Vitamin D3 (human) ELISA Kit

8/15

(Catalog # K4806-100, 100 assays; Store at 4°C)

I. Introduction:

Vitamin D3 (Cholecalciferol) is found in human epidermis and dermis. BioVision's human Vit. D3 ELISA Kit is based on the standard principle of a sandwich enzyme-linked immunosorbent assay. Human Vit. D3 antibody is coated on a 96-well plate. Standards and test samples are added to the wells and Vit. D3 present in a sample is bound by the immobilized antibody. An HRP-conjugate reagent is added subsequently. After washing away the unbound antibody/HRP conjugates, HRP substrate TMB is used to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue product that changes into yellow after adding acidic stop solution. The density of yellow color is proportional to the human Vit. D3 captured onto the plate. This ELISA kit shows no species cross-reactivity. Detection Range: 20 – 800 µg/L.

II. Application:

Quantitative detection of Vit. D3, establishing normal range etc.

III. Specificity:

Human Vit. D3

IV. Sample Type:

- Serum & plasma (EDTA/Citrate), Urine
- Cell culture medium, Tissue homogenates and cell lysates

V. Kit Contents:

Components	K4806-100	Part No.
96 wells coated with anti-human Vit. D3 antibody, 1 Microplate	12 strips x 8 wells	K4806-100-1
Human Vit. D3 standard (0.9 ng/µl)	0.5 ml	K4806-100-2
HRP-conjugate reagent	6 ml	K4806-100-3
Standard Diluent	1.5 ml	K4806-100-4
Sample diluent	6 ml	K4806-100-5
Chromogen Solution A	6 ml	K4806-100-6
Chromogen Solution B	6 ml	K4806-100-7
Stop Solution	6 ml	K4806-100-8
Wash Solution (30x stock)	20 ml	K4806-100-9

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended for large number of samples.

VII. Storage Conditions and Reagent Preparation:

Store kit at 4°C for 12 months, protected from light. The antibody-coated microplate must be stored in a dry place at 4°C in the sealed bag provided. Equilibrate all components to Room Temperature before starting the assay.

- **Preparation of 1x wash solution**: Dilute the 30x concentrated stock 1:30 with distilled water and mix thoroughly. Prepare 0.35 ml of working wash solution for a single wash for each well. The 20 ml stock will make 600 ml of working wash solution.
  Note: If there is precipitation in the wash solution, gently warm to 37°C to dissolve.
- **Human Vit. D3 Standard Preparation**: 450 ng of Vit. D3 Standard is included in each kit. Use 10 wells on the Microplate (5 concentrations in duplicate) to prepare Standards with 600 ng/ml, 400 ng/ml, 200 ng/ml, 100 ng/ml, 50 ng/ml of Vit. D3.

Well 1: Add 100 µl of the Human Vit. D3 Standard and 50 µl Standard Diluent, mix.

Well 2: Add 100 µl from Well 1 and 50 µl Standard Diluent, mix; Discard 50 µl.

Well 3: Add 50 µl from Well 2 and 50 µl Standard Diluent, mix.

Well 4: Add 50 µl from Well 3 and 50 µl Standard Diluent, mix.

Well 5: Add 50 µl from Well 4 and 50 µl Standard Diluent, mix; Discard 50 µl.

Samples can be spiked into the standards if desired.

VIII. Sample Preparation and Storage:

a. Centrifuge cell culture media, cerebrospinal fluid or urine samples for 20 mins at 2000-3000 rpm to remove particulates. For serum samples, clot in a serum separator tube (20-30 mins) at room temperature. Centrifuge at approximately 2000-3000 rpm for 20 min. Collect plasma using EDTA or Citrate, mix for 10 mins. Centrifuge for 20 min. at 2000-3000 rpm. For cells and tissues, homogenize in PBS (pH 7.2 – 7.4), spin at top-speed in a table-top centrifuge and collect supernatant. Tissue samples frozen in Liquid-Nitrogen can be ground and used to prepare homogenates.

