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# **Human VDBP (GC Globulin) ELISA Assay Kit**

Catalog Number:

**DBP31-K01 (1 x 96 wells)**

*For Research Use Only. Not for use in diagnostic procedures.*

*v. 1.0*

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## INTENDED USE

Eagle Biosciences Human VDBP (GC Globulin) ELISA Assay Kit is intended for use in 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. The Human VDBP (GC Globulin) ELISA Assay Kit is for research use only and not intended for diagnostic procedures.

## 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<sub>2/3</sub> and 1,25-(OH)<sub>2</sub>-D<sub>3</sub>, regulates the bioavailability of active vitamin D, and transports them to target organs. There is only less than one percent 25-OH-D<sub>2/3</sub> 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.

## PRINCIPLE OF THE ASSAY

The Eagle Biosciences Human VDBP (GC Globulin) quantitative VDBP ELISA Assay Kit 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

The Eagle Biosciences Human VDBP (GC Globulin) quantitative VDBP ELISA 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.

**Prior to use, allow all reagents to equalize to room temperature.** Reagents from different kit lot numbers should not be combined or interchanged.

### 1. VDBP Antibody Coated Microplate

One microplate with twelve by eight well-breakable strips (96 wells total) coated with anti-VDBP antibody. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2-8°C and is stable until the expiration date on the kit box.

### 2. HRP Conjugated VDBP

One bottle containing **12 mL** ready to use HRP labeled GC Globulin in a stabilized protein matrix. This reagent should be stored in 2-8°C and is stable until the expiration date on the kit box.



#### 4. VDBP Concentrated Sample Diluent

One bottle containing **30 mL** of **6-fold** concentrated buffer matrix with protein stabilizers and preservative. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box. Before use the concentrated buffer must be diluted by mixing with **150 mL** of demineralized water or distilled water to make the **1x VDBP Sample Dilution Buffer**.

#### 5. ELISA Wash Concentrate

One bottle containing **30 mL** of 30-fold concentrate. Before use, the contents must be diluted with **870 mL** of demineralized water and mixed well. Upon dilution, this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide, non-mercury preservative. The diluted wash solution may be stored at room temperature and is stable until the expiration date on the kit box.

#### 6. ELISA HRP Substrate

One bottle containing **15 mL** of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2-8°C and is stable until the expiration date on the kit box.

#### 7. ELISA Stop Solution

One bottle containing **15 mL** of stop solution. This reagent may be stored at 2-8°C or room temperature and is stable until the expiration date on the kit box.

#### 8. VDBP Calibrators 1 to 6

Six vials contain **0.5 mL** each of liquid GC Globulin in a bovine serum albumin-based matrix with a non-azide preservative. **Refer to the vial for exact concentration.** This reagent should be stored in 2-8°C and is stable until the expiration date on the kit box.

#### 9. VDBP Controls

Two vials each contain **0.5 mL** of liquid GC Globulin in a bovine serum albumin-based matrix with a non-azide preservative **Refer to vials for concentration range for each control.** This reagent should be stored in 2-8°C and is stable until the expiration date on the kit box.

### SAFETY PRECAUTIONS

The reagents from the Human VDBP (GC Globulin) ELISA Assay Kit must be used in professional laboratory. Source material for reagents containing bovine serum 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 are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. 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

- Precision single channel pipettes capable of delivering 25 µl, 100 µl, 500 µL, etc.
- Disposable pipette tips suitable for above volume dispensing.
- DT-water or DI-water
- Aluminum foil.
- Plastic microtiter well cover or polyethylene film.



- ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
- Spectrophotometric microplate reader capable of reading absorbance at 450 nm.
- Oven or water bath with adjustable temperature to 37°C.

## SPECIMEN COLLECTION

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 – 15°C. Avoid more than three freeze-thaw cycles of specimen.

## ASSAY PROCEDURES

### 1. Reagent Preparation

- (1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
- (2) ELISA Wash Concentrate and sample diluent must be diluted to working solution prior to use. Please see REAGENTS section for details.
- (3) Each unknown sample needs to be diluted **1:200** using **1x VDBP Sample Dilution Buffer** as a sample diluent.
  - Mark 13 x 73 mm tubes accordingly
  - Add 2 mL of VDBP Sample Dilution Buffer into each plastic tube.
  - Add 10 µL unknown specimen to the designated tube and mix well.

### 2. Assay Procedure

Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3	STRIP 4
A	CAL 1	CAL 5	SAMPLE 1	SAMPLE 5
B	CAL 1	CAL 5	SAMPLE 1	SAMPLE 5
C	CAL 2	CAL 6	SAMPLE 2	SAMPLE 6
D	CAL 2	CAL 6	SAMPLE 2	SAMPLE 6
E	CAL 3	C 1	SAMPLE 3	
F	CAL 3	C 1	SAMPLE 3	
G	CAL 4	C 2	SAMPLE 4	
H	CAL 4	C 2	SAMPLE 4	

- (1) Add **15 µl** of calibrators, controls and diluted 1:200 test samples into the designated microwells.
- (2) Add **100 µl HRP Conjugated VDBP** to each well.
- (3) Mix contents of wells gently for 5 - 10 seconds. Seal the plate securely, cover with aluminum foil, and incubate at **room temperature for 60 minutes**.
- (4) Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
- (5) Add **100 µl TMB** reagent to each of the wells.
- (6) Cover plate with aluminum foil, and incubate at **room temperature for 20 minutes**.



