Human 25-Hydroxy Vitamin D CLIA Kit

Chemiluminescence Immunoassay for the quantitative determination of 25-Hydroxy Vitamin D2 and D3 in human serum

REF SKT-041 CE IVD 100, 150 +2 +8 °C

INTENDED USE

Human 25-Hydroxy Vitamin D CLIA Kit is a Chemiluminescence Immunoassay (CLIA) intended for the quantitative measurement of total 25-hydroxy vitamin D2 and D3 concentration in human serum.

For in-vitro diagnostics purposes only.

SUMMARY OF PHYSIOLOGY

The group of compounds referred to as Vitamin D, are actually fat soluble steroidal pre-hormones. The main forms which occur in the body are Vitamin D2 (ergocalciferol) and Vitamin D3 (cholecalciferol). The active form of these molecules is Dihydroxyvitamin D3 (1, 25(OH) 2 D3). Vitamin D3 is formed in the skin by photolysis of 7-dehydrocholesterol by ultraviolet radiation from the sunlight. It is transported in blood circulation bound to proteins to the liver where it is hydroxylated. Further hydroxylation occurs in the kidneys to produce the most active form. Vitamin D levels are highest in newborns and decrease exponentially throughout the life. Sufficient circulating levels of vitamin D are necessary for healthy bone maintenance and cell metabolism. Recent studies have shown that it may also lower incidents of certain cancers. Insufficient levels of Vitamin D can result in osteoporosis and bone fracture in the elderly, secondary hyperparathyroidism, abnormal cell metabolism and even increased incidents of cancer. Severe deficiency may lead to rickets in children and osteomalacia in adults. Disease associated with Vitamin D deficiency may also include impaired immunity, increased autoimmunity, myopathy, diabetes mellitus, and an increased risk of colon, breast, and prostate cancers. An abnormally high level (> 200 ng/mL) of Vitamin D leads to Vitamin D toxicity and may cause hypercalcaemia.

ASSAY PRINCIPLE

The Human 25-Hydroxy Vitamin D CLIA Kit is designed, developed, and produced for the quantitative measurement of human 25-OH VD level in serum samples. The assay utilizes competitive binding mechanisms for testing.

The calibrator, control or sample, and acridine ester labeled 25-OH VD monoclonal antibody are mixed and incubated to form antigen-antibody immune complex.
Streptavidin coated particles and biotinylated 25-OH VD were added under culture conditions. The unbound acridine ester labeled 25-OH VD monoclonal antibody of the sample binds to biotinylated 25-OH VD and binds to the microparticle through the biotin-streptavidin reaction.
After precipitation in a magnetic field, the supernatant is decanted, and then a wash cycle is performed to remove any remaining substances that are not bound to the magnetic microbeads. Subsequently, the washed compound is sent into the measurement chamber where trigger solution is automatically added to initiate a chemiluminescence reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is inversely proportional to the concentration of 25-OH VD present in the sample.
The test result is automatically calculated by the system according to the working curve.

REAGENTS: PREPARATION AND STORAGE

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date. It can be stored for 1 month at 2℃~8℃ after kit opening.

1. Streptavidin coated microparticles (04101)
Qty: 3.0mL (100/kit), 4.0mL (150/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

2. Biotinylated 25-OH VD antibody (04102)
Qty: 3.5mL (100/kit), 4.8mL (150/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

3. Acridinium ester 25-OH VD antibody (04103)
Qty: 6.0mL (100/kit),8.5mL (150/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

4. 25-OH VD Calibrators (04106-04107)
Qty: 2 x vials
Storage: 2 – 8°C
Preparation: Ready to Use
After the first use, it is recommended to storage at 2 - 8℃ and can be used within one month. Do not freeze.

5. 25-OH VD Controls (04108-04109)
Qty: 2 x vials
Storage: 2 – 8°C
Preparation: Ready to Use
After the first use, it is recommended to storage at 2 - 8℃ and can be used within one month. Do not freeze.

