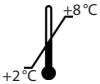
Manual

VDBP ELISA

For the in vitro determination of VDBP (vitamin D binding protein) in serum, plasma and urine

Valid from 2019-01-01

REF **K 2314**



IVD CE

Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
Tel.: +49 6251 70190-0 Fax: + 49 6251 849430
e.mail: info@immundiagnostik.com www.immundiagnostik.com

Table of Contents

1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of free and not actin complex bound vitamin D binding protein (VDBP) in serum, plasma and urine. For *in vitro* diagnostic use only.

2. INTRODUCTION

Vitamin D binding protein (VDBP, also known as group-specific component / Gc protein) is a multifunctional serum protein which is formed in the liver. High estrogen levels, caused e.g. by pregnancy or hormonal birth control, stimulate its synthesis. VDBP can be found in plasma, ascites, liquor, urine and at the surface of various cell types.

In the blood, VDBP binds the bigger part of the circulating 25-OH vitamin D and brings it to the kidneys, where it is transformed into the hormone 1,25-$(OH)_2$ vitamin D.

In addition, VDBP binds monomeric actin at the ratio of 1:1. Actin is an intracellular protein which is available as monomer or filament. Massive tissue destruction or cell death cause the plasma level of actin to rise significantly, whereupon VDBP-actin complexes are formed which are removed quickly. Hence the VDBP level of trauma or sepsis patients decreases quickly, especially in patients with a high level of multiorgan failure.

Furthermore VDBP is a precursor of the immunomodulating protein Gc-MAF (Gc protein-derived macrophage activating factor) which increases the activity of macrophages against tumours.

Indications

- Prognostic factor for trauma patients
- Nephritic syndrome
- Tumours

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 2314	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml
K 2314	CONJ	Conjugate concentrate, peroxidase-labelled (rabbit-anti-VDBP)	1 x 200 µl

Cat. No.	Label	Kit components	Quantity
K 2314	STD	Calibrators, lyophilised (60; 20; 6,6; 2,2; 0 ng/ml)	2 x 5 vials
K 2314	STDBUF	Standard dilution buffer, ready-to-use	1 x 20 ml
K 2314	CTRL1	Control, lyophilised (see specification for range)	2 x 1 vial
K 2314	CTRL2	Control, lyophilised (see specification for range)	2 x 1 vial
K 2314	SAMPLEBUF	Sample dilution buffer, ready-to-use	2 x 100 ml
K 0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure 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 (≥ 18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** 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 **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** is stable at **2–8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.
- The **lyophilised standards (STD)** and **controls (CTRL)** are stable at **2–8 °C** until the expiry date stated on the label. Before use, the STD and CTRL have to be reconstituted with **500 µl of standard dilution buffer (STDBUF)** and mixed by gentle inversion to ensure complete reconstitution. Allow the vial content to dissolve for 10 minutes and then mix thoroughly. **Standards and controls** (reconstituted STD and CTRL) **can be stored at 2–8 °C for 4 weeks or at -20 °C for 4 weeks.**
- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in wash buffer (100 µl CONJ + 10 ml wash buffer). The CONJ is stable at **2–8 °C** until expiry date stated on the label. **Conjugate** (1:101 diluted CONJ) **is not stable and cannot be stored.**
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

Serum and plasma samples

Dilute all plasma and serum samples 1:40 000 with sample dilution buffer (SAMPLEBUF). 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, pipet **100 µl** of **dilution III** per well.

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

Serum and plasma samples can be stored for 3 months at -20 °C. Avoid more than 3 freeze thaw cycles.

Urine

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

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

For testing in duplicates, pipette **2 x 100 µl** of each prepared sample per well.

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

7. ASSAY PROCEDURE

Principle of the test

This enzyme immunoassay is a sandwich assay for the quantitative determination of VDBP in serum, plasma and urine samples. The wells of the microtiterplate are coated with polyclonal anti-VDBP antibodies. In a first incubation step, the VDBP 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-VDBP 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 colour converts to yellow. The intensity of the yellow colour is directly proportional to the VDBP concentration in the sample. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. VDBP, present in the patient samples, is determined directly from this curve.

Test procedure

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

Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 °C. Strips are stable until expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	**Before use**, wash the wells **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add each **100 µl standards/controls/diluted samples** into the respective wells.
3.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker***.
4.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
5.	Add **100 µl conjugate** (diluted CONJ) into each well.
6.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker***.
7.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8.	Add **100 µl substrate** (SUB) into each well.
9.	Incubate for **10–20 min**** at room temperature (15–30 °C) in the **dark**.
10.	Add **100 µl stop solution** (STOP) into each well and mix well.
11.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at **405 nm** against 620 nm as a reference.

* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

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

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the 4 parameter algorithm.

1. **4 parameter algorithm**

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

2. **Point-to-point calculation**

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

3. **Spline algorithm**

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

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

Serum/plasma samples

The obtained VDBP levels of plasma and serum samples have to be multplied with the dilution factor of 40 000.

Urine samples

The obtained VDBP levels of urine samples have to be multplied with the dilution factor of 10.

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

9. LIMITATIONS

Samples with concentrations above the measurement range can be further diluted and re-assayed. Please consider this greater dilution when calculating the 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*

Analytical sensitivity see chapter "Performance Characteristics".

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. 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.

Reference range

Plasma / serum samples

200–550 mg/l

L. Thomas, 1982

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. Normalbuminuria 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.

11. PERFORMANCE CHARACTERISTICS

Accuracy – Precision

Repeatability (Intra-Assay); n=16

The repeatability was assessed with 2 serum samples under constant parameters (same operator, measurement system, day and kit lot).

Sample	Mean value [mg/l]	CV [%]
1	242.4	5.0
2	429.0	3.3

Reproducibility (Inter-Assay); n=17

The reproducibility was assessed with a serum and a control sample under varying parameters (different operators, measurement systems, days and kit lots).

Sample	Mean value [mg/l]	CV [%]
1	128.3	13.9
2	383.2	3.3

Accuracy – Trueness

The trueness states the closeness of the agreement between the result of a measurement and the true value of the measurand. Therefore, VDBP spikes with known concentrations were added to 4 serum samples. The results below were obtained without consideration of the sample dilution factor.

Sample [ng/ml]	Spike [ng/ml]	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
2.25	5	7.25	8.00	110.27
	10	12.25	13.17	107.45
	20	22.25	25.55	114.83
6.91	2.5	9.41	9.07	96.45
	7.5	14.41	13.58	94.28
	15	21.91	23.39	106.76

Sample [ng/ml]	Spike [ng/ml]	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
2.21	5	7.21	7.78	107.96
	10	12.21	12.72	104.22
	20	22.21	22.87	102.99
6.74	2.5	9.24	8.79	95.16
	7.5	14.24	13.11	92.13
	15	21.74	22.53	103.66

Linearity

The linearity states the ability of a method to provide results proportional to the concentration of analyte in the test sample within a given range. This was assessed according to CLSI guideline EP6-A with a serial dilution of 2 different serum samples.

For VDBP in serum, plasma and urine, the method has been demonstrated to be linear from 2.38 to 46.20 ng/ml based on the standard curve without considering possibly used sample dilution factors, showing a non-linear behaviour of less than ±20 % in this interval.

Sample	Dilution	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
A	1:5000	46.20	46.20	100.00
	1:10000	23.10	23.30	100.87
	1:20000	11.55	10.50	90.91
	1:40000	5.78	5.83	100.87
	1:80000	2.89	2.80	96.97
B	1:10000	38.00	38.00	100.00
	1:20000	19.00	20.25	106.58
	1:40000	9.50	8.75	92.11
	1:80000	4.75	4.30	90.53
	1:160000	2.38	2.49	104.74

Analytical sensitivity

The following values have been estimated based on the concentrations of the standard without considering possibly used sample dilution factors.

Limit of blank, LoB	0.154 ng/ml
Limit of detection, LoD	0.944 ng/ml
Limit of quantitation, LoQ	0.944 ng/ml

The evaluation was performed according to the CLSI guideline EP17-A2. The specified accuracy goal for the LoQ was 20 % CV.

Analytical specificity

The specificity of the antibody was tested by measuring the cross-reactivity against a range of compounds with structural similarity to VDBP. There was no cross-reactivity observed.

Substance tested	Concentration added	Concentration obtained [ng/ml]	Conclusion
h-albumin	10 µg/ml	< 0.154	< LoB
CRP	10 µg/ml	< 0.154	< LoB
RBP	10 µg/ml	< 0.154	< LoB

12. PRECAUTIONS

- All reagents in the kit package are 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 ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.

- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

15. REFERENCES

General literature

1. Bouillon, R., van Baelen, H. & de Moor, P., 1977. The measurement of the vitamin D-binding protein in human serum. *The Journal of clinical endocrinology and metabolism*, **45**(2), pp.225–31.
2. Fu, L. et al., 2009. Common genetic variants of the vitamin D binding protein (DBP) predict differences in response of serum 25-hydroxyvitamin D [25(OH)D] to vitamin D supplementation. *Clinical biochemistry*, **42**(10-11), pp.1174–7.
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.

5. Jørgensen, C.S. et al., 2004. Gc globulin (vitamin D-binding protein) levels: an inhibition ELISA assay for determination of the total concentration of Gc globulin in plasma and serum. *Scandinavian journal of clinical and laboratory investigation*, **64**(2), pp.157–66.

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. **1**st ed., Frankfurt am Main: *Behringwerke, Medizinische Information und Vertrieb*.

Literature using the Immundiagnostik AG VDBP ELISA

9. Cauley, J.A. et al., 2011. Serum 25-hydroxyvitamin D and clinical fracture risk in a multiethnic cohort of women: the Women's Health Initiative (WHI). *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*, **26**(10), pp.2378–88.

10. Comabella, M. et al., 2010. Cerebrospinal fluid chitinase 3-like 1 levels are associated with conversion to multiple sclerosis. *Brain : a journal of neurology*, **133**(Pt 4), pp.1082–93.

11. Correale, J., Ysrraelit, M.C. & Gaitán, M.I., 2010. Gender differences in 1,25 dihydroxyvitamin D3 immunomodulatory effects in multiple sclerosis patients and healthy subjects. *Journal of immunology (Baltimore, Md. : 1950)*, **185**(8), pp.4948–58.

12. Doorenbos, C.R.C. et al., 2012. Antiproteinuric treatment reduces urinary loss of vitamin D-binding protein but does not affect vitamin D status in patients with chronic kidney disease. *The Journal of steroid biochemistry and molecular biology*, **128**(1-2), pp.56–61.

13. Gressner, O. et al., 2007. Gc-globulin concentrations and C5 haplotype-tagging polymorphisms contribute to variations in serum activity of complement factor C5. *Clinical biochemistry*, **40**(11), pp.771–5.

14. Jeffery, L.E. et al., 2012. Availability of 25-hydroxyvitamin D(3) to APCs controls the balance between regulatory and inflammatory T cell responses. *Journal of immunology (Baltimore, Md. : 1950)*, **189**(11), pp.5155–64.

15. Jeng, L. et al., 2009. Alterations in vitamin D status and anti-microbial peptide levels in patients in the intensive care unit with sepsis. *Journal of translational medicine*, **7**, p.28.

16. Lee, S.-H. et al., 2011. Relationship between group-specific component protein and the development of asthma. *American journal of respiratory and critical care medicine*, **184**(5), pp.528–36.

17. Meguro, S. et al., 2011. Plasma 25-hydroxyvitamin d is independently associated with hemoglobin concentration in male subjects with type 2 diabetes mellitus. *International journal of endocrinology*, 2011, p.362981.

18. Mirković, K. et al., 2013. Urinary vitamin D binding protein: a potential novel marker of renal interstitial inflammation and fibrosis. *PloS one*, **8**(2), p.e55887.

19. Prytuła, A. et al., 2012. Urinary and dialysate losses of vitamin D-binding protein in children on chronic peritoneal dialysis. *Pediatric nephrology (Berlin, Germany),* **27**(4), pp.643–9.

20. Turner, A.M. et al., 2013. Circulating DBP level and prognosis in operated lung cancer: an exploration of pathophysiology. *The European respiratory journal,* **41**(2), pp.410–6.

21. Wagner, D. et al., 2013. Randomized clinical trial of vitamin D3 doses on prostatic vitamin D metabolite levels and ki67 labeling in prostate cancer patients. *The Journal of clinical endocrinology and metabolism*, **98**(4), pp.1498–507.

22. Wang, X. et al., 2013. Vitamin D-binding protein levels in female patients with primary hyperparathyroidism. *Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists*, **19**(4), pp.609–13.

23. Wood, A.M. et al., 2011. Vitamin D-binding protein contributes to COPD by activation of alveolar macrophages. *Thorax*, **66**(3), pp.205–10.

Used symbols:

 Temperature limitation

In Vitro Diagnostic Medical Device

 Manufacturer

Lot number

 Attention

Consult specification data sheet

Catalogue Number

To be used with

Contains sufficient for <n> tests

 Use by

 Consult instructions for use