Notes:

b. For all samples, aliquot and freeze samples at -80°C. Avoid repeated freeze-thaw cycles.

c. Sodium Azide is incompatible with this assay.

d. Sample dilution guidelines: The user needs to estimate the concentration of Vit. D3 in the sample and select a proper dilution factor so that the diluted Vit. D3 concentration falls near the middle of the linear regime of the standard curve. Dilute the sample using the provided sample diluent. The sample must be well mixed with the diluent buffer. Several trials may be necessary to optimize sample dilution. Suggested dilution: 1:5. Add 10 µl sample and 40 µl Sample diluent, mix gently without touching the walls of the plate.

IX. Assay Protocol:

The 96-well plate should not be dry at any time, as drying will inactivate the active components on the plate.

1. There is 50 µl per well of the human Vit. D3 standard solutions in the pre-coated 96-well plate. Add 50 µl sample diluent buffer into the sample control well (Zero well). Add 50 µl each of the 1:5 or properly diluted samples of human cell culture medium, cell or tissue lysate, urine, serum or plasma (EDTA/Citrate) to each empty well. See "Sample Dilution Guideline" for details.

   Notes:

   a. We recommend that each human Vit. D3 standard solution and each sample be measured in duplicate.

   b. We recommend doing a pilot experiment using standards and a small number of samples to validate the assay procedure with the specific samples and optimize the appropriate sample dilution.
2. Seal the plate with the cover and incubate at 37°C for 30 min. Remove the cover, discard plate contents, and blot the plate onto paper towels or other absorbent material. Do not let the wells completely dry at any time.
3. Add 0.35 ml of prepared working wash solution into each well. Wash plate 5 times, each time leave washing buffer in the wells for 1-2 mins. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. Always drain excess wash solution without drying the wells.
4. Add 50 µl of HRP-conjugate reagent into each well (except the Zero well) and incubate plate at 37°C in dark for 30 min.

   Note: These guidelines are for reference only; the optimal incubation time should be determined by end user. The shades of blue can be seen in the wells with the most concentrated human Vit. D3 standard solutions; the other wells might not show any obvious color.
5. Discard the HRP solution and wash the wells as described in Step 3.
6. Add 50 µl of Chromogen solution A and 50 µl of Chromogen solution B into each well. Incubate plate at 37°C in dark for 15 mins. or as required.
7. Add 50 µl of stop solution into each well. The color changes from blue to yellow immediately.
8. Read absorbance at 450 nm in a microplate reader within 15 min. after adding the stop solution.
9. Calculation: Relative $O.D._{450}$ = $O.D._{450}$ of each well – $O.D._{450}$ of Zero well. The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human Vit. D3 concentration of the samples can be interpolated from the standard curve. **Note:** if the samples were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the original concentration in the sample.

Typical Data Obtained from Human Vitamin D3
(Reaction incubated at 37°C for 30 min.)

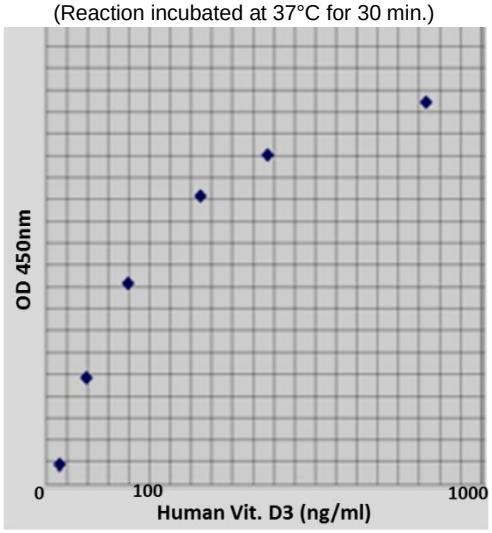

Figure: Standard Curve: This standard curve is for demonstration only. A standard curve must be run with each assay.

X. RELATED PRODUCTS:

Calcitriol, Activator of Vitamin D Receptor (1880-50, -250) CRSP1 Antibody (3735-100)

FOR RESEARCH USE ONLY! Not to be used on humans.