Retinol-binding protein (RBP) / RBP4 ELISA

For the determination des RBP/RBP4 in plasma, serum and urine.

For research use only, not for use in diagnostic procedures.

Catalog Number: MBS495091 (96T)
Storage: 2-8°C

1. INTENDED USE

The MyBioSource Assay is intended for the determination of **free Retinol-binding protein (RBP)/RBP4** as well as **RBP4 complexed with transthyretin** in plasma, serum and urine. For research use only, not for use in diagnostic procedures.

2. SUMMARY AND EXPLANATION OF THE TEST

Retinol-binding protein (RBP)/RBP4 is a small (21kD) transport protein for vitamin A which forms a complex with prealbumin in blood but loses its affinity for prealbumin once the vitamin has been delivered to the target cells. The free RBP/RBP4 molecule is rapidly filtered at the glomerulus and catabolized in the renal tubules after resorption by the proximal tubular cells (like other small molecules, e. g. ß-2 microglobulin). In kidney disease with prevailing tubular changes, these proteins are not reabsorbed and appear in the urine.

As published by Yang et al. (2005), the retinol-binding protein **(RBP)/RBP4** seems to play a key role in the development of insulin resistance. The fat cell derived peptide RBP/RBP4 also modulates the glucose homeostasis and impairs the insulin sensitivity as well as insulin resistance. The elevation of serum RBP/RBP4 causes systemic insulin resistance, whereas its reduction improves the insulin action.

As a conclusion from the results, the authors suggest that **RBP/RBP4** alters insulin sensitivity in part by affecting insulin signaling in muscle through alterations in the amount of tyrosine-phosphorylated IRS-1 and PI(3)K activation. Thus, RBP/RBP4 may contribute to the pathogenesis of type 2 diabetes, and lowering RBP/RBP4 could be a new strategy for treating type 2 diabetes.

3. MATERIAL SUPPLIED

Part No.	Label	Kit components	Quantity
K 6120MTP	PLATE	Holder with precoated strips	12 x 8 wells
K 6120WP	WASHBUF	ELISA wash buffer concentrate, 10x	2 x 100 ml
K 6120PV	SAMPLEBUF	Sample dilution buffer, ready to use	100 ml

Part No.	Label	Kit components	Quantity
K 6120K	CONJ	Conjugate (rabbit anti RBP/RBP4, peroxidase-labeled)	200 µl
K 6120CAL	CAL	Calibrator, lyophilized	2 x 1 vial
K 6120KO1	CTRL	Control, lyophilized	2 vials
K 6120KO2	CTRL	Control, lyophilized	2 vials
K 6120TMB	SUB	TMB substrate (tetramethylbenzidine), ready to use	15 ml
K 6120AC	STOP	ELISA stop solution, ready to use	15 ml

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- Calibrated precision pipettors and 5–1000 µl tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge, 3000 g
- Vortex
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader

* MyBioSource recommends 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 (≥ 18.2 MΩ cm).

5. PREPARATION AND STORAGE 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.
- The **ELISA wash buffer concentrate** (WASHBUF) should be diluted **1:10 in ultra pure water** before use (100 ml concentrate + 900 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at room temperature or 37 °C before

dilution of the buffer solutions. The **buffer concentrate** is stable at **2–8 °C** until the expiry date stated on the label. **Diluted buffer solution** (wash buffer) can be stored in a closed flask at **2–8 °C for one month**.

- Use **100 µl of diluted wash buffer as a BLANK**. Pipet into the respective well.
- The **lyophilized calibrator** (CAL) and **controls** (CTRL) are stable at **2–8 °C** until the expiry date stated on the label. Before use, the **CAL** (calibrator) and **CTRL** (controls) must be reconstituted with **500 µl of ultra pure water**. Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to ensure complete reconstitution. **Reconstituted calibrators and controls can be stored at 2–8 °C for two weeks.**
- The **conjugate** (CONJ) must be diluted **1:101 in wash buffer** (100 µl CONJ + 10 ml wash buffer). The undiluted conjugate is stable at **2–8 °C** until the expiry date stated on the label. **Diluted conjugate 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 of test package) when stored at **2–8 °C**.

6. 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 pH between 6 and 8 with 1 N NaOH. Samples are stable at 2–8 °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

7. ASSAY PROCEDURE

Principle of the test

This ELISA can be used for determination 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 microtiter plate. A peroxidase-conjugated anti RBP/RBP4 antibody is used for detection and determination, and tetramethylbenzidine (TMB) as a peroxidase substrate. Samples are determined by referring their optical density to a lot-dependent master calibration curve and the use of a calibrator that is run with each test.