6. 25-OH VD Assay Diluent (04110)
Qty: 13.0mL (100/kit),
19.0mL (150/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

SAFETY PRECAUTIONS

The reagents must be used in a professional laboratory environment and are for in vitro diagnostic use. Source material which contains reagents of bovine serum albumin was derived in New Zealand. It was obtained only from healthy donor animals, maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing hydrogen peroxide. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Exercise Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

1. ECL100 Immunoassay Analyzer (ECL100) or ECL25 Immunoassay Analyzer (ECL25)
2. CL011 Cuvettes (for ECL100) or CL010 Cuvettes (for ECL25)
3. EDI™ Wash Reagent (P-594)
4. EDI™ Trigger Solutions A and B (P-595A, P-595B)

The instrument must operate using materials supplied by Epitope Biotechnology, Co., Ltd. or Epitope Diagnostics, Inc. When materials sourced from third-party suppliers are being used, Epitope Biotechnology, Co., Ltd. and Epitope Diagnostics, Inc. takes no responsibility for the validity of obtained results. Materials are available to purchase from Epitope Biotechnology, Co., Ltd. and Epitope Diagnostics, Inc. Please contact your distributor for more information.

SPECIMEN COLLECTION AND PREPARATION

1. Blood sample should be collected under sterile conditions.
2. For human serum samples only; other body fluids and samples may not yield accurate results.
3. Clinical samples should be tested within 2 hours after collection. If the measurement cannot be completed within 2 hours, please store under the following conditions:
   - storage at low temperature and away from light (2 ℃~8℃) for 2 days,
   - storage at -20℃ or below for 30 days
   - Freeze and thaw three times
4. Avoid heat-inactivated samples. Mixed, contaminated and hemolysis samples should be discarded.
5. Samples should be restored to room temperature before testing. Frozen samples should be completely melted and mixed well before use. Due to possible volatilization, samples, calibrators and controls on the ECL platform should be tested within 2 hours.
6. Some substances in the samples will interfere with the test results. The common interfering substances and maximum allowable concentrations are as follows:
   - bilirubin 50 mg/dL
   - triglycerides 2000 mg/dL
   - hemoglobin 1000 mg/dL
   - biotin 400 nmol/L
   - For patients receiving high-dose biotin therapy (5 mg/ day), samples must be collected 8 hours after taking the last dose of biotin
7. A single assay of this item requires 15 µL sample. This quantity does not include the amount of dead volume in the sample container, the capacity required for retesting, and other measurement items. For the definition of minimum required sample size, refer to the equipment manual.

CALIBRATION

An active calibration curve is required for all tests. Calibration is required for the first time use of a reagent lot and every 14 days thereafter or when either kit control is out of range. Refer to appropriate system manuals for configuring calibrators.

QUALITY CONTROL

The characteristics of patient samples are simulated through controls and are critical to validate the performance of CLIA assays due to the random access format. Use of controls is left to the discretion of the user based on good laboratory practices, requirements, and applicable laws. We suggest performing a control test once every day. Quality control results that do not fall within acceptable ranges may indicate invalid test results.

ASSAY PROCEDURE

1. Reagents from different kit lot numbers should not be combined or interchanged. Make sure that there are no air bubbles in any reagents, calibrator and control vials.

2. Reagent Preparation

2.1 Remove reagent cartridges from packaging and replace the solid caps with the provided soft caps for ECL100. For ECL25, carefully remove the aluminum foil seal on each container on the cartridges.

2.2 For the ECL100, take out the Magnetic Particle bottle make sure to roll between hands and gently but thoroughly mix until the magnetic particle solution is homogenous. The solution should be uniform with no clumps of magnetic particles visible; this step is vital for assay performance. For ECL25, mix the magnetic beads by moving back and forth the bottom part of the cartridge at upright position. Make sure to look inside the cartridge until the solution is uniform with no clumps of magnetic particles visible and no air bubbles. Recap the bottle. Open the top soft cap of all reagent bottles, leaving only the hollow soft rubber. The reagents are now ready to be loaded into the ECL100 or ECL 25 for calibration.

3. Assay Program

The following table illustrates the protocol used by the ECL100 or ECL25 for instrument operation.

Component	Quality Control Hole (µL)	Sample Hole (µL)
25-OH VD Controls (04108-04109)	15	-
Samples	-	15
25-OH VD Assay Diluent (04110)	120	120
Incubate at 37°C for 8 minutes		
25-OH VD Magnetic Particle Solution (04101)	20	20
Biotinylated 25-OH VD (04102)	25	25
Acridinium ester 25-OH VD antibody (04103)	50	50
Incubate at 37°C for 10 minutes		
Wash the reaction cuvette 3 times with wash reagent.		
Trigger Solution A (P-595)	100-200	100-200
Trigger Solution B (P-595)	100-200	100-200

NOTE FOR ASSAY PROCEDURE

All the reagents in this kit are ready-to-use. Make sure that there is no air bubble in any reagents, calibrator and control vials. Reagents from different kit lot numbers must not be combined or interchanged.

