# Mouse 25-Dihydroxy vitamin D3 (HVD3) ELISA Kit

## Catalogue Number: OM441889

## (96Tests)

## Store all reagents at 2-8°C

## Collect sample: serum or blood plasma

## Assay range ： 1.0ng/ml -120ng/ml

**FOR LABORATORY RESEARCH USE ONLY.**
**NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!**
**PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!**

## INTENDED USE

This Member 2 (HVD3 ELISA kit is intended Laboratory for Research use only and is not for use in diagnostic or therapeutic procedures.The Stop Solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of Member 2 (HVD3 in the sample, this Member 2 (HVD3 ELISA Kit includes a set of calibration standards. The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density versus Member 2 (HVD3 concentration. The concentration of Member 2 (HVD3 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

## PRINCIPLE OF THE ASSAY

The kit assay Member 2 (HVD3 level in the sample， use Purified antibody to coat microtiter plate wells, make solid-phase antibody, Samples which

including standards of known concentrations and unknowns are pipetted into coated microtiter wells, after Incubating ,add Biotinylated anti-IgG,and Combined Streptavidin-HRP, become antibody – antigen - enzyme- antibody complex, after washing Completely, Add TMB substrate solution, TMB Chromogen Solution Becomes blue color At HRP enzyme-catalyzed, And at the effect of acid the color finally become yellow, The intensity of this coloured product is directly proportional to the concentration of Member 2 (HVD3 present in the samples. measure the optical densit (OD) at 450 nm with microtiter plate reader, calculate Member 2 (HVD3 concentration by standard curve.

## REAGENTS PROVIDED

All reagents provided are stored at 2-8° C. Refer to the expiration date on the label.

1. MICROTITER PLATE 96 wells
2. Biotinylated anti-IgG 6.0 mL 1 tube
3. STANDARD(120ng/ml) 0.5ml 1 tube
4. STREPTAVIDIN-HRP 6.0 mL 1 tube
5. STANDARD DILUENT 1.5ml 1 tube
6. Chromogen Solution A 6.0 mL 1 vial
7. Chromogen Solution B 6.0 mL 1 vial
8. STOP SOLUTION 6.0 mL 1 vial
9. WASH SOLUTION x30 20 mL 1 vial
10. Instruction 1

## SAMPLE COLLECTION AND STORAGE

Serum- Use a serum separator tube(SST) and allow samples to clot for 30minutes before centrifugation for 15minutes at approximately 1000 x g.Remove serum and assay immediately or aliquot and store samples at -20 °C or -80°C.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant.Centrifuge samples for 15 minutes at 1000 x g at 2-8°C within 30minutes of collection.Store samples at -20°C or -80°C.Avoid repeated freeze-thaw cycles.

Cell culture fluid and other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C.Avoid repeated freeze-thaw cycles.

NOTE: Serum, plasma, and cell culture fluid samples to be used within 7 days may be stored at 2-8°C, otherwise samples must stored at -20°C(≤2months) or -80°C(≤6months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles .When performing the assay slowly bring samples to room temperature.

DO NOT USE HEAT-TREATED SPECIMENS.

## MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 ml to 1 ml volumes. .
3. Adjustable 10ml -100ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
6. Absorbent paper.
7. 37°C incubator.
8. Distilled or deionized water.
9. Data analysis and graphing software..
10. Tubes to prepare standard or sample dilutions.

## PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from Canine blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate viruses.
10. Solid Waste: Autoclave 60 min. at 121°C.
11. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
12. Substrate Solution is easily contaminated. If bluish prior to use, do not use.
13. Chromogen Solution B contains 20% acetone, keep this reagent away from sources of heat or flame.
14. Remove all kit reagents from refrigerator and allow them to reach room

temperature ( 20-25°C).

## REAGENT PREPARATION

Standard -The Kit provides a stock standard（120ng/ml）. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.Pipette 150µL of Standard Dilution into each tube.
(total 5 tubes) Use the 150µL of stock solution to produce a 2-fold dilution series (including 60 **ng/ml,30ng/ml，15ng/ml,7.5ng/ml ,3.75ng/ml**). Mix each tube thoroughly before the next transfer.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, add 20mL Wash Buffer Concentrate into deionized or distilled water to prepare 600mL of Wash Buffer.

## ASSAY PROCEDURE

Prepare all Standards before starting assay procedure (see Preparation Reagents). It is recommended that all Standards and Samples be added in duplicate to the Microtiter Plate.

1. Determine the number of microwell strips required to test the desired number of samples, Each sample, standard and blank should be assayed in duplicate.
2. add sample: Set blank wells separately (blank comparison wells don’t add sample and ELISA reagent, other each step operation is same). Add 50 µL of Standards or Samples to the appropriate well of the antibody pre-coated Microtiter Plate, and Gently mix. Incubate for 45 min at 37℃
3. Configurate liquid: 30 times of wash solution diluted 30 times with distilled water and reserve.
4. washing: remove Liquid, dry by swing, add washing buffer to every well, still for 30 second then remove, repeat 4 times.
5. add Biotinylated anti-IgG: Add diluted Biotinylated anti-IgG 50ul to the sample wells((the standard has been pre-integrated biotin antibody, so do not add biotin antibodies);Incubate for 30 min at 37℃
6. washing: Operation with 4.
7. add streptavidin-HRP: Add streptavidin-HRP 50ul to all wells, Gently mix Incubate for 30 min at 37℃
8. washing：Operation with 4.
9. color: Add Chromogen Solution A 50ul and Chromogen Solution B 50ul to each well,Incubate for 15 min at 37℃
10. Stop the reaction: Add Stop Solution50 µl to each well, Stop the reaction(the blue color change to yellow color Immediately).
11. assay: take blank well as zero , measure the optical densit (OD) at 450 nm

after Adding Stop Solution and within 15min.

## TYPICAL DATA

## CALCULATION OF RESULTS

Average the duplicate readings for each standard,control, and sample and subtract the average zero standard optical density. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

