EPITOPE DIAGNOSTICS, INC.

# EDI™ Human VDBP (GC Globulin) ELISA Kit

***Enzyme Linked ImmunoSorbent Assay (ELISA) for the Quantitative Measurement of Vitamin D Binding Protein Level in Serum or Plasma***

REF KT-896 CE IVD 96

## INTENDED USE

This ELISA (enzyme-linked immunosorbent assay) kit is produced for the quantitative determination of free and not actin complex bound Vitamin D Binding Protein (VDBP, also known as GC Globulin) in human serum and plasma. This kit is for in vitro diagnostic use only.

## SUMMARY OF PHYSIOLOGY

Vitamin D-binding protein (DBP) is a 58 kDa circulating glycoprotein secreted by the liver. It binds about 85% to 90% of circulating 25-OH-$D_{2/3}$ and 1,25-$(OH)_2$-$D_3$, regulates the bioavailability of active vitamin D, and transports them to target organs. There is only less than one percent 25-OH-$D_{2/3}$ is in the free form in the circulation. The full length DBP contains 476-amino acids, including a 16-amino acid signal sequence. Biologically, DBP may involve directly and indirectly in regulating bone metabolism.

Circulating DBP also binds to actin at 1:1 molecule ratio, while this protein complex is removed by kidney. In patient with trauma, sepsis or multiple organ failure, DBP concentration decreases.

Urine DBP is reported to be a biomarker of major renal event in patient undergoing coronary angiography.

## ASSAY PRINCIPLE

The quantitative VDBP ELISA is a solid phase *competitive* immunoassay designed to detect VDBP. Microwells are coated with anti-VDBP antibody. Assay calibrators, control and diluted unknown serum or plasma specimens are added to the microwells along with GC Globulin HRP conjugated antibody. After an incubation period, the immunocomplex of solid phase bound *"VDBP Antibody-VDBP" is formed, which inhibits the formation of "VDBP Antibody – HRP Conjugated VDBP"*. Unbound HRP Conjugated VDBP are removed with a washing step. During a second incubation with TMB substrate, a blue color is developed. The enzyme-substrate reaction is stopped by the addition of sulfuric acid. The absorbance of assay calibrators, controls and unknown specimens are measured by an ELISA plate reader with wavelength set at 450 nm. The color intensity is inversely proportional to the amount of VDBP present in the calibrators controls and specimens.

## REAGENTS: PREPARATION AND STORAGE

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

### 1. VDBP Antibody Coated Microplate (30974)

Microplate coated with anti-VDBP antibody.

Qty: 1 x 96 well microplate
Storage: 2 – 8°C
Preparation: Ready to Use.

### 2. HRP Conjugated VDBP (30975)

HRP labeled GC Globulin in a stabilized protein matrix.

Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: This reagent must be diluted with tracer antibody diluent (30052) prior to use.

### 3. VDBP Concentrated Sample Diluent (30976)

Concentrated buffer matrix with protein stabilizers and preservative.

Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: 6X Concentrate. The contents must be diluted with 150 mL of deionized water.

### 4. ELISA Wash Concentrate (10010)

Surfactant in a phosphate buffered saline with non-azide preservative.

Qty: 1 x 30 mL
Storage: 2 – 25°C
Preparation: 30X Concentrate. The contents must be diluted with 870 mL deionized water and mixed well before use. The diluted wash solution may be stored at room temperature and is stable until the expiration date on the kit box.

### 5. ELISA HRP Substrate (10020)

Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.

Qty: 1 x 15 mL
Storage: 2 – 8°C
Preparation: Ready to Use.

### 6. ELISA Stop Solution (10030)

0.5 M sulfuric acid.

Qty: 1 x 12 mL
Storage: 2 – 25°C
Preparation: Ready to Use

### 7. VDBP Calibrators Levels 1 to 6 (30981 - 30986)

Liquid GC Globulin in a bovine serum albumin-based matrix with a non-azide preservative. Refer to each vial for exact concentration.

Qty: 6 x Vials
Storage: 2 – 8°C
Preparation: Ready to Use.

### 8. VDBP Controls (30987, 30988)

Liquid GC Globulin in a bovine serum albumin-based matrix with a non-azide preservative. Refer to each vial for exact concentration.

Qty: 2 x Vials
Storage: 2 – 8°C
Preparation: Ready to Use.

