

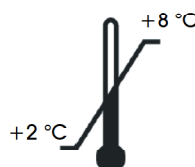
# Retinol-binding protein (RBP)/ RBP4 ELISA Kit

*For the in vitro determination of RBP/RBP4 in plasma, serum and  
urine*

Gültig ab / Valid from 31.03.2010



K 6110



## 1. INTENDED USE

The *Immundiagnostik* Assay is intended for the quantitative determination of **free Retinol-binding protein (RBP)/RBP4** as well as **RBP4 complexed with transthyretin** in plasma, serum and urine. For *in vitro* diagnostic use only. of free as well as complexed

## 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.  $\beta$ -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 signalling 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.

### Indications

- Early detection of tubular proteinuria
- Chronic liver diseases
- Cadmium poisoning
- Studies of insulin resistance

### 3. PRINCIPLE OF THE TEST

This Enzyme-Linked Immunosorbent Assay (ELISA) can be used for quantitative 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 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 patient samples is determined directly from this curve.

### 4. MATERIAL SUPPLIED

Cat. No	Content	Kit Components	Quantity
K 6110MTP	PLATE	One holder with precoated strips	12 x 8 wells
K 6110WP	WASHBUF	ELISA wash buffer concentrate 10x	2 x 100 ml
K 6110K	CONJ	Conjugate, (rabbit anti RBP/RBP4, peroxidase-labeled)	200 µl
K 6110KO1	CTRL	Control, lyophilized	2 vials
K 6110KO2	CTRL	Control, lyophilized	2 vials
K 6110PV	SAMPLEBUF	Sample dilution buffer, ready-to-use	100 ml
K 6110ST	STD	Calibrators, lyophilized (0; 1.1; 3.3; 11; 33 µg/l)	2 x 5 vials
K 6110TMB	SUB	TMB Substrate (Tetramethylbenzidin), ready-to-use	15 ml
K 6110AC	STOP	ELISA Stop solution, ready-to-use	15 ml

## 5. 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)

## 6. 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 **2-8°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**.
- 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 - 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 -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 **2-8°C.**

## 7. PRECAUTIONS

- For *in vitro* diagnostic use only.
- The calibrators 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.

## 8. 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

## 9. ASSAY PROCEDURE

### *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. Immundiagnostik can therefore not be held reliable for any damage resulting from this.
- Carry out the assay with the actual manual delivered with the kit.

## 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** (controls) and **prediluted patient 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.



## 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 or Plasma

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

### Urine

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

## 11. LIMITATIONS

Samples with RBP/RBP4 levels greater than the highest calibrator value, should be diluted and re-assayed.



## 12. QUALITY CONTROL

**Immundiagnostik recommends commercially control samples as internal quality control.**

**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 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.

### *Expected values*

#### **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

It is recommended for each laboratory to establish its own normal range.

## 13. PERFORMANCE CHARACTERISTICS

### *Precision and reproducibility*

The precision (intra-assay variation) of the Immundiagnostik 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

The total precision (inter-assay variation) of the Immundiagnostik 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

### *Sensitivity*

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

### *Sample dilution*

Linearity n= 1

One patient sample was diluted. The results are shown below:

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

## 14. 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

## 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.
- All reagents in the kit package are for *in vitro* diagnostic 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.
- 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. Immundiagnostik AG 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 Immundiagnostik AG along with a written complaint.

**Used symbols:**

Temperature limitation



Catalogue Number



In Vitro Diagnostic Medical Device



Contains sufficient for &lt;n&gt; tests



Manufacturer



Use by



Lot number