-OH-VD derivative, and is washed off after a magnet is used to capture the microparticle

(4) A photomultiplier tube is used to measure photons generated from the reaction. The count is inversely proportional to the quantity of 25-OH-VD in the sample. Concentration of 25-OH-VD is determined by an internal calibration curve.

[Main Components]

Packing Size

Component \ Packing Size		Fill Volume		
		24×1 Tests/Pkg (Calibrators included)	60×1 Tests/Pkg (Calibrators included)	60×1 Tests/Pkg
25-OH-VD Reagent Cartridge	Microparticle (R1)	24×50 μL	60×50 μL	60×50 μL
	Conjugate (R2)	24×100 μL	60×100 μL	60×100 μL
	Pretreatment reagent 1 (PT1)	24×50 μL	60×50 μL	60×50 μL
	Pretreatment reagent 1 (PT2)	24×25 μL	60×25 μL	60×25 μL
25-OH-VD Calibrator	25-OH-VD Calibrator C1	1×1.0 mL	1×1.0 mL	/
	25-OH-VD Calibrator C2	1×1.0 mL	1×1.0 mL	/
Calibration Card	Calibration curve and calibrator information	1 pcs	1 pcs	/

Main Composition

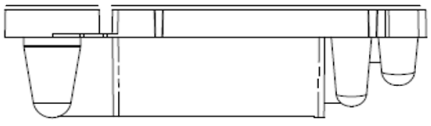
Component		Main Composition
25-OH-VD Reagent Cartridge	Microparticle (R1)	Magnetic microparticle coated with a 25-OH-VD derivative, ~0.3 g/L; Tris buffer, 50 mmol/L; ProClin 300, 0.5 g/L
	Conjugate (R2)	Acridinium labeled anti-25-OH-VD monoclonal antibody, ~10 μg/L; MES buffer, 50 mmol/L; ProClin 300, 0.5 g/L
	Pretreatment reagent 1 (PT1)	Tris buffer solution, 50 mmol/L; ProClin 300, 0.5 g/L
	Pretreatment reagent 2 (PT2)	NaOH solution, 0.5 mol/L
25-OH-VD Calibrator C1		25-OH-VD; Tris buffer solution, 25 mmol/L; ProClin 300, 0.5 g/L
25-OH-VD Calibrator C2		25-OH-VD; Tris buffer, 25 mmol/L; ProClin 300, 0.5 g/L
Calibration Card		Calibration curve and calibrator information

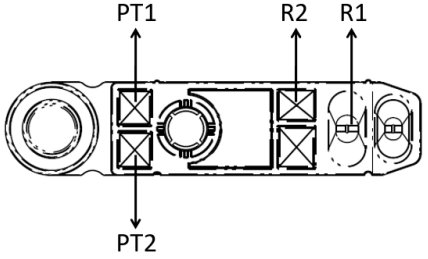
Note: (1) Components in different lots of reagent cannot be mixed or exchanged for use.

(2) Traceability: This quantification method can be traced back to the internationally agreed standard substance NIST SRM 2972a.

(3) Information about calibrators can be found in the instrument after scanning the Calibrator Card (such as lot number and concentration etc.)

The position of each component is shown in the front view (Upper) and vertical views (Down) of the reagent pack.





Instruments and accessories needed but not supplied (available from

Medcaptain)

(1) Medcaptain Immu F6/F6S automatic chemiluminescent immunoassay analyzers;

(2) Pre-trigger solution;

(3) Trigger solution;

(4) Washing solution;

(5) 500μL pipette tips;

(6) Metabolite Controls.

[Storage and Shelf-life]

Storage: Store sealed reagent cartridge and calibrators at 2~8℃ in upright position, and avoid freezing.

Shelf life: 14 months.

Stability of Calibrators: Sealed vial of calibrators can be kept at 2~8℃ in dark for 14 months. After calibrator C1 and C2 is uncapped, it can be stored at 10~30℃ for 5 days, and at 2~8℃ for 60 days.

The dates of manufacturing and expiration can be found on the labels.

