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Sunlong Medical™ Vitamin D (VD) Quantitative ELISA Kit

Cat No.:EL0320Ge

Kit Performance

Physical Performance:

All liquid components should be clear and transparent, without precipitation or flocculent substances. The microplate aluminum foil bag should be vacuum-sealed without damage or leakage.

Standard Curve Linearity:

The correlation coefficient (r value) of the calibrator dose-response curve should be ≥ 0.99.

Precision:

Intra-assay coefficient of variation (CV%) is less than 10%;

Inter-assay coefficient of variation (CV%) is less than 15%.

Sensitivity: The minimum detectable dose is less than 3.125 ng/mL.

Recovery Rate: Recovery rate is between 85% and 115%.

Specificity:

This kit recognizes both natural and recombinant Vitamin D (VD). No cross-reactivity with structurally similar compounds.

Stability: Store at 2-8°C. The validity period is 6 months.

Detection Range: 25 ng/mL-400 ng/mL.

Intended Use:

For the determination of Vitamin D (VD) concentration in samples such as serum, plasma, and cell culture supernatant.

Assay Principle

This kit employs a competitive ELISA method. Samples/calibrators and Assay Diluent are added sequentially to the microplate wells pre-coated with Vitamin D (VD) antibody. After incubation and washing, SA-HRP is added and incubated. Following another wash, the substrate TMB is added for color development. The reaction is stopped with stop solution after color development at room

temperature, and the Optical Density (OD) is measured. The OD value is inversely proportional to the Vitamin D (VD) concentration in the sample.

This kit features high sensitivity, strong specificity, good reproducibility, simple and quick operation, and provides reliable detection performance for decreases or increases of VD in serum.

Kit Components and Storage

Store the unopened kit at 2-8°C. Do not use the kit beyond its expiration date.

Component	**Quantity (96T/48T)**	**Main Composition**
Calibrator	0.2 mL × 6 vials	6 concentrations (0 – 400 ng/mL)
Pre-coated Microplate	96/48 wells	Pre-coated solid-phase antibody
SA-HRP	12 mL	HRP-conjugated streptavidin
Assay Diluent	12 mL	Releases VD from binding proteins
TMB Substrate	12 mL	Ready-to-use TMB solution
Stop Solution	12 mL	Acidic solution
20× Wash Buffer	30 mL	PBS + 0.15 % Tween 20
Instruction Manual	1 copy	--
zip-lock bag	1 piece	--
adhesive seals	2 pieces	--

Calibrator concentrations are: 400, 200, 100, 50, 25, 0 ng/mL.

Note:

1. *Before use, please check that the labels and quantities of reagents in the kit match the table.*
2. *If kit components are to be reused, ensure they have not been contaminated after previous use.*

3. *If the microplate is not used completely in one session, remember to seal it and store at 2-8°C.*

Materials Required but Not Supplied

1. Standard microplate reader.
2. Automatic plate washer.
3. Plate shaker.
4. Adjustable pipettes and tips. For large sample numbers, a multi-channel pipette is recommended.

Kit Limitations

1. For research use only. Not for use in diagnostic procedures.
2. Use within the validity period indicated on the kit. Do not use expired products.
3. Do not mix with components from kits of other manufacturers.
4. Use the sample diluent provided with the kit.
5. If a sample value exceeds the highest calibrator concentration, dilute the sample appropriately and re-assay.
6. Heterophilic antibodies such as human anti-mouse antibodies present in test samples may interfere with detection. Eliminate this factor before testing.
7. Results obtained by other methods are not directly comparable to the results obtained with this kit.

Precautions

1. This kit is for in vitro research use only. Not for clinical diagnosis.
2. Wear a lab coat and latex gloves during experiments for protection. Especially when testing blood or other body fluid samples, follow national laboratory biosafety regulations.
3. Strictly adhere to specified incubation times and temperatures for accurate results. All reagents must reach room temperature (20-25°C) before use. Refrigerate reagents immediately after use.
4. Incorrect washing can lead to inaccurate results. Ensure wells are thoroughly drained before adding substrate. Do not let wells dry out during incubations.