## SAFETY PRECAUTIONS

The reagents are for in vitro diagnostic use only. Source material which contains reagents of bovine serum albumin was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

## MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 25 µL, 50 µL, 100 µL, and 1000 µL etc.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm or 13 x 100 glass tubes.
5. Disposable plastic 100 mL and 1000 mL bottle with caps.
6. Aluminum foil.
7. Deionized or distilled water.
8. Plastic microtiter well cover or polyethylene film.
9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

## SPECIMEN COLLECTION & STORAGE

Both serum and EDTA-plasma can be used with this test kit. Only 10 µL total of human EDTA-plasma or serum is required for the VDBP measurement in duplicate. No special preparation of individual is necessary prior to specimen collection. Collect whole blood with Vacutainer and separate the serum or plasma from cells according to manufacturer's instruction. Sample can be kept at – 15ºC. Avoid more than three freeze-thaw cycles of specimen.

## ASSAY PROCEDURE

**1. Reagent Preparation**

1. Prior to use allow all reagents to come to room temperature (20-25 ºC). Reagents from different kit lot numbers should not be combined or interchanged.
2. ELISA Wash Concentrate (10010) must be diluted to working solution prior to use. Please see REAGENTS section for details.
3. VDBP Concentrated Sample Diluent (30976) must be diluted to working solution prior to use. Please see REAGENTS section for more details.

**2. Sample Preparation**

1. Samples must be diluted 1:200 using diluted VDBP Concentrated Sample Diluent (30976)
2. Add 2 mL of diluted VDBP Concentrated Sample Diluent (30976) to a labeled disposable tube.
3. Add 10 µL of the sample to the tube and mix well.

**3. Assay Procedure**

1. Place a sufficient number of VDBP Antibody Coated Microplate (30974) in a holder to run calibrators (30981 - 30986), controls (30987, 30988), and samples in duplicate.
2. Test Configuration

| Row | Strip 1 | Strip 2 | Strip 3 |
|---|---|---|---|
| A | Calibrator Level 1 | Calibrator Level 5 | SAMPLE 1 |
| B | Calibrator Level 1 | Calibrator Level 5 | SAMPLE 1 |
| C | Calibrator Level 2 | Calibrator Level 6 | SAMPLE 2 |
| D | Calibrator Level 2 | Calibrator Level 6 | SAMPLE 2 |
| E | Calibrator Level 3 | Control 1 | SAMPLE 3 |
| F | Calibrator Level 3 | Control 1 | SAMPLE 3 |
| G | Calibrator Level 4 | Control 2 | SAMPLE 4 |
| H | Calibrator Level 4 | Control 2 | SAMPLE 4 |

1. Add **15 µL** of calibrators (30064 - 30068), controls (30987, 30988), and diluted samples into the designated microwells.
2. Add **100 µL** of HRP Conjugated VDBP (30975) into each microwell. Mix by gently tapping the plate.
3. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **60 minutes.**
4. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
5. Add **100 µL** of ELISA HRP Substrate (10020) into each of the wells. Mix by gently tapping the plate.
6. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **20 minutes.**
7. Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution (10030) into each of the wells. Mix by gently tapping the plate.
8. Read the absorbance at **450 nm** within **10 minutes** with a microplate reader. It is recommended to use a 4-parameter curve fit to calculate the results.

## PROCEDURAL NOTES

1. It is recommended that all calibrators, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original amber bottles.
3. Store any unused VDBP Antibody Coated Microplate (30974) strips in the foil Ziploc bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
7. All reagents should be mixed gently and thoroughly prior use. Avoid foaming.
8. It is important to seal the plate properly to avoid evaporation.

## INTERPRETION OF RESULTS

1. Calculate the average absorbance for each pair of duplicate test results.
2. The calibration curve is generated by the corrected absorbance of all calibrator levels on the ordinate against the calibrator concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results. We recommend using **4-Parameter** curve fit.
3. The concentrations for the controls and patient samples are read directly from the calibration curve.

## LIMITATIONS OF THE PROCEDURE

1. This assay requires serum or plasma sample for testing.
2. Serum or plasma samples from different species may show different matrix background.

3. For sample values greater than 12.5 µg/mL, it is recommended to re-assay samples with dilution (i.e. 1:10 or 1:100. The best dilution matrix is vitamin D free human serum.
4. Cell culture or tissue culture samples should be validated with total binding and other performance specifications before being used.
5. Severely hemolyzed samples, icteric or lipaemic sample should not be used

## QUALITY CONTROL

To assure the validity of the results, each assay should include adequate controls with known positive levels of VDBP. We recommend that all assays include the laboratory's own control samples in addition to those provided with this kit.

