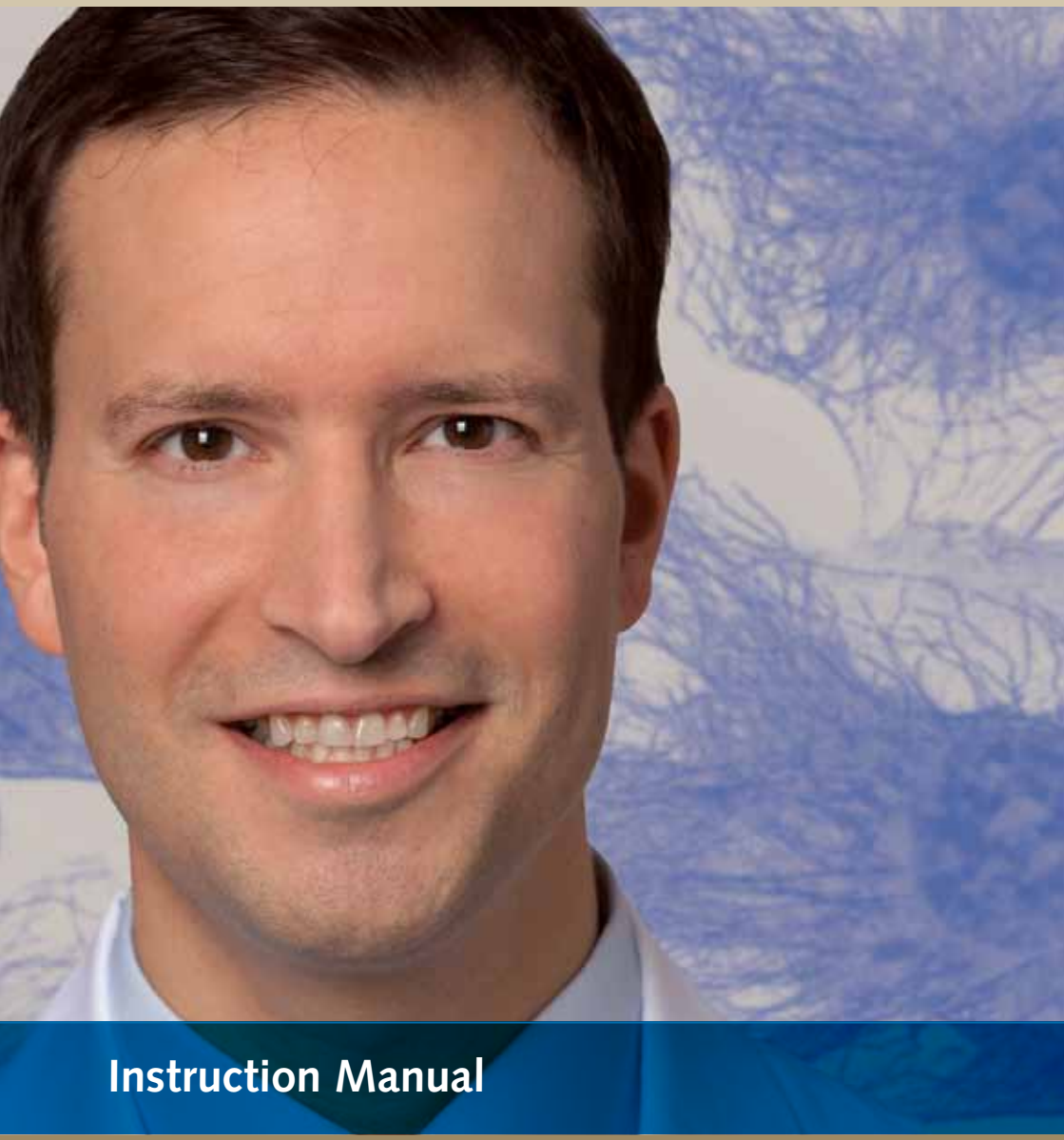
Human VDBP ELISA Kit



Instruction Manual

PromoKine

Contents

Intended Use

The PromoKine Assay is intended for the quantitative determination of free, not Actin-bound, Vitamin-D binding protein in serum, plasma and urine. For *in vitro* diagnostic use only.

Clinical Relevance

Vitamin D-binding protein (VDB; MW = 51 243 Da, positions 17–474, 458 amino acids, P02774 VTDB_HUMAN) or Gc-globulin is a multifunctional serum protein synthesized in the liver. It is structurally related to albumin and is similar in size. The majority of vitamin D in the blood circulates bound to the VDB. Gc-globulin has been reported to be a macrophage-stimulating factor, to possess chemotaxin activity and to have endotoxin-binding capacity. Furthermore, Gc-globulin has one actin-binding site and forms 1:1 complexes with monomeric actin. Actin is an intracellular protein that can polymerise and form filaments. The mobility and the shape of cells depends on this ability.

Upon massive cell death and tissue destruction, the release of actin may lead to a significant decrease in the components of the extracellular actin scavenger system. Decreased VDB levels were found in serum samples from several patients groups at risk of developing multi-organ failure, e.g. trauma, spesis etc.

Indications

- Risk factor for traumatic injury
- Nephrotic syndrom

Principle of the Test

This Enzyme Immuno Assay is a sandwich assay for VDB determination in serum, plasma and urine samples. The wells of the micro titer plate are coated with polyclonal anti-VDB antibodies. In a first incubation step, the VDB in the samples is bound to the coated polyclonal rabbit antibodies (in excess). To remove all unbound substances, a washing step is carried out. In a second incubation step, a polyclonal Peroxidase-labeled rabbit-anti-VDB antibody is added. After another washing step, to remove all unbound substances, the

solid phase is incubated with the substrate, Tetramethylbenzidine. An acidic stopping solution is then added. The color converts to yellow. The intensity of the yellow color is directly proportional to the VDB concentration in the sample. A dose response curve of the absorbance (at 450 nm) unit vs. concentration is generated.

Material Supplied

Content	Kit Components	Quantity
PLATE	One holder with precoated strips	12 x 8 wells
WASHBUF	ELISA wash concentrate 10x	2 x 100 ml
CONJ	POD antibody (rabbit-anti-VDB, Peroxidase-labeled), pre-diluted	1 x 200 µl
STD	Calibrators, lyophilized (60; 20; 6,6; 2,2; 0 ng/ml)	4 x 5 vials
STDBUF	Standard Dilution Buffer	1 x 20 ml
CTRL 1	Control 1, lyophilized	4 vials
CTRL 2	Control 2, lyophilized	4 vials
SAMPLEBUF	Dilution buffer, ready to use	2 x 100 ml
SUB	TMB substrate (Tetramethylbenzidine), ready to use	1 x 15 ml
STOP	ELISA stop solution, ready to use	1 x 15 ml

Material Required but not Supplied

- Ultra-pure water*
- Calibrated precision pipettors and 10-1000 µl tips
- Multi-channel pipets or repeater pipets
- Horizontal microtiter plate shaker
- Vortex-Mixer
- Laboratory balance
- Centrifuge, 3000*g*
- Standard laboratory glass or plastic vials, cups, etc. (one time products)
- Microtiter plate reader 450 nm
  (reference wave length 620 or 690 nm)
- Foil to cover the microtiter plate

*We recommend the use of Ultra Pure Water (Water Type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25°C (≥18.2MΩ cm).

Preparation and Storage of Reagents

- To run the assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each assay.** The kit can be used up to 4 times within the expiry date stated on the label.

- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.

- Preparation of the wash buffer: The **WASHBUF** (wash buffer concentrate) should be diluted with ultra-pure water **1:10** before use (100 ml WASHBUF + 900 ml ultra-pure), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at 37°C in a water bath before dilution. The WASHBUF (wash buffer concentrate) is stable at 2-8°C until the expiry date stated on the label. Diluted wash buffer (1:10 diluted WASHBUF) can be stored in a closed flask at 2-8°C for one month.

- The lyophilized **STD** (standards) and **CTRL** (controls) are stable at 2-8°C until the expiry date stated on the label or the CoA. Before use, they must be reconstituted with **500 µl STDBUF**. Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to insure complete reconstitution. **Reconstituted standards and controls are not stable and cannot be stored.**

- Preparation of the conjugate: Before use, the conjugate concentrate (**CONJ**) must be diluted **1:100** in wash buffer (100 µl CONJ + 10 ml wash buffer). The undiluted CONJ (conjugate) is stable at 2-8 °C until the expiry date stated on the label. Diluted conjugate (1:101 diluted CONJ) is not stable and can not be stored.

- All other test reagents are ready to use. Test reagents are stable at 2-8°C until the expiry date stated on the label of kit.

Precautions

- For *in vitro* diagnostic use only.

- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.

- Kit reagents contain sodium azide or thimerosal as bactericides. Sodium azide and thimerosal are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.

- Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.

- Reagents should not be used beyond the expiration date stated on kit label.

