

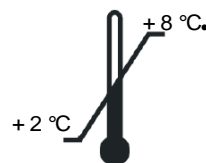
Vitamin D binding protein

For the in vitro determination of Vitamin D binding protein in serum, plasma and urine

Gültig ab / Valid from 09.07.2010



IMM-K 2314



1. INTENDED USE

The *Immundiagnostik* 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.

2. 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, sepsis etc.

Indications

- Risk factor for traumatic injury
- Nephrotic syndrom

3. 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.

4. MATERIAL SUPPLIED

Cat. No.	Label	Kit Components	Quantity
K 2314MTP	PLATE	one holder with precoated strips	96
K 2314WP	WASHBUF	ELISA wash buffer concentrate 10x	2 x 100 ml
K 2314K	CONJ	POD antibody, (rabbit-anti-VDB, Peroxidase-labeled), pre-diluted	1 x 200 μ l
K 2314ST	STD	Calibrators, lyophilized (60; 20; 6,6; 2,2; 0 ng/ml)	4 x 5 vials
K 2314KO1	CTRL	Control, lyophilized	4 vials
K 2314KO2	CTRL	Control, lyophilized	4 vials
K 2314PV	SAMPLEBUF	Dilution buffer, ready to use	2 x 100 ml
K 2314TMB	SUB	TMB substrate (Tetramethylbenzidine), ready to use	1 x 15 ml
K 2314AC	STOP	ELISA stop solution, ready to use	1 x 15 ml

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Distilled water
- Bidistilled (aqua bidest.) or deionized water
- Deep freezer -20 °C
- Precision pipettors calibrated to deliver 10-1000 μ l
- Horizontal microtiter plate shaker
- Multi-channel dispenser or repeating dispenser
- Vortex-Mixer
- Water bath or heating block
- Standard laboratory glass or plastic vials, cups, etc. (one time products)
- Microtiter plate reader 450 nm (reference wave length 620 or 690 nm)
- Seal cover for microtiter plates

6. 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.
- The **WASHBUF** (wash buffer concentrate) should be diluted with aqua bidest. **1:10** before use (100 ml WASHBUF + 900 ml aqua bidest.), 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 buffer solution can be stored in a closed flask at 2-8°C for one month.**
- The lyophilized **STD** (standards) and **CTRL** (controls) must be reconstituted with **500 µl** aqua bidest. 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.
- The **CONJ** (conjugate, POD-antibody) 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 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.

7. 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.

8. SPECIMEN COLLECTION AND PREPARATION

Serum, plasma

Dilute all plasma and serum samples **1:40000** with **SAMPLEBUF** (sample dilution buffer). For example:

10 µl Sample + 990 µl SAMPLEBUF=1:100 (**Dilution I**)

10 µl Dilution I + 990 µl SAMPLEBUF=1:10 000 (**Dilution II**)

250 µl Dilution II + 750 µl SAMPLEBUF=1:40 000 (**Dilution III**)

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

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

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

9. 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. Immundiagnostik can therefore not be held responsible for any damage.
- The assay should always be performed according the enclosed manual.

Test procedure

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

Carry out the tests in duplicate.

1. Add **100 µl STD** (Standard), **CTRL** (Controls) and pre-diluted sample into respective well.
2. Incubate for **1 hour** shaking on a horizontal mixer at room temperature.
3. Decant the content of the PLATE and wash the wells **5 x with 250µl** diluted wash buffer.
4. Add **100 µl** diluted **CONJ** (Peroxidase-labeled antibody).
5. Incubate for **1 hour** shaking on a horizontal mixer at room temperature.
6. Decant the content of the PLATE and wash the wells **5 x with 250µl** diluted wash buffer.
7. Add **100 µl SUB** (TMB substrate).
8. Incubate for **10-20 minutes** at room temperature.
9. Add **50 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 (or 690 nm) as reference.

10. 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 used program, a control of the paired values should be done manually.

Serum/plasma samples

For the calculation of the VDBP concentration in serum or plasma samples, the result must be multiplied by **40000**.

Urine samples

For the calculation of the VDBP concentration in urine samples, the result must be multiplied by **10**.

11. LIMITATIONS

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

12. QUALITY CONTROL

Immundiagnostik recommends commercial control samples for internal quality control.

Control samples or serum pools should be analyzed with each run of calibrators and patient samples. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. In assays in which one or more of the quality control sample values lie outside the acceptable limits, the results for the patient sample may not be valid.

Expected values

Normal range 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 20-55 mg/dl
(MW = 51 243 Da, positions 17-474, 458 amino acids, P02774 VTDB_HUMAN)

Deviations of the reference range may be present in the case of

- **pregnancy** (elevated) or 124.9 mg/dl^[2]
- **acute disorders of the liver** (decreased) 23 mg/dl^[2]

Urine < 200 µg/l

Elevated levels may indicate a **nephritic syndrome**

Urine 0.8 - 34 mg/dl^[1]

13. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

The precision (intra-assay variation) of the Immundiagnostik 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 Immundiagnostik 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

Sensitivity

Sensitivity: n=22

Sample	VDB Mean value [OD]	Standard variation	Detection limit [ng/ml]
1	0.025	0.003	1.23

Recovery

Two samples were spiked with VDB calibrator and measured with this assay.

Recovery 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:

Linearity: n=2

Sample	Dilution	Expected [mg/dl]	Measured [mg/dl]
	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
	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

14. REFERENCES

1. Schmidt-Gayk H et al. (1977) The Lancet 16:105-108
2. Houghton M et al (1992) Clin Chem 38:1796-1801
3. Bouillon R et al. (1977) JCE & M 45:225-231
4. Feldmann et al. Vitamin D (1997) by Academic Press
5. Ray R (1996) P. S. E. B. M. 212:305-312
6. Cooke N et al. (1989) Endocrine Reviews 10:294-307
7. 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. Scand J Clin Lab Invest 64: 157–166

15. 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. Immundiagnostik can therefore not be held responsible for any damage.

Used symbols:

Temperature limitation



Catalogue Number



In Vitro Diagnostic Medical Device



Contains sufficient for <n> tests



Manufacturer



Use by



Lot number

Li StarFish distribuisce: