Human RBP ELISA Kit

Instruction Manual

PromoKine