Human 25-hydroxy vitamin D (25-OH-VD) Detection Reagent (ELISA)

Kit Name	Pack Size	Catalogue No
GB LISA vitamin D	96 TEST / KIT	

GENUINE BIOSYSTEM

INTENDED USE

This 25-OH-VD ELISA kit is a solid-phase ELISA designed for the quantitative determination of Human 25-OH-VD.

PRINCIPLE

The kit uses a competitive ELISA method in which the sample/calibrator and Assay Diluent diluted VD-biotin are added sequentially to a microtiter plate pre-coated with 25-OH-VD antibody, washed with WASHING solution, and then incubated with SA-HRP. The OD value was negatively correlated with the 25-OH-VD in the sample.

KIT COMPONENTS

Size	96 servings/box
Pre-coated microliter plates	96-well (12x8)
VD-biotin-concentrate	6mL×1bottle
Enzyme reagent	12mL×1bottle
Asaay Diluent	12mL×1bottle
Calibrators	6vials, A-F
Wash buffer concentrate	25 mL×1bottle
TMB substrate	12mL×1bottle
Stopping solution	6mL×1bottle

STORAGE AND STABILITY

The kit should be dry and stored at 2℃~8℃, avoid heavy pressure and pay attention to moisture, light and heat. It is valid for 12 months. After opening the microporous reaction plate, it should be sealed and dried in aluminum foil bag, and stored at 2℃~8℃, and the validity period is 14 days.

APPLICABLE INSTRUMENT

The enzyme label instrument with 450nm/620-630nm wavelength.

SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells. Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing.

METHOD

Specimen Pretreatment requirement: No need.

Bring all reagents, calibrators, specimen and controls to room temperature before use. Calibrators, patient specimen and controls should be assayed in duplicate.VD-biotin working solution preparation: Prepared by the customer according to the assay dosage. For example: 10μL VD-biotin-100X solution + 1mL Asaay Diluent, mix well and reserve.

Test procedure should be performed by a skilled individual or trained professional.

1.Remove the desired slats from the aluminum foil bag after equilibration at room temperature for 20 min, and return the remaining slats to 4°C in a self-sealing bag.

2.Set up calibrator and sample wells and add 15 μL of calibrator/sample to each well.

3.Add the prepared VD-biotin working solution, 100μL/well, to each well above; gently tap the side of the plate for 30s to mix, seal the reaction wells with sealing film, and incubate for 60min at 37℃ in a thermostat.

4.Discard the liquid, pat dry on blotting paper, fill each well with Wash Buffer, gently tap the side of the plate for 30 s, shake off the wash solution and pat dry on blotting paper, and repeat washing the plate 3 times.

5.Add 100 μL of SA-HRP to each well and incubate for 30 min in a 37°C incubator.

6.Repeat step 4 for washing.

7.Add 100 μL of TMB to each well and react at room temperature and avoid light for 10-15 min. Then add 50 μL of termination solution to each well and measure the OD value of each well at 450 nm.

TRACEABILITY

This kit is traceable to the Roche 25-hydroxyvitamin D (Vitamin D total) assay kit (electrochemiluminescence method).

REFERENCE INTERVALS

The following reference intervals were obtained for information purposes only by applying this kit to healthy adult serum samples. Each laboratory should establish its own reference range.

Reference range (2.5 th-97.5 th): 9.6-48ng/mL (n=72)

CALCULATIONS

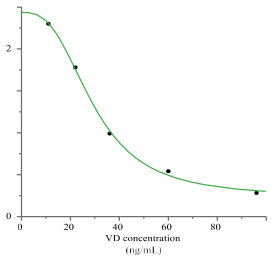
1. Calibrator data processing: Reading the each calibrator OD value and calculate the average optical density of each calibrator duplicate. Draw a calibrator curve on semi-log paper with the mean OD on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a

4-parameter or 5-parameter curve is recommended.

2. Unknow sample assay: Calculate the mean optical density of each unknown duplicate and read the values of the unknowns directly off the calibrator curve (or can utilize the fitted curve formula to calculate).

NO.	OD1	CON. ng/mL
Cal. A	2.40	0
Cal. B	2.10	11
Cal. C	1.78	22
Cal. D	0.991	36
Cal. E	0.542	60
Cal. F	0.283	96

EXAMPLE 1

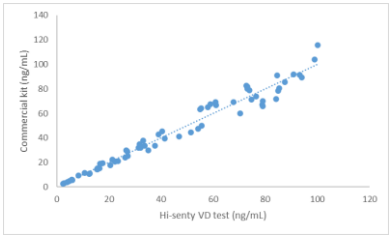


*The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a dose response curve prepared with each assay.

PERFORMANCE CHARACTERISTICS

METHODS COMPARISON

Representative data for methods comparison are provided for illustration only. Performance obtained in individual laboratories may vary. A comparison of 158 samples using GB VD ELISA Kit and a commercially available immunoassay kit gave the following statistical data using Pearson's correlation, following the CLSI EP9-A3 guideline: the R^2=0.9622。



Only slight amounts of bias between the GB VD ELISA method and the reference method. The least square regression equation and correlation coefficient indicates excellent method agreement.

SENSITIVITY

The sensitivity of the GB VD ELISA kit is 2.04ng/mL. The analytical sensitivity was ascertained by determining the variability of the 0ng/mL calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

RECOVERY

Spiked a samples were prepared by adding defined amounts of VD to two patient serum samples (1:1). The results (in ng/mL) are as below :

Sample	Test result	Expectancy result	Recovery
S1+ (ng/mL)	-	15	-
15	15.46	15	103.07%
30	21.64	22.5	96.18%
60	35.21	37.5	93.89%
90	53.94	52.5	102.74%
120	65.42	67.5	96.92%

Sample	Test result	Expectancy result	Recovery
S2+ (ng/mL)	-	32	-
15	25.64	23.5	109.11%
30	33	31	106.45%
60	48.1	46	104.57%
90	59.23	61	97.10%
120	75.09	76	98.80%

LIMITATIONS

1. Analysis range: Samples can be accurately measured within the analytical range of the Limit of Detection (LoD) and the highest calibrator value (approximately 3-100ng/mL).
2. The test results are for clinical reference only and cannot be used as the basis for diagnosis or exclusion of cases alone.
3. For diagnostic purposes, the test results of this kit should be combined with the patient's clinical presentation, history, and other clinical indicators.
4. Severe hemolysis, lipid blood, or clouded samples may result in incorrect results.
5. The reliability of this product for the determination of serum and plasma samples has not been fully established for the determination of other body fluid samples.

WARNINGS AND PRECAUTIONS

1.This product is only used for in vitro diagnosis, not for other purposes, the operation should be strictly in accordance with the instructions.
2.Do not use expired kits; Different batches of kits should not be mixed; Do not mix with reagents from other manufacturers.
3.All human derived materials used in the preparation of this reagent were tested negative for HBsAg, HBsAb, HAV-IgM, HCV, TP, and CMV (using an experimental method approved by the CFDA). Because there is no clear test methods can ensure no HBsAg detection of negative samples completely, HAV and other infectious virus, so all material derived from the human body, especially the clinical samples should be treat by infectious samples, and according to the ministry of health, Ministry of Science and Technology, the state food and drug administration issued by the relevant laboratory specifications and requirements.
4.During the operation, special attention should be paid to all samples and waste liquid and other materials such as test tubes, buffer solution and suction head, which may contain infectious substances. During the operation, work clothes and gloves should be worn. It is strictly prohibited to suck samples by mouth. In case of contact with any wound, consult a doctor in time. If any liquid spills during the experiment, it should be immediately disinfected with disinfectant. At the end of the experiment, all used samples and laboratory items should be disposed of as medical waste.
5.Some of the ingredients contained in this reagent, such as Proclin300, may cause allergic reactions in a very small number of people. Long-term contact with skin should be avoided and hands should be washed thoroughly after contact.
6.When conducting laboratory operations according to the product specifications, the manufacturer only warrants that the assay kit functions as an in vitro diagnostic function within the specified range described in the specifications. The manufacturer assumes no responsibility for any other warranty or implication, including commercial value, or other use within the scope of application. The responsibility of the manufacturer is limited to the replacement or return of the product.

IVD CE

Genuine Biosystem Private Limited
Plot 97 & 98, Kattabomman street, Parvathy nagar Extn, Old Perungalatur, Chennai-600063.TN.India
Ph: 044-48681845
Email: info@genuinebiosystem.com
Web: www.genuinebiosystem.com