[Matched Instruments]

Medcaptain Immu F6/F6S automatic chemiluminescent immunoassay analyzers

[Specimen Types]

Serum, and plasma (collected with EDTA-K_2, EDTA-K_3, lithium heparin, and sodium heparin as the anti-coagulants) can be used in the testing.

Sample volume for each test: 20μL

The collected sample should be tested as soon as possible.

Serum and plasma can be kept at 10~30℃ for 24 hours, at 2～8℃ for 4 days, and at -20℃ or below for 180 days. Frequent freeze-thaw cycle should be avoided, and three freeze-thaw cycles are allowed. If the sample contains precipitate or frozen floccule, centrifugation is needed to clear the sample before testing.

Sample collection tubes from different manufacturers may lead to variation in testing results, due to the difference in tube material and additives in the tubes. Medcaptain has not evaluated all types of sample collection tubes from different manufacturers. Each laboratory should make its own judgment about the usability of sample collection tubes.

[Test Procedure]

Reagent Preparation

Reagent: 25-OH-VD reagent cartridge (containing magnetic microparticle R1, acridinium labeled antibody conjugate R2, pretreatment reagent PT1, and pretreatment reagent PT2) is ready for use. It can be loaded directly into instrument after opening the package.

Calibrators: Calibrator C1 and C2 are ready for use. Each calibrator can be added to a sample cup. The cups are loaded to a sample rack, and then the rack is directly pushed into instrument for testing.

Calibration

Refer to the relevant chapter in the operation manual of each chemiluminescent immunoassay analyzer for system calibration.

Calibration tests should be ordered before the first time use of 25-OH-VD assay. Medcaptain provides 25-OH-VD reagent pack and matched calibrators to calibrate the instrument.

Before calibration, scan the calibration card provided in the kit, and the calibration curve and calibrator information will be scanned into the system.

Before calibration testing, take out reagent cartridges from the package, and load them into the instrument. The instrument scans two-dimensional barcode on the reagent pack automatically to obtain information of the reagent (reagent name, Lot No., and expiration date etc.).

Put calibrators on a sample rack, load the sample rack into the instrument.

On the screen interface of “Reagent > Request Calibration”, select test name and lot number to request a calibration.

Select the position of each calibrator on the sample rack, set the number of repeat tests, start calibration.

Automatic immunoassay analyzer makes use of calibration data to validate the calibration, and adjust the calibration curve automatically.

Instrument calibration is effective for 60 days.

A new calibration is needed in the following situation:

(1) Change into a new lot of reagent;

(2) Controls are out of the acceptable range;

(3) The lot of reagent has been used on the same instrument for more than 60 days.

Refer to the Chapter of “Calibration” in the instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about calibration.

Control Testing

Metabolite Controls are matched with 25-OH-VD reagent pack. There are two levels of controls: Low Control (L) and High Control (H).

These two-level controls should be tested in accordance with any local applicable regulations. Control testing is highly recommended every time the lot of reagent has been changed, the instrument has been re-calibrated, or after trouble shooting/ maintenance service.

Before the control testing, take out reagent cartridges from the package, and load them into the instrument. The instrument scans two-dimensional barcode on the reagent pack automatically to obtain information of the reagent (reagent name, Lot No., and expiration date etc.).

Put controls on a sample rack, and load the sample rack into the instrument;

Select “Control” on the interface of test menu, select test name and control lot;

Click on “Start” and begin the testing. Check the results after control test is finished.

Control test results should fall into a specific range. If it is out of target range, the user should check the system, such as expiration date of the controls, storage condition, instrument performance and status. After root cause analysis and correction, the user should test controls again. If the same problem exists, please contact customer service of Medcaptain.

Each laboratory should set up its own control range and frequency of control testing, based on its own practice.

Refer to the Chapter of “Control Testing” in instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about control testing.

Sample Testing

Before sample testing, take out reagent cartridges from the package, and load them into the instrument. The instrument scans two-dimensional barcode on the reagent pack automatically to obtain information of the reagent (reagent name, Lot No., and expiration date etc.);

If a sample collection tube is directly loaded to the instrument for testing, the sample volume should be at least 1.0mL;

Uncap sample collection tubes, put samples on a sample rack, and push it into the instrument;

Select “Sample” on the interface of test menu, enter information of samples, select test name;

Click on “Start” and begin the testing. Check the results after sample test is finished.