5. Remove any residual liquid or fingerprints from the plate bottom, as they can affect OD values.
6. Substrate solution should be colorless or very light in color.
7. Avoid cross-contamination of reagents and specimens to prevent erroneous results.
8. Avoid direct exposure to strong light during storage and incubation.
9. Allow the sealed bag to reach room temperature before opening to prevent condensation on the cold strips.
10. Do not allow any reaction reagents to contact bleach solvents or strong fumes from bleach. Any bleach component can destroy the biological activity of the kit reagents.
11. The microplate reader used must be equipped with a filter capable of detecting 450±10nm wavelength, with an optical density range of 0-3.5. Preheat for 15 minutes before use is recommended.
12. Do not mix or substitute reagents in this kit with reagents from other batches or sources.
13. EP tubes and pipette tips used in the experiment are for single use only. Do not reuse them.
14. Do not use expired reagents.

Sample Preparation and Storage

The following are general guidelines for sample collection and storage. Do not use sodium azide as a preservative during sample collection and storage. If samples are not analyzed immediately, aliquot and store frozen, avoiding repeated freeze-thaw cycles.

Cell Culture Supernatant ------ Centrifuge to remove particulates. Analyze immediately or aliquot and store at -20°C.

Serum ------ Collect blood in a clean tube, allow to clot at room temperature for 30 minutes, then centrifuge at 2000×g for 20 minutes to collect serum. Analyze immediately or aliquot and store at -20°C.

Plasma ------ Use heparin, citrate, or EDTA as anticoagulant. Centrifuge at 2000×g for 20 minutes at 2-8°C within 30 minutes of blood collection. To eliminate platelet effects, further centrifugation at 10000×g for 10 minutes at 2-8°C is recommended. Analyze immediately or aliquot and store at -20°C.

Cell Lysate ------ For adherent cells, remove culture medium and wash once with PBS, saline, or serum-free medium. Add appropriate amount of lysis buffer and pipette several times to ensure full contact. Cells are usually lysed within 10 seconds. For suspension cells, centrifuge to collect cells, wash once with PBS, saline, or serum-free medium. Add appropriate amount of lysis buffer, pipette to disperse cells, and tap gently to ensure complete lysis. After complete lysis, centrifuge at

10000---14000×g for 3-5 minutes, and collect the supernatant. Analyze immediately or aliquot and store at -20°C.

Urine ------ Collect in a sterile tube and centrifuge at 2000×g for 20 minutes. Carefully collect the supernatant. If precipitation forms, centrifuge again.

Reagent Preparation

1. Before use, all components should be equilibrated to room temperature for at least 120 minutes.
2. Wash Concentrate (20x): Crystals may form in the concentrate after storage in the refrigerator. This is normal. Heat in a water bath to dissolve crystals completely. Dilute the Wash Concentrate with distilled water at a ratio of 1:20 (i.e., 1 part concentrate to 19 parts distilled water).

Assay Procedure

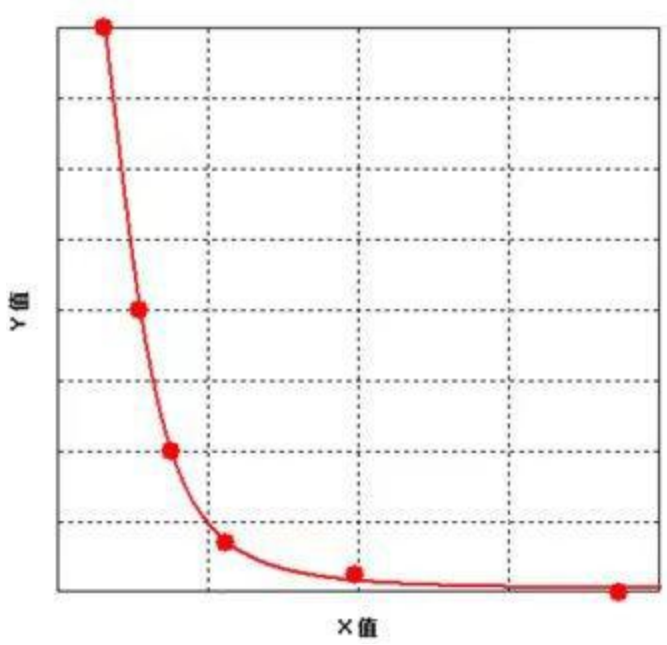
1. Equilibrate all reagents to room temperature for two hours. Prepare Wash Buffer according to the batch size: dilute the Wash Concentrate 1:20 with distilled water, mix well, and set aside.
2. Remove the pre-coated plate from the sealed bag. Set up one blank control well (add nothing). Set up 2 wells for each calibrator and add 15µl of the corresponding calibrator to each. For remaining test wells, directly add 15µl of the test serum or control.
3. Add 100µl of Assay Diluent to all wells except the blank control well. Mix gently, cover with plate sealer, and incubate at room temperature (20°C-25°C) for 60 minutes.
4. Manual Wash: Discard the liquid, fill each well with Wash Buffer, let stand for 10 seconds, discard, and repeat 3 times. Blot dry on absorbent paper.