Test procedure

Wash the pre-coated PLATE (microtiter plate) **5 x with 250 µl ELISA wash buffer before use**. After the final washing step, the inverted microtiter plate should be tapped on absorbent paper.

Carry out the tests in duplicate.

1.	Add **100 µl of BLANK/CAL/CTRL/SAMPLE** (blank/calibrator/controls/ sample) in duplicate into respective well.
2.	Incubate for **1 hour at room temperature** (15–30 °C) 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 **100 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.

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

8. RESULTS

For result evaluation, please use a four parametric logit-log model based on the standard curve of the respective kit lot and the calibrator value (CAL). All essential information on the standard curve is provided on the QC data sheet of the respective product lot.

The calibration curve can be expressed either by the concentration of each standard with its corresponding optical density or by the four parameters A, B, C and D. In both cases, the optical density of the calibrator (CAL) is essential.

Depending on your evaluation software program, either the one or the other kind of data described above should be entered.

Caution: Please make sure that all parameters and values are transferred accurately into your software as minor deviations can cause severe errors during evaluation.

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

Serum or Plasma

Multiply the result **by the dilution factor 5000** to get the real concentration.

Urine

To obtain the real concentration, **multiply** the result **by the dilution factor used**.

9. LIMITATIONS

Samples with concentrations above the measurement range must 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 determined.

Measurement range

In due consideration of the specified sample preparation the applicable measurement range for

- serum / plasma samples is 4.5 to 165 mg/L.
- urine samples is 0.009 to 0.330 mg/L.

10. QUALITY CONTROL

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

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

Normal range

Plasma or Serum:

Adults	20–75 mg/l
Newborn	11–34 mg/l
Age 6 months	18–50 mg/l
Urine:	0.01–0.54 mg/l

We recommend each laboratory to establish its own norm concentration range.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay

The precision (intra-assay variation) of the MyBioSource RBP/RBP4 ELISA test

was calculated from 16 determinations on each of two samples. Intra-Assay CV n= 16

Sample	RBP/RBP4 mean value [µg/l]	Intra-Assay CV [%]
1	24.1	5
2	11.1	5

Inter-Assay

The total precision (inter-assay variation) of the RBP/RBP4 ELISA test was calculated from data on 2 samples obtained by different technicians on different days.

Inter-Assay CV n= 25

Sample	RBP/RBP4 mean value [µg/l]	Inter-Assay CV [%]
1	4.4	9.8
2	6.9	9.7

Dilution recovery

Two samples were diluted and analyzed. The results are shown below (n = 2):

Sample	Dilution	expected [µg/l]	measured [µg/l]
A	1:7000	4.8	4.8
	1:14000	2.8	2.4
	1:28000	1.2	1.2
	1:56000	0.6	0.8

Analytical Sensitivity

The detection limit was defined as B_0 + 2 SD and determined to be 0.9 µg/l.

12. PRECAUTIONS

- All reagents in the kit package are for research use only, not for use in diagnostic procedures.
- Control samples should be analyzed with each run.
- 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 color 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 vapor 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 as wells from already opened microtiter plates are exposed to different conditions than sealed ones.
- Reagents should not be used beyond the expiration date stated on kit label.
- Substrate solution should remain colorless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- The assay should always be performed according 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.
- Quality control guidelines 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. MyBioSource 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 MyBioSource along with a written complaint.

15. REFERENCES

1. Graham, T. E., Wason, C. J., Blüher, M. & Kahn, B. B. Shortcomings in methodology complicate measurements of serum retinol binding protein (RBP4) in insulin-resistant human subjects. *Diabetologia* **50**, 814–23 (2007).
2. Graham, T. E. et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *N. Engl. J. Med*. **354**, 2552–63 (2006).
3. Yang, Q. et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* **436**, 356–62 (2005).
4. Blumsohn, A., Morris, B. W., Griffiths, H. & Ramsey, C. F. Stability of β2-microglobulin and retinol binding protein at different values of pH and temperature in normal and pathological urine. *Clin. Chim. Acta* **195**, 133–137 (1991).
5. Bernard, A. M., Moreau, D. & Lauwerys, R. Comparison of retinol-binding protein and β2-microglobulin determination in urine for the early detection of tubular proteinuria. *Clin. Chim. Acta* **126**, 1–7 (1982).