Human 25-hydroxy vitamin D3 (25HVD3) ELISA Kit

Catalog Number. CSB-E08097h

For the quantitative determination of human 25-hydroxy vitamin D3 (25HVD3) concentrations in serum.

This package insert must be read in its entirety before using this product.

If You Have Problems

Technical Service Contact information

Phone: 86-27-87582341
Fax: 86-27-87196150
Email: tech@cusabio.com
Web: www.cusabio.com

In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).

PRINCIPLE OF THE ASSAY

This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with an antibody. Standards or samples are added to the appropriate microtiter plate wells with Horseradish Peroxidase (HRP) conjugated 25HVD3 Hapten. The competitive inhibition reaction is launched between with HRP-conjugated 25HVD3 Hapten and 25HVD3 in samples. A substrate solution is added to the wells and the color develops in opposite to the amount of 25HVD3 in the sample. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

20 μg/L-100 μg/L.

SENSITIVITY

The minimum detectable dose of human 25HVD3 is typically less than 5 μg/L.
The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest human 25HVD3 concentration that could be differentiated from zero.

SPECIFICITY

This assay has high sensitivity and excellent specificity for detection of human 25HVD3. No significant cross-reactivity or interference between human 25HVD3 and analogues was observed.

Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between human 25HVD3 and all the analogues, therefore, cross reaction may still exist.

PRECISION

Intra-assay Precision (Precision within an assay): CV%<10%
Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<15%
Three samples of known concentration were tested in twenty assays to assess.

LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in Sample Diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Reagents	Quantity
Assay plate	1(96 wells)
Standard	5 x 0.5 ml
High Value Control	1 x 0.5 ml
Middle Value Control	1 x 0.5 ml
Sample Diluent	1 x 50 ml
HRP-conjugate	1 x 7 ml
Wash Buffer (20 x concentrate)	1 x 30 ml
Substrate A	1 x 7 ml
Substrate B	1 x 7 ml
Stop Solution	1 x 7 ml
Adhesive Strip (For 96 wells)	4
Instruction manual	1

STANDARD CONCENTRATION

Tube	S4	S3	S2	S1	S0
Concentration (μg/L)	100	80	40	20	0

STORAGE

<table>
<tr><td>Unopened kit</td><td colspan="2">Store at 2 - 8°C. Do not use the kit beyond the expiration date.</td></tr>
<tr><td rowspan="8">Opened kit</td><td>Coated assay plate</td><td>May be stored for up to 1 month at 2 - 8°C. Try to keep it in a sealed aluminum foil bag, and avoid the damp.</td></tr>
<tr><td>Standard</td><td rowspan="3">May be stored for up to one week at 2 - 8°C. If don’t make recent use, better keep it store at -20°C.</td></tr>
<tr><td>High Value Control</td></tr>
<tr><td>Middle Value Control</td></tr>
<tr><td>HRP-conjugate</td><td rowspan="5">May be stored for up to 1 month at 2 - 8°C.</td></tr>
<tr><td>Sample Diluent</td></tr>
<tr><td>Wash Buffer</td></tr>
<tr><td>Substrate A</td></tr>
<tr><td>Substrate B</td></tr>
<tr><td>Stop Solution</td></tr>
</table>

***Provided this is within the expiration date of the kit.**

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630 nm.
- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100ml and 500ml graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

SAMPLE COLLECTION AND STORAGE

- **Serum** Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000 ×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

- Serum samples require a 40-fold dilution into Sample Diluent. The suggested 40-fold dilution can be achieved by adding 6μl sample to 234μl of Sample Diluent.

Note:

1. CUSABIO is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤1month) or -80°C (≤2month) to avoid loss of bioactivity and contamination.
3. Grossly hemolyzed samples are not suitable for use in this assay.
4. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
5. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

6. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
7. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
8. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

REAGENT PREPARATION

Note:

- **Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.**
- Bring all reagents to room temperature (18-25°C) before use for 30min.
- Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

Wash Buffer(1x)- If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 ml of Wash Buffer Concentrate (20 x) into deionized or distilled water to prepare 600 ml of Wash Buffer (1 x).

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples, standards and controls be assayed in duplicate.

1. Prepare all reagents and samples as directed in the previous sections.
2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Set a **Blank** well without any solution.
4. Add 50µl of **standard, control** and diluted **sample** per well. Add 50µl **HRP-conjugate** to each well immediately (not to Blank well). Mix well with the pipette or shake the plate gently for 60 seconds. A plate layout is provided to record standards and samples assayed.
5. Cover with the adhesive strip provided. Incubate for 60 minutes at 37°C.
6. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with **Wash Buffer** (300µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 10 seconds, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7. Add 50µl of **Substrate A** and 50µl of **Substrate B** to each well. Incubate for 15 minutes at 37°C. **Protect from light.**
8. Add 50µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 630 nm. Subtract readings at 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

***Samples may require dilution. Please refer to Sample Preparation section.**

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), TMB Substrate should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. Substrates are easily contaminated. Substrates should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

CALCULATION OF RESULTS

Using the professional soft "Curve Expert" to make a standard curve is recommended, which can be downloaded from our web.

Average the duplicate readings for each standard and sample and subtract the average optical density of Blank.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the 25HVD3 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

The concentration read from the standard curve is the actual concentration of the samples.