The reagent usage for each test is: R1 50 μL, R2 100 μL, PT1 50 μL, PT2 25 μL. The instrument aspirates and mixes each component in the reagent cartridge, and incubates at 37℃. Time duration from sampling to result is about 30 min.

Refer to the Chapter of "Sample Testing" in instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about sample testing.

Result Calculation

Based on the built-in calibration curve, the instrument automatically calculate 25-OH VD concentration of each sample, in a unit of ng/mL.

[**Reference Intervals**]

Samples for the study of reference intervals come from local area in Guangdong Province. A total of 278 healthy and normal people have been recruited. Serum tests give a reference interval of 2.5% - 97.5% population in the following table.

25-OH-VD	Mean	2.5^{th}-97.5^{th} Percentile
Healthy and normal Individuals	25.8 ng/mL	10.6~49.7 ng/mL

Vitamin D levels are affected by many factors. Due to the differences in ultraviolet radiation, dietary supplements, obesity, geography, race, sex, age and so on of tested population, the reference interval may vary in different laboratories. It is highly recommended for each clinical lab to establish its own reference intervals.

[Interpretation of Test Results]

The test data is for clinical reference only. It cannot be used as the only confirmatory evidence nor to eliminate the possibility of diseases. Clinical diagnosis of patients should take clinical symptoms, body sign, disease history, other lab test results, and treatment response into comprehensive consideration.

The measurement range of this assay is: 4.0~150 ng/mL. If 25-OH-VD concentration is lower than LoD, it will be reported as <4.0 ng/mL; if 25-OH-VD concentration is over the upper limit, it will be reported as >150 ng/mL.

When the instrument shows a warning sign of "SMPL", it means there is insufficient sample volume. Make sure enough sample is added for repeating the test. When the instrument shows a warning sign of "SMPJ", it means the sample probe has been blocked. Clean sample clot in the probe before repeating the test.

Some results are tagged with other signs. Refer to the Chapter of "Result Signs" in the instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about results tagged with signs.

[Limitation of the Test Method]

The test data is for clinical reference only. It cannot be used individually as the evidence to confirm or eliminate the possibility of diseases.

Measurement of 25-OH-VD uses a competition method, which will not generate HOOK effect theoretically.

For endogenous interference substances with concentration less than the value shown in the table below, measurement error caused by the interference is within ±10%.

Endogenous interference substance	Concentration of interference substance
Total Protein	≤12 g/dL
Bilirubin	≤40 mg/dL
Hemoglobin	≤200 mg/dL
Triglyceride	≤500 mg/dL

For potential cross-reacting interference substances with concentration shown in the table below, potential cross-reaction rates is more over 90%.

Cross-reacting interference substances	Concentration of interference substance
25-OH -Vitamin D_2	≤100 ng/mL
25-OH-Vitamin D_3	≤100 ng/mL
24,25-$(OH)_2$-Vitamin D_3	≤100 ng/mL
3-epi-25-OH-Vitamin D_3	≤100 ng/mL

For potential cross-reacting interference substances with concentration shown in the table below, the test results is less than or equal to 4 ng/mL.

Cross-reacting interference substances	Concentration of interference substance
Vitamin D_2	100 ng/mL
Vitamin D_3	100 ng/mL
1,25-$(OH)_2$-Vitamin D_2	100 ng/mL
1,25-$(OH)_2$-Vitamin D_3	100 ng/mL

Heterophilic antibodies in human serum may react with immunoglobulin in the reagent or sample, and interfere with immunoassay in vitro. More clinical or diagnostic information is needed to confirm disease diagnosis of patients.