## EXPECTED VALUES

18 sera and plasma from normal human samples were measured using this ELISA. The test resulted with an average level of 375 µg/mL (1.875 µg/mL measured directly from the calibrator curve), ranges from 275.6 – 505.2 µg/mL, Std Dev of 60.4 µg/mL).
We strongly recommend for each clinical laboratory to establish its own normal range by measuring EDTA plasma and/or serum with this kit.

*Reference Range:*
200 – 550 µg/mL (L.Thomas, 1982)

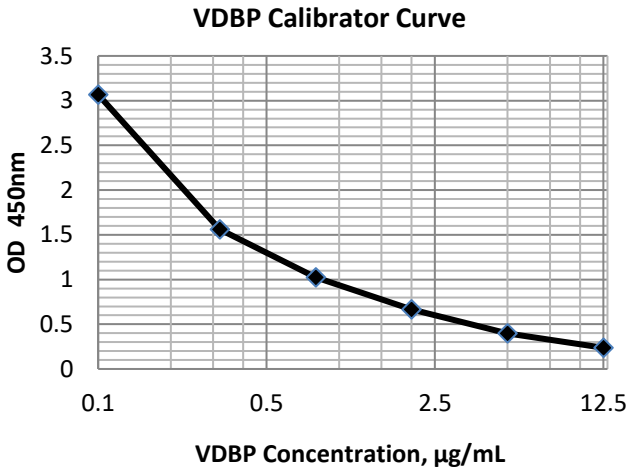
*Additional Reference Ranges:*
Pregnant woman
Samples of pregnant women were measured to have a 30-80% higher reference range than the control groups.
Liver diseases
According to Haughton et al., the reference range of patients with liver diseases is 35% lower than the one of healthy controls.

## EXAMPLE DATA

A typical absorbance data and the resulting calibration curve from this 25-OH Vitamin D EIA is represented .

*Note: This curve should not be used in lieu of calibration curve run with each assay.*

| Well I.D | OD 450 nm Absorbance | | VDBP concentration (µg/mL) | B/B0 |
|---|---|---|---|---|
| | Readings | Average | | |
| Calibrator Level 1: 0 µg/mL | 3.116 | 3.141 | | 100% |
| | 3.165 | | | |
| Calibrator Level 2: 100 µg/mL | 1.540 | 1.533 | | 49% |
| | 1.527 | | | |
| Calibrator Level 3: 260 µg/mL | 1.154 | 1.095 | | 35% |
| | 1.036 | | | |
| Calibrator Level 4 : 640 µg/mL | 0.568 | 0.598 | | 19% |
| | 0.629 | | | |
| Calibrator Level 5 : 1600 µg/mL | 0.373 | 0.373 | | 12% |
| | 0.373 | | | |
| Calibrator Level 6: 4020 µg/mL | 0.208 | 0.202 | | 6% |
| | 0.196 | | | |
| Control 1 | 1.290 | 1.061 | 250.2 | |
| | 1.211 | | | |
| Control 2 | 6.614 | 6.422 | 1294.6 | |
| | 6.331 | | | |




## PERFORMANCE CHARACTERISTICS

### Sensitivity

The analytical sensitivity (LLOD) of this VDBP ELISA as determined by the 2 times standard deviation below the mean of $B_0$ ($B_0$ - 2SD) on 16 duplicate determinations of calibrator 1 ($B_0$) and calibrator 2 with a concentration of 0.205µg/mL . The LLOD of this test is approximately 0.0637 µg/mL.

### Reproducibility and Precision

The intra-assay precision was validated by measuring three diluted 1:200 samples with 16 replicate determinations. The results are as follows:

| Sample | VDBP Value (µg/mL) | CV (%) |
|---|---|---|
| 1 | 264.2 | 8.8 |
| 2 | 1008.6 | 8.1 |
| 3 | 1945.4 | 9.1 |

The inter-assay precision was validated by measuring two control levels in duplicate in 13 individual assays.

| Sample | Mean VDBP Value (µg/mL) | CV (%) |
|---|---|---|
| 1 | 184.8 | 8.7 |
| 2 | 1164.9 | 5.9 |

