

Retinol-binding protein RBP/RBP4 ELISA

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

Valid from 2022-02-09

REF KR6110

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96

+2°C
+8°C

RUO



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1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay 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 research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

Retinol-binding protein RBP/RBP4 is a small (21 kD) 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 catabolised in the renal tubules after resorption by the proximal tubular cells (like other small molecules, e.g. β -2 microglobulin). In kidney disease with prevailing tubular changes, these proteins are not reabsorbed and appear in the urine.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR6110	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10x	2 x 100 ml
KR6110	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 100 ml
KR6110	CONJ	Conjugate concentrate (rabbit anti RBP/RBP4, peroxidase-labelled)	1 x 200 μ l
KR6110	STD	Standards, lyophilised (0; 1.1; 3.3; 11; 33 μ g/l)	2 x 5 vials
KR6110	CTRL 1	Control, lyophilised (see specification for range)	2 x 1 vial
KR6110	CTRL 2	Control, lyophilised (see specification for range)	2 x 1 vial
KR0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
KR0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge, 3000 g
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure 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. STORAGE AND PREPARATION 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.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** is stable at **2–8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.
- The **lyophilised standards (STD)** and **controls (CTRL)** are stable at **2–8 °C** until the expiry date stated on the label. **Reconstitution** details are given in the **specification data sheet**. **Standards and controls** (reconstituted STD and CTRL) **can be stored at 2–8 °C for 2 weeks**.
- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in wash buffer (100 µl CONJ + 10 ml wash buffer). The CONJ is stable at **2–8 °C** until the expiry date stated on the label. **Conjugate** (1:101 diluted CONJ) **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) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

Plasma and serum

Samples can be stored for two weeks at 4 °C. For longer storage, freeze at or below -20 °C.

Dilute samples **1:5 000 in sample dilution buffer** (SAMPLEBUF) before use. For example:

- **20 µl** sample + **980 µl** SAMPLEBUF, mix well = **1:50 (dilution I)**
- **50 µl** dilution I + **450 µl** SAMPLEBUF, mix well = **1:10 (dilution II)**
- **50 µl** dilution II + **450 µl** SAMPLEBUF, mix well = **1:10 (dilution III)**.

This results in a final dilution of **1:5 000**.

For analysis, pipet **100 µl** of **dilution III** per well.

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 sample dilution buffer** (SAMPLEBUF), for example:

100 µl urine + 900 µl SAMPLEBUF, mix well = **1:10**

Urine with an **RBP4 concentration > 330 µg/l must be diluted 1:100**, for example:

10 µl urine + 990 µl SAMPLEBUF, mix well = **1:100**

For analysis, pipet **100 µl** of the **diluted sample** per well.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the 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, immobilised 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 samples is determined directly from this curve.

Test procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 °C. Strips are stable until expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	Before use , wash the wells 5 times with 250 µl wash buffer . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add each 100 µl standards/controls/ diluted samples into the respective wells.
3.	Cover the strips and incubate for 1 hour at room temperature (15–30 °C) on a horizontal shaker* .
4.	Discard the content of each well and wash 5 times with 250 µl wash buffer . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
5.	Add 100 µl conjugate (diluted CONJ) into each well.
6.	Cover the strips and incubate for 1 hour at room temperature (15–30 °C) on a horizontal shaker* .
7.	Discard the content of each well and wash 5 times with 250 µl wash buffer . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8.	Add 100 µl substrate (SUB) into each well.
9.	Incubate for 10–20 min** at room temperature (15–30 °C) in the dark .
10.	Add 100 µl stop solution (STOP) into each well and mix well.

11.

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.

* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

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

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the "4 parameter algorithm".

1. 4 parameter algorithm

It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e.g. 0.001).

2. Point-to-point calculation

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3. Spline algorithm

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

Serum or plasma

The obtained results have to be multiplied by the **dilution factor of 5000** to get the actual concentrations.

Urine

The obtained results have to be multiplied by the **dilution factor used** to get the actual concentrations.

In case **another dilution factor** has been used, multiply the obtained result by the dilution factor used.

9. LIMITATIONS

Samples with concentrations above the measurement range (see definition below) can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

Samples with concentrations lower than the measurement range (see definition below) cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × sample dilution factor to be used

The lower limit of the measurement range can be calculated as:

LoB × sample dilution factor to be used

LoB see chapter "Performance Characteristics".

10. QUALITY CONTROL

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

Control samples should be analysed 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.

Reference range

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n = 16)

The precision (intra-assay variation) of the Immundiagnostik RBP/RBP4 ELISA test was calculated from 16 determinations on each of two samples.

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

Inter-Assay (n = 14)

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.

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

Analytical Sensitivity

The following values have been estimated based on the concentrations of the standard curve without considering possibly used sample dilution factors.

Limit of blank, LoB

0.2 µg/l

12. PRECAUTIONS

- All reagents in the kit package are 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 ProClin as bactericides. Sodium azide or ProClin are hazardous to health and the environment. Substrates for enzymatic colour reactions may also cause skin and/or respiratory irritation. Any contact with the substances must be avoided. Further safety information can be found in the safety data sheet, which is available from Immundiagnostik AG on request.
- The 10x Wash buffer concentrate (WASHBUF) contains surfactants which may cause severe eye irritation in case of eye contact.

Warning: Causes serious eye irritation. **IF IN EYES:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: get medical Advice/attention.

- 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 vapour 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.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for laboratories 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. Immundiagnostik AG 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 Immundiagnostik AG 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 β 2-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 β 2-microglobulin determination in urine for the early detection of tubular proteinuria. *Clin. Chim. Acta* **126**, 1–7 (1982).

Used symbols:

	Temperature limitation		Catalogue number
	For research use only		To be used with
	Manufacturer		Contains sufficient for <n> tests
	Lot number		Use by
	Attention		Consult instructions for use
	Consult specification data sheet		Irritant