   Automatic Wash: Wash 3 times using a plate washer, then blot dry.
5. Add 100µl of SA-HRP to each well (except the blank control well). Mix gently, cover with plate sealer, and incubate at room temperature (20°C-25°C) for 30 minutes.
6. Manual Wash: Discard the liquid, fill each well with Wash Buffer, let stand for 10 seconds, discard, and repeat 3 times. Blot dry on absorbent paper.

   Automatic Wash: Wash 3 times using a plate washer, then blot dry.
7. Add 100µl of TMB to each well. Mix by gentle shaking, then incubate at 37°C in the dark for 15 minutes for color development. Add 100µl of Stop Solution to each well.
8. Read the Optical Density (OD) at 450nm using a microplate reader. First, use the blank control well to zero the instrument, then measure the OD value of each well.

Calculation of Results

9. Using the calibrator concentrations as the X-axis and the corresponding OD values as the Y-axis, create a standard curve equation using computer software and a four-parameter logistic (4-PL) curve fit. Calculate the sample concentration from its OD value using this equation. [Use ELISA Calc software for calculation].
10. If the sample was diluted, multiply the concentration obtained by the dilution factor to obtain the final sample concentration.



(Schematic diagram, for reference only)

Troubleshooting

If the experimental results are unsatisfactory, please promptly photograph the color development results, save the experimental data, retain the used strips and unused reagents, and then contact our technical support for assistance. You can also refer to the following information:

Problem Description	Possible Cause	Corresponding Solution
Poor standard curve gradient	Inaccurate pipetting or dispensing	Check pipettes and tips

	Insufficient equilibration time	Ensure adequate equilibration time
	Incomplete washing	Ensure washing time, number of washes, and volume per well
Weak or no color	Insufficient incubation time	Ensure adequate incubation time
	Incorrect incubation temperature	Use recommended incubation temperature
	Insufficient reagent volume or	Check pipetting and dispensing procedures to ensure all reagents are added in correct order and sufficient volume
	missed addition	Incorrect dilution
	Inactivated enzyme conjugate or expired substrate	Mix enzyme conjugate and substrate to check for rapid color development
Low readings	Incorrect microplate reader setup	Check wavelength and filter settings on the microplate reader
		Preheat the microplate reader in advance
High CV%	Incorrect dispensing	Check dispensing procedures
High background	Detection antibody concentration too high	Use recommended dilution factor
	Incomplete washing of microplate	Ensure each wash step is complete; if using an automatic washer, check all ports for blockage; use the wash buffer supplied with the kit
	supplied with the kit	Prepare fresh wash buffer
Low sensitivity	Improper storage of ELISA kit	Store reagents as specified in the manual
	No stop solution added before reading	Add stop solution to each well before OD reading

Disclaimer

1. Due to current conditions and technological limitations, comprehensive identification and analysis of all raw materials is not possible. This product may carry certain quality and technical risks.
2. During development, some endogenous interfering factors in biological samples were removed/reduced, but not all potential interfering factors may have been eliminated.
3. Final experimental results are closely related to reagent validity, operator technique, and experimental conditions. Our company is only responsible for the kit itself, not for sample consumption resulting from its use. Users should consider potential sample requirements and reserve sufficient samples before use.
4. To achieve optimal results, use only the reagents provided within this kit. Do not mix with products from other manufacturers. Follow the instructions strictly.
5. Incorrect reagent preparation or microplate reader parameter settings may lead to abnormal results. Read the manual carefully and adjust instruments accordingly before the experiment.
6. Even the same operator may obtain different results in two independent experiments. To ensure result reproducibility, control every step of the operation.
7. Kits undergo strict quality control before shipment. However, due to factors such as transportation conditions and experimental equipment differences, user results may differ from factory data.
8. This kit has not been compared with similar kits from other manufacturers or products detecting the same analyte by different methods. Therefore, discrepancies in detection results cannot be ruled out.
9. This kit is for research use only. If used for clinical diagnosis or any other purpose, our company will not be responsible for any resulting issues and will not bear any legal liability.