Human DBP / Vitamin D binding protein ELISA Kit

Human DBP / Vitamin D binding protein ELISA Kit is an Enzyme Immunoassay kit for the quantification of Human DBP / Vitamin D binding protein in serum and plasma.

Catalog number: ARG82260

Package: 96 wells

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INTRODUCTION

Vitamin D-binding protein (DBP), also/originally known as gc-globulin (group-specific component), is a protein that in humans is encoded by the GC gene.

Vitamin D-binding protein belongs to the albumin gene family, together with human serum albumin and alpha-fetoprotein. It is a multifunctional protein found in plasma, ascitic fluid, cerebrospinal fluid and on the surface of many cell types.

It is able to bind the various forms of vitamin D including ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3), the 25-hydroxylated forms (calcifediol), and the active hormonal product, 1,25-dihydroxyvitamin D (calcitriol). The major proportion of vitamin D in blood is bound to this protein. It transports vitamin D metabolites between skin, liver and kidney, and then on to the various target tissues.

As Gc protein-derived macrophage activating factor it is a Macrophage Activating Factor (MAF) that has been tested for use as a cancer treatment that would activate macrophages against cancer cells. [Provide by Wikipedia: Vitamin D binding protein]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A capture antibody specific for Human Vitamin D Binding Protein (DBP) has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any DBP present is bound by the immobilized antibody. After washing away any unbound substances, added antibody-conjugate specific for DBP to each well and incubate. After washing away any unbound substances, the TMB substrate is added to the wells and color develops in proportion to the amount of DBP bound in the initial step. The color development is stopped by the addition of stop solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of DBP in the samples is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 x 12 strip	4°C
Standards (lyophilized)	1 vial	4°C
100X Antibody Conjugate	150 µL	4°C (protect from light)
5X Diluent Buffer	50 mL	4°C
20X Wash Buffer	50 mL	4°C
TMB Substrate	12 mL (ready to use)	4°C (protect from light)
Stop Solution	12 mL (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm
- Centrifuge and centrifuge tube
- Deionized or distilled water
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Return any unused microplate strips to the plate pouch with desiccant.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (16-25°C).
- The reagent preparation method might be different from lot to lot, so please check the lot and follow the instructions given in this manual.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 20X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 1,000 x g for 15 minutes at 4°C. Recommended starting dilution is 1/40,000. To prepare a 1/40,000 dilution of a sample, transfer 5 µL of sample to 495 µL of 1X Diluent Buffer. This gives you a 1/100 dilution. Next, dilute the 1/100 by transferring 2 µL into 798 µL of 1X Diluent Buffer. This gives you a 1/40,000 dilution. Mix thoroughly each stage.

Plasma: Collect blood with EDTA or citrate and centrifuge at 1,000 x g for 15 minutes at 4°C. Recommended starting dilution is 1/40,000. To prepare a 1/40,000 dilution of a sample, transfer 5 µL of sample to 495 µL of 1X Diluent Buffer. This gives you a 1/100 dilution. Next, dilute the 1/100 by transferring 2 µL into 798 µL of 1X Diluent Buffer. This gives you a 1/40,000 dilution. Mix thoroughly each stage.

Urine: Collect mid-stream using sterile or clean urine collector. Centrifuge to remove debris.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.
3. Store all samples on ice after preparation and use immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 20X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 25 mL of 10X Wash Buffer into 475 mL of distilled water to a final volume of 500 mL)
- 1X Diluent Buffer: Dilute 5X Diluent Buffer into distilled water to yield 1X Diluent Buffer. (E.g., add 10 mL of 5X Wash Buffer into 40 mL of distilled water to a final volume of 50 mL)
- 1X Antibody Conjugate: For each strip to be used for testing, add 10 µL of 100X Antibody Conjugate to 990 µL of 1X Diluent Buffer. Dilute immediately before use and protect from light.
- Standards: Prepare according to the lot specific Certificate of Analysis.

Standard tube	Final DBP conc. (ng/mL)
S1	200
S2	100
S3	50
S4	25
S5	12.5
S6	6.25
S7	3.13
S0	0

Note: S0 (Diluent Buffer only) to determine background.

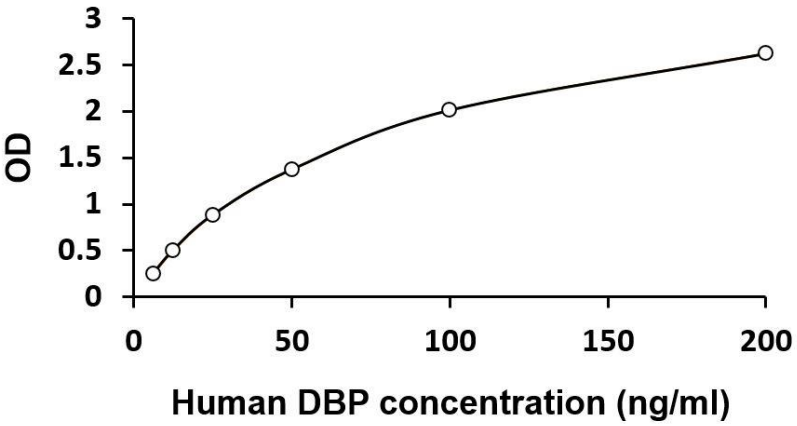
ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 16-25°C) before use. Standards and samples should be assayed in duplicates.

1. Add 100 µL of samples and each diluted Standards to the Antibody Coated Microplate. Cover the plate and incubate for 30 minutes at room temperature.
2. Aspirate each well and wash, repeating the process 3 times for a total 4 washes. Wash by filling each well with 1X Wash Buffer (300 µL) using a squirt bottle, manifold dispenser. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
3. Add 100 µL of the 1X Antibody Conjugate per well. Then cover the plate and incubate for 30 minutes at room temperature in the dark.
4. Aspirate and wash plate as in step 2.
5. Add 100 µL of TMB Substrate to each well. Incubate for 10 minutes at room temperature in the dark.
6. Immediately Add 100 µL of Stop Solution to each well. The color of the solution should change from blue to yellow.
7. Read the absorbance at O.D. 450 nm within 30 minutes.

EXAMPLE OF TYPICAL STANDARD VALUES

The following figures demonstrate typical results with the Human DBP / Vitamin D binding protein ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



CALCULATION OF RESULTS

1. Subtract zero point (S0) from all standards and unknowns to determine corrected absorbance.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter

Logistics is the preferred method. Other data reduction functions may give slightly different results.

4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Sensitivity

3 ng/mL