Specimen Collection and Preparation

Serum and plasma samples

Dilute all plasma and serum samples 1:40,000 with **SAMPLEBUF** (sample dilution buffer). For example:

20 µl Sample + 980 µl SAMPLEBUF, mix well = 1:50 (Dilution I)
20 µl Dilution I + 980 µl SAMPLEBUF, mix well = 1:50 (Dilution II)
20 µl Dilution II + 300 µl SAMPLEBUF, mix well = 1:16 (Dilution III)
This results in a final dilution of 1:40,000.

For analysis, pipette **100 µl of Dilution III** per well.

Other sample collectives should be diluted according to the expected VDBP concentration.

Samples with VDB levels greater than the highest calibrator should be further diluted and re-assayed.

Urine

Urine samples have to be diluted 1:10 with **SAMPLEBUF** (sample dilution buffer). For example:

100 µl Sample + 900 µl SAMPLEBUF, mix well = **1:10**

Samples with VDB levels greater than the highest calibrator should be further diluted and re-assayed.

Assay Procedure

Procedural notes

- Do not mix different lot numbers of any kit component within the same assay.
- Quality control guidelines should be observed.
- Incubation time, incubation temperature and pipetting volumes of the different components are defined by the producer. Any variations of the test procedure, that are not coordinated with the producer, may influence the test results. PromoCell can therefore not be held responsible for any damage.
- The assay should always be performed according the enclosed manual.

Test procedure

Bring all reagents and samples to room temperature (15-30°C) and mix well.

Wash the precoated PLATE (microtiter plate) **5 times with 250 µl diluted wash buffer** per well. After the final washing step, the inverted PLATE should be firmly tapped on absorbent paper to remove excess solution.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. Please contact your supplier for further information.

Carry out the tests in duplicate.

1. Add **100 µl STD** (Standard), **CTRL** (Control) and **pre-diluted sample** into the respective wells.
2. Incubate for **1 hour shaking** on a horizontal mixer at room temperature (15-30°C).
3. Discard the content of the plate. Wash **5 times** with **250 µl** diluted wash buffer per well.
4. Add **100 µl diluted CONJ** (peroxidase-labeled antibody).
5. Incubate for **1 hour shaking** on a horizontal mixer at room temperature (15-30°C).
6. Discard the content of the plate. Wash **5 times** with **250 µl** diluted wash buffer per well.
7. Add **100 µl SUB** (TMB substrate).
8. Incubate for 10-20 minutes at room temperature (15-30°C)*.
9. Add **100 µl STOP** (stop solution) and mix shortly.
10. Determine absorption immediately with an ELISA reader at 450 nm against 620 nm (or 690 nm) as reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the measurement range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as a reference.

*The intensity of the color change is temperature-sensitive. We recommend observing the color change and stopping the reaction upon good differentiation.

Results

The following algorithms can be used alternatively to calculate the results. We recommend to use the "4-Parameter-algorithm".

1. 4-parameter-algorithm

   It is recommended to use a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.001).

2. Point-to-point-calculation

   We recommend a linear ordinate for optical density and a linear abscissa for concentration.

3. Spline-algorithm

   We recommend a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.001).

The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the program used, the duplicate values should be evaluated manually.

Serum/plasma samples

For the calculation of the VDBP concentration in serum or plasma samples, the obtained VDBP levels have to be multiplied with the dilution factor of **40,000**.

Urine samples

For the calculation of the VDBP concentration in urine samples, the obtained VDBP levels have to be multiplied with the dilution factor of **10**.

In case another dilution factor has been used, multiply the obtained result with this dilution factor.

Limitations

Samples with VDB levels greater than the highest calibrator must be further diluted in dilution buffer and re-assayed. Consider the greater dilution when calculating your results.
Samples with concentrations lower than the measurement range cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:
highest concentration of the standard curve × sample dilution factor to be used
The lower limit of the measurement range can be calculated as:
Analytical sensitivity × sample dilution factor to be used

Quality Control

PromoCell recommends commercial control samples for internal quality control.
Control samples should be analyzed with each. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Expected values
The levels listed should be used as a guideline only. It is recommended that each laboratory establishes an own expected range for its patient population.

Plasma or serum	200-550 mg/L (Thomas, 1982)
	1 mg/L = 1.94 µmol/L
	1 µg/L = 1.94 nmol/L

(MW = 51,335 Da, Tietz Textbook of Clinical Chemistry, 3rd edition, p.1418)

You can find further reference ranges in the following publications in the references section: Bouillon (1977), Haughton (1992), Jorgensen (2004), Heijboer (2012).

We recommend each laboratory to establish its own reference range.

