DRG® Vitamin D Binding Protein (EIA-5088)

USA: RUO

Revised 7 Dec. 2010 rm (Vers. 2.1)

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only. Not for use in diagnostic procedures.

1 INTENDED USE

The Assay is intended for determination of free, not Actin-bound, Vitamin-D binding protein in serum, plasma and urine.

2 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.

3 MATERIAL SUPPLIED

Label	Kit Components	Quantity
PLATE	one holder with precoated strips	96
WASHBUF	ELISA wash buffer 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
CTRL1	Control 1, lyophilized	4 vials
CTRL2	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

4 MATERIAL REQUIRED BUT NOT SUPPLIED

Distilled water
Bidistilled (aqua bidest.)or deionized water
Deep freezer -20 °C
Precision pipettors calibrated to deliver 10-1000 µl
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

5 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.

6 PRECAUTIONS

For research 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.

7 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.

8 ASSAY PROCEDURE

8.1 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. The producer can therefore not be held responsible for any damage.
The assay should always be performed according the enclosed manual.

8.2 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 250 µl** 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 250 µl** 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.

9 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**.

10 LIMITATIONS

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

REFERENCES / LITERATURE

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

11 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 research 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. The producer can therefore not be held responsible for any damage.