- (7) Immediately add **100 µL of ELISA Stop Solution** into each of the wells. Mix gently.
- (8) Read the absorbance at 450 nm. It is recommended to use 4-parameter curve fit to calculate the sample results.

### PROCEDURAL NOTES

1. It is recommended that all calibrators 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. It is recommended to add external controls to each assay.
2. Keep light sensitive reagents in the original amber bottles.
3. Store any unused antibody coated strips in the foil zipper 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 to use. Avoid foaming.
8. It is important to seal the plate properly to avoid evaporation.

### INTERPRETATION OF RESULTS

It is recommended to use a 4-parameter calibration curve fitting.

1. Calculate the average absorbance for each pair of duplicate test results.
2. The calibration curve is generated by the corrected absorbance of all calibration levels on the ordinate against the calibrator concentration. Appropriate computer assisted data reduction programs should be used for the calculation of results.

**VDBP concentrations for the control and samples must be multiplied by the dilution factor, which is 200.**

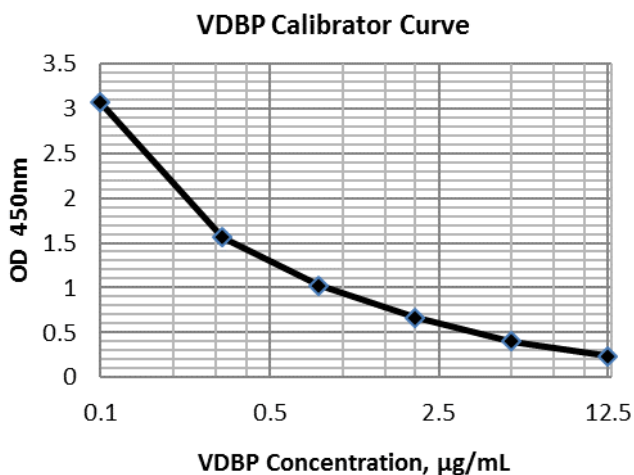
### EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting calibration curve from this 25-OH Vitamin D EIA is represented. **This curve should not be used in lieu of calibration curve generated with each assay.**

Well I.D	OD 450 nm Absorbance		B/B0
	Readings	Average	
Cal - 1: 0 µg/mL	3.116	3.141	100%
	3.165		
Cal - 2: 0.5 µg/mL	1.540	1.533	49%
	1.527		
Cal - 3: 1.3 µg/mL	1.154	1.095	35%
	1.036		
Cal - 4 : 3.2 µg/mL	0.568	0.598	19%
	0.629		
Cal - 5 : 8.0 µg/mL	0.373	0.373	12%
	0.373		
Cal - 6: 20.1 µg/mL	0.208	0.202	6%
	0.196		



	OD	Mean OD	Result	VDBP concentration (µg/mL)
<b>Control 1</b>	1.290	1.061	1.251	250.2
	1.211			
<b>Control 2</b>	6.614	0.422	6.473	1294.6
	6.331			



### EXPECTED VALUE

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.



## LIMITATION 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.

## 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.

### Precision

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

Sample 1:200	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:



<b>DILUTION 1:200</b>	<b>VDBP VALUE (<math>\mu\text{g/mL}</math>)</b>	<b>RECOVERY %</b>
<b>Sample A</b>	2030	-
1:2	1006	100
1:4	495.4	99
1:8	226.4	90
<b>Sample B</b>	750.4	-
1:2	402.8	107
1:4	208.6	111
1:8	90.6	97
<b>Sample C</b>	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  $\mu\text{g/mL}$ ) were combined at equal volumes and tested. The results are as follows:

<b>DILUTION 1:200</b>	<b>VDBP VALUE (<math>\text{ng/mL}</math>)</b>	<b>RECOVERY %</b>
<b>Sample A</b>	247.8	-
0.8 $\mu\text{g/mL}$	203.8	100
2.0 $\mu\text{g/mL}$	361	111
5.0 $\mu\text{g/mL}$	634.2	102
<b>Sample B</b>	297.2	-
0.8 $\mu\text{g/mL}$	234.6	103
2.0 $\mu\text{g/mL}$	403.2	115
5.0 $\mu\text{g/mL}$	662.2	102
<b>Sample C</b>	222	-
0.8 $\mu\text{g/mL}$	189.8	99
2.0 $\mu\text{g/mL}$	337.6	109
5.0 $\mu\text{g/mL}$	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	Amt 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

### REFERENCES

1. Haddad JG1. Plasma vitamin D-binding protein (Gc-globulin): multiple tasks. J Steroid Biochem Mol Biol. 1995 Jun;53(1-6):579-82.
2. Carpenter TO1, 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.

### Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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