Urine samples

Doorenbos et al. analysed the urinary loss of VDBP in 24 h urine samples. In the healthy control group, they reported a value of 64 µg/24 h (23–111 µg/24 h).

Mirkovic et al. normalised the measured VDBP levels for albuminuria. Norm albuminuria was reported as 0.44 mg/mg albumin (0.22–0.77 mg/mg albumin).

We recommend each laboratory to establish its own reference range.

Additional reference ranges

For some patient groups, other reference ranges of serum / plasma samples have.been reported.

Pregnant women

Samples of pregnant women were measured to have a 30–80 % higher reference.range than the control groups (see publications cited for the serum reference range).

Liver diseases

According to Haughton et al., the reference range of patients with liver diseases is 35 % lower than the one of healthy controls.

Performance Characteristics

Precision and Reproducibility
The precision (intra-assay variation) of the PromoKine VDB ELISA test was calculated from 16 replicate determinations on each of one samples.

Intra-Assay CV: n= 16

Sample	VDB Mean Value [mg/dl]	Intra-Assay CV [%]
1	24.2	5.0
2	42.9	3.2

The total precision (inter-assay variation) of the PromoKine VDB ELISA test was calculated from data on 1 sample obtained in 14 different assays by three technicians on two different lots of reagents over a period of three months.

Inter-Assay CV: n= 14

Sample	VDB Mean Value [mg/dl]	Inter-Assay CV [%]
1	19.3	12.7

Analytical Sensitivity

The Zero-standard was measured 20 times. The detection limit was set as B_0 + 2 SD and estimated to be 1.23 ng/ml.

Recovery
Two samples were spiked with VDB calibrator and measured with this assay (n=2):

Sample [ng/ml]	Spike [ng/ml]	VDB expected [ng/ml]	VDB measured [ng/ml]
2.2	5	7.5	7.7
2.2	10	12.2	12.7
2.2	20	22.2	25.3
6.7	2.5	9.2	8.7
6.7	7.5	14.2	13.1
6.7	15	21.7	22.5

Sample dilution
Two patient samples were diluted with sample dilution buffer. The results are shown below (n=2):

Sample	Dilution	Expected [pg/ml]	Measured [pg/ml]
A	1:5000	46.2	46.2
	1:10000	23.1	23.3
	1:20000	11.5	10.4
	1:40000	5.7	5.8
	1:80000	2.8	2.7
B	1:5000	38	38
	1:10000	19	20.2
	1:20000	9.5	8.7
	1:40000	4.7	4.3
	1:80000	2.3	2.4

References

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3. Haughton, M.A. & Mason, R.S., 1992. Immunonephelometric assay of vitamin D binding protein. *Clinical chemistry*, **38**(9), pp.1796–801.
4. Heijboer, A.C. et al., 2012. Accuracy of 6 Routine 25-Hydroxyvitamin D Assays: Influence of Vitamin D Binding Protein Concentration. *Clinical Chemistry*, **58**(3), pp.543–548.
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6. Malik, S. et al., 2013. Common variants of the vitamin D binding protein gene and adverse health outcomes. *Critical reviews in clinical laboratory sciences*.
7. Schmidt-Gayk, H. et al., 1977. 25-hydroxy-vitamin-D in nephrotic syndrome. *Lancet*, **2**(8029), pp.105–8.
8. Thomas, L., 1982. Proteindiagnostik: Diagnose, Therapiekontrolle. 1st ed., Frankfurt am Main: Behringwerke, Medizinische Information und Vertrieb.

General Notes on the Test and Test Procedure

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.
- The test components which are made of human serum are tested for HVB and HIV and found to be negative. However, since no test method can offer complete assurance that infectious agents are absent, these reagents should be handled as recommended for any potentially infectious human serum or blood specimen.

The normal precautions for laboratory working should be observed.

- Reagents of the test package contain sodium azide as a bactericide.
- Contact with skin or mucous membranes has to be avoided.
- All reagents in the test package are to be used for in-vitro diagnostics only.
- The reagents should not be used after the date of expiry (see label on the test package).
- Single components with different lot numbers should not be mixed or exchanged.
- The guidelines for medical laboratories should be observed.
- Incubation time, incubation temperature and pipetting volumes of the different components have been defined by the producer. Any alterations of the test procedure, that are not coordinated with the producer, may influence the results of the test. PromoCell can therefore not be held responsible for any damage.

Ordering Information

Product Name	Product Description	Size	Catalog Number
VDBP ELISA Kit, human	Human Vitamin D Binding Protein ELISA Kit	96 Tests	PK-EL-K2314

For in vitro research use only.
Not for diagnostic or therapeutic procedures.