DRG® Retinol Binding Protein (RBP) ELISA (EIA-4391)

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This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

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

1 INTENDED USE

The DRG RBP ELISA is intended for measurement of free Retinol-binding protein (RBP)/RBP4 as well as RBP4 complexed with transthyretin in plasma, serum and urine.

2 PRINCIPLE OF THE TEST

This Enzyme-Linked Immunosorbent Assay (ELISA) can be used measurement of Retinol-binding protein (RBP)/RBP4 in plasma, serum and urine.
In a first incubation step, RBP/RBP4 in the samples is bound to polyclonal rabbit anti RBP/RBP4 antibodies, immobilized on the microtitre plate. A peroxidase-conjugated anti RBP/RBP4 antibody is used for detection and quantification, and tetramethylbenzidine (TMB) as a peroxidase substrate. A dose response curve of absorbance unit (optical density at 450 nm) vs. concentration is generated using the values obtained from standard. RBP/RBP4 present in the specimen samples is determined directly from this curve.

3 MATERIAL SUPPLIED

Content	Kit Components	Quantity
PLATE	One holder with precoated strips	12 x 8 wells
WASHBUF	ELISA wash buffer concentrate, 10x	2 x 100 mL
CONJ	Conjugate, (rabbit anti RBP/RBP4, peroxidase-labeled)	200 µL
CTRL 1	Control, lyophilized	2 vials
CTRL 2	Control, lyophilized	2 vials
SAMPLEBUF	Sample dilution buffer, ready-to-use	100 mL
STD	Calibrators, lyophilized (0; 1.1; 3.3; 11; 33 µg/L)	2 x 5 vials
SUB	TMB Substrate (Tetramethylbenzidin), ready-to-use	15 mL
STOP	ELISA Stop solution, ready-to-use	15 mL

4 MATERIAL REQUIRED BUT NOT SUPPLIED

- Bidistilled water (aqua bidest.)
- Precision pipettors calibrated and tips to deliver 5-1000 µL
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker

- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 450 or 405 nm (reference wave length 620 or 690 nm)

5 PREPARATION AND STORAGE OF REAGENTS

- To run 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 ELISA **WASHBUF** (wash buffer concentrate) must 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 room temperature or at 37 °C before dilution of the buffer solutions. The **buffer concentrate** is stable at **2 °C - 8 °C** until the expiry date stated on the label. Diluted **buffer solution** can be stored in a closed flask at **2 °C - 8 °C for one month**.
- Lyophilized **STD** (calibrators) and **CTRL** (controls) must be reconstituted with **500 µL** aqua bidest. Allow the vial to stand for minimum 10 minutes and then mix thoroughly by gentle inversion to insure complete reconstitution. Reconstituted Calibrators and Controls can be stored for two weeks at 2 °C - 8 °C.
- **CONJ** (conjugate) (POD-Antibody) must be diluted **1:100** in wash buffer (100 µL CONJ + 10 mL wash buffer). The antibody is stable at 2 °C - 8 °C until expiry date stated on the label. **Diluted antibody solution is not stable and can not be stored.**
- All other test reagents are ready to use. Test reagents are stable until the expiry date stated on the label of test package when stored at **2 °C - 8 °C**.

6 PRECAUTIONS

- The standards and controls contain human source material which was tested and found to be non-reactive to HBsAg, anti-HIV-1/2, and anti-HCV. Since no method can offer complete assurance that hepatitis B virus, HIV-1/2, HVC or other infectious agents are absent, these reagents should be handled as if potentially infectious.
- Stop Solution consists of diluted Sulfuric Acid. This is 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 out immediately with copious quantities of water. Do not breath vapor and avoid inhalation.
- Reagents should not be used beyond the expiration date shown on kit label.

7 SPECIMEN COLLECTION AND PREPARATION

Plasma and serum
Samples can be stored for two weeks at 4°C. For longer storage, freeze at or below -20°C.
Dilute samples **1:5000 in SAMPLEBUF** (sample dilution buffer) before use.

Dilution I: 20 µL sample + 980 µL SAMPLEBUF = 1:50

Dilution II: 10 µL Dilution I + 990 µL SAMPLEBUF = 1:100.

To avoid some potential for error in RBP/RBP4 estimation due to small volumes, we recommend the following dilution steps:

Dilution I: 20 µL Sample + 980 µL SAMPLEBUF = 1:50
Dilution II: 50 µL Dilution I + 450 µL SAMPLEBUF = 1:10
Dilution III: 50 µL Dilution II + 450 µL SAMPLEBUF = 1:10

Urine
Adjust the urine to a pH of 6 to 8 with 1 N NaOH. Samples are stable at 2 °C - 8 °C for 2 weeks.
For longer storage, freeze at or below -20 °C.
Before use, dilute urine **1:10 in SAMPLEBUF** (sample dilution buffer), e.g. 100 µL urine + 900 µL SAMPLEBUF
Urine with a **RBP4 concentration > 330 µg/l** must be diluted **1:100**, e.g. 10 µL urine + 990 µL SAMPLEBUF

8 ASSAY PROCEDURE

8.1 Procedural notes

Do not interchange different lot numbers of any kit component within the same assay.
The 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. DRG can therefore not be held reliable for any damage resulting from this.
Carry out the assay with the actual manual delivered with the kit.

8.2 Test procedure

Wash the precoated **PLATE** (microtiter plate) **5 x with 250 µL** ELISA wash buffer. Carry out the tests in duplicate.

1. Add **100 µL** of **STD** (standard), **CTRL** (control) and **prediluted specimen samples** into the wells.
2. Incubate for **1 hour at room temperature** shaking on a horizontal mixer.
3. Decant the content of the plate and wash the wells **5 x with 250 µL** of washing buffer.
4. Add **100 µL** of diluted **CONJ** (conjugate) into each well.
5. Incubate for **1 hour** at room temperature**,** shaking on a horizontal mixer.
6. Decant the content of the plate and wash the wells **5 x with 250 µL** of washing buffer.
7. Add **100 µL** of **SUB** (TMB substrate solution).
8. Incubate for **10-20 minutes** at room temperature, shaking slightly until color differences are sufficient.
9. Add **50 µL** of **STOP** (stop solution) and mix shortly.
10. 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.

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 or Plasma
Multiply the result **with 5000** to get the real concentration.

Urine
Multiply the result **with the dilution factor** to get the real concentration.

10 REFERENCES

1. Graham TE, Wason CJ, Blüher M, Kahn BB (2007) Shortcomings in methodology complicate measurements of serum retinol binding protein (RBP4) in insulin-resistant human subjects. Diabetologia DOI 10.1007/s00125-006-0557-0
2. Graham TE, Yang Q, Bluher M, Hammarstedt A, Ciaraldi TP, Henry RR, Wason CJ, Oberbach A, Jansson PA, Smith U, Kahn BB (2006) Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med 354(24):2552-63
3. Yang et al. (2005), Nature 436:356-62
4. Blumsohn A et al. (1990) Clinica Chimica Acta 195 :133-38
5. Bernard AM et al. (1982) Clinica Chimica Acta 126 :1-7

11 GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- All reagents in the kit package are for research use only.
- Guidelines for medical laboratories should be observed.
- 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.

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- 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 shown on the kit label.
- 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. DRG can therefore not be held responsible for any damage resulting from wrong use.
- Warranty claims and complaints in respect of deficiencies must be logged within 14 days after receipt of the product. The product shall be send to DRG along with a written complaint.

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