Please read the reagent instructions and equipment instructions carefully before using this kit and perform the test according to relevant requirements. When reagents are loaded, the equipment will automatically stir the magnetic particles to resuspend them. Allow the regent to mix for minimum 15 min before starting the assay program.

INTERPRETATION OF RESULTS

1. The default concentration unit of this assay is ng/mL or nmol/L.
   - *conversion factor:*
     - ng/mL x 2.5=nmol/L
     - nmol/L x 0.4= ng/mL

2. Due to methodological differences or antibody specificity, there may be deviations between the test results of reagents from different manufacturers. Therefore, direct comparisons should not be made to avoid false interpretation.

3. When the 25-OH VD concentration in the sample exceeds 100.00 ng/mL, a sample dilution could be performed before measurement (recommended 2-times dilution)

4. Any result below the minimum detection limit will be reported as <3.00 ng/mL; any result above the maximum detection limit will be reported as >100.00 ng/mL

EXPECTED VALUES

Normal reference value of this assay is ⩾30.00ng/mL

Note: each Laboratory is recommended to determine and establish its own reference range with local population.

LIMITATIONS OF THE PROCEDURE

1. This product is for use on the ECL100 Immunoassay Analyzer or ECL 25 Immunoassay Analyzer only. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, operation, system performance, instructions, calibration, precautions, hazards, maintenance, and troubleshooting.
2. Reagents from different kit lot numbers should not be combined or interchanged.
3. Test results obtained from the proposed kit should not be served as a sole basis for clinical diagnosis or patient management.
4. If the test sample result is higher than the upper limit of the calibration curve, it is recommended to re-measure after dilution according to a certain ratio. The measured value is recalculated according to the dilution ratio to ensure the accuracy of the result.

PERFORMANCE CHARACTERISTICS

- **Limit of Detection (LoD):**
  - 3.00 ng/mL
- **Linearity:**
  - 3.00 ng/mL~100.00 ng/mL
  - linearity correlation coefficient R ≥0.990
- **Accuracy:**
  - relative deviation within ±15%
- **Precision:**
  - Intra-assay repeatability: CV≤8%
  - Inter-assay reproducibility: CV≤15%
- **Repeatability:**
  - CV⩽8%

NOTES

1. Read the instructions carefully and gently but thoroughly mix the reagent before use. Remove any air bubbles before loading the reagents onto the equipment.
2. Keep the reagent in the storage conditions indicated in this IFU and on the reagent label. Do not freeze reagents.
3. Avoid contact with skin, eyes and mucous membrane. Upon contact, flush the area with clean water immediately.
4. All patient samples must be treated as potential infectious material.
5. Components in different kits cannot be mixed.
6. All waste must be disposed of in compliance with local regulations and laws.

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Biotechnology Co,Ltd and its distributors DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Biotechnology Co, Ltd..be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

REFERENCE

1. Zerwekh JE. Blood biomarkers of Vitamin D status. Am J ClinNutr, 2008, 87(suppl): 1087S-1091S.
2. Chapuy MC, Preziosi P, Maamer M, et al. Prevalence of Vitamin D insufficiency in an adult normal population. Osteoporosis Int, 1997, 7(5):439-443.
3. Bischoff-Ferrari HA, Giovannucci E, Willett WC, et al. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J ClinNutr, 2006, 84: 18-28.
4. TahaNM,Vieth R. The problem of an optimal target level for 25-Hydroxyvitamin D, the test for vitamin D nutritional status. Clinical Laboratory International, 2010, 34: 28-30.

TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or to place an order, please contact Epitope Diagnostics, Inc. in USA at +1 858-693-7877 or email to cs@epitopediagnostics.com

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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)

IVD In Vitro Diagnostic Device	CE European Conformity	LOT Lot Number
REF Catalog Number	 Read Instructions before Use	 Number of Tests
 Store at	 Use by	 Keep Away from Heat and Direct Sun light
 Manufacturer	EC REP Authorized Representative in Europe	 Distributor