### Linearity

Three samples were collected, diluted 1:200 and spiked with various amounts of VDBP, diluted with standard zero matrix and tested. The results of VDBP percent recovery value in VDBP concentration µg/mL are as follows:

| Dilution 1:200 | VDBP Value (µg/mL) | Recovery (%) |
|---|---|---|
| Sample A | 2030 | - |
| 1:2 | 1006 | 100 |
| 1:4 | 495.4 | 99 |
| 1:8 | 226.4 | 90 |
| Sample B | 750.4 | - |
| 1:2 | 402.8 | 107 |

| | | |
|---|---|---|
| 1:4 | 208.6 | 111 |
| 1:8 | 90.6 | 97 |
| Sample C | 220.8 | - |
| 1:2 | 103.6 | 94 |
| 1:4 | 55.4 | 100 |
| 1:8 | 21.8 | 79 |

### Spike Recovery

Three diluted 1:200 samples and three assay calibrators (0.8, 2.0 and 5 µg/mL) were combined at equal volumes and tested. The results are as follows:

| Dilution 1:200 | VDBP Value (ng/mL) | Recovery % |
|---|---|---|
| Sample A | 247.8 | - |
| 0.8 µg/mL | 203.8 | 100 |
| 2.0 µg/mL | 361 | 111 |
| 5.0 µg/mL | 634.2 | 102 |
| Sample B | 297.2 | - |
| 0.8 µg/mL | 234.6 | 103 |
| 2.0 µg/mL | 403.2 | 115 |
| 5.0 µg/mL | 662.2 | 102 |
| Sample C | 222 | - |
| 0.8 µg/mL | 189.8 | 99 |
| 2.0 µg/mL | 337.6 | 109 |
| 5.0 µg/mL | 587.4 | 96 |

### Interference

One positive sample is added with 5% volume of interference materials to reach a final concentration shown in the table below. All samples are tested in an assay in duplicate.

| | Mean OD 450 nm | | |
|---|---|---|---|
| | **Additive** | **Volume Added (mg/mL)** | **Sample** |
| 1 | Test Control | - | 405.5 |
| 2 | Bilirubin - L | 0.4 | 433.0 |
| 3 | Bilirubin - H | 10 | 394.7 |
| 4 | Test Control | - | 476.2 |
| 5 | Hb - L | 0.26 | 461.0 |
| 6 | Hb - H | 6.5 | 478.2 |
| 7 | Lipid – L | 8 | 368.4 |
| 8 | Lipid -H | 200 | 456.3 |

## WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

## REFERENCES


1. Haddad JG[1]. Plasma vitamin D-binding protein (Gc-globulin): multiple tasks. J Steroid Biochem Mol Biol. 1995 Jun;53(1-6):579-82.
2. Carpenter TO[1], Zhang JH, Parra E, Ellis BK, Simpson C, Lee WM, Balko J, Fu L, Wong BY, Cole DE. Vitamin D binding protein is a key determinant of 25-hydroxyvitamin D levels in infants and toddlers. J Bone Miner Res. 2013 Jan;28(1):213-21. doi: 10.1002/jbmr.1735.


## TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.

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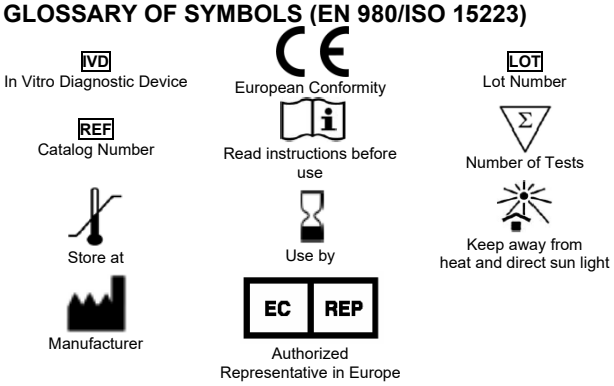
## GLOSSARY OF SYMBOLS (EN 980/ISO 15223)




## SHORT ASSAY PROCEDURE

1. Add **5 µL** of the calibrators, controls, and diluted samples into the designated microwells.
2. Add **100 µL** of the tracer antibody to each well.
3. Mix, cover, and incubate at **room temperature (20-25 ºC)** for **60 minutes**.
4. Wash each well five times.
5. Add **100 µL** of substrate to each well.
6. Cover and incubate at **room temperature (20-25 ºC)** for **20 minutes**.
7. Add **100 µL** of the stop solution to each well.
8. Read the absorbance at **450 nm.**