Some patients have frequent contact with animals, or have been treated or diagnosed with mouse monoclonal antibodies. They may have generated heterophilic antibodies. For example, some patients under monoclonal antibody treatment may have human anti-mouse antibodies (HAMA) in blood circulation, leading to false positive or false negative results. Anti-interference components are added to this reagent formulation to minimize the impact of HAMA and ANA, but the problem may not be totally eliminated, and some sample testing may still be impacted. More clinical and diagnostic information is needed to make a solid conclusion.

Samples with the titer of no less than 1:1000 by anti-nuclear IgG test kit (indirect immune-fluorescence method) were studied in the interference tests. It has shown less than ±10% error in the test results.

For RF at a concentration of less than 1500 IU/mL, and for HAMA at a concentration of less than 120 ng/mL, the measurement error of 25-OH-VD is within ±10 %.

[**Property and Performance]**

1 Limit of Blank

LoB ≤ 2.0 ng/mL.

2 Limit of Detection

LoD ≤ 4.0 ng/mL.

3 Accuracy

Accuracy should meet at least one of the following criteria:

a) Relative Deviation: use international reference material as the test sample that can be used to evaluate conventional methods, relative deviation of the measurement result from the target value should not exceed ±15.0 %.

b) Relative Deviation: test the accuracy reference samples at two concentration levels multiple times. Relative deviation between the measurement result and the target value must not exceed 15.0%.

c) Spike 25-OH-VD of a known concentration into real samples at different levels of 25-OH-VD. Spiked recovery should be 100±15 %.

4 Linearity

Test 25-OH-VD samples with concentration in the range of 4.0～150 ng/mL, the linearity correlation coefficient r ≥ 0.990.

5 Repeatability

Coefficient of variation (CV) for the test results of low (14±6 ng/mL) and high (40±10 ng/mL) corporate reference sample is less than 10.0 %.

6 Lot-to-lot Variation

Coefficient of variation (CV) for the test results of low (14±6 ng/mL) and high (40±10 ng/mL) corporate reference sample with three batches of reagent is less than 15.0 %.

7 Accuracy of Calibrator Value Assignment

Use primary calibrators with assigned values from higher level measurement procedure, calibrate the immunoassay analyzer, and use the same lot of reagent to measure the value of each product calibrator. The measured value of Calibrator C1 and C2 has a relative deviation within ±10.0 % from its assigned value.

8 Homogeneity of Calibrators

8.1 Within-vial Homogeneity

Within-vial homogeneity of Calibrator C1 or C2 is represented with coefficient of variation, and CV≤ 8.0 %.

8.2 Between-vial Homogeneity

Between-vial homogeneity of calibrator C1 or C2 is represented with coefficient of variation, and CV≤ 8.0 %.

[Attention Notes]

1 It is for in-vitro diagnosis only.
2 It can only be used by professionals.
3 Never use expired reagent kit.
4 Never mix components from different kits, or from different reagent lots.
5 Do not put the reagent cartridges upside down.
6 Measurement of 25-OH-VD in a sample using different detection systems may yield different results, due to the difference in test methods, assay specificity, and factors of interference. The measured values from different systems should not be directly compared to avoid inappropriate clinical interpretation.
7 Strictly follow the protocol in the package insert, and operate according to the lab guidelines.
8 The test results can only be used for clinical reference. Clinical diagnosis of patients should take symptoms, body sign, disease history, other laboratory test results, and response to treatment for comprehensive assessment.
9 User should wear gloves and lab coat. Rinse with water if the skin is in contact with the reagent. Flush eyes with copious of water if eyes are in touch with the reagent, and see a doctor immediately.
10 Take all samples and reaction waste as potential biohazards. All waste must be handled following the local government regulation.
11 This product is a single-use cartridge. Reagent cartridge should be put back into refrigerator and stored at 2~8℃ if they have been placed at room temperature but not opened yet.

[Interpretation of Signs]

	Temperature limit.		Date of manufacturing
IVD	*In vitro* diagnostic medical device	REF	Catalogue number
LOT	Batch Code		Consult instructions for use or consult electronic instructions for use
	Use-by Date	EC REP	Authorized representative in the European Community/European Union
	This way up	CE	CE marking
	Manufacturer	UDI	Unique device identifier
	Biological risks		

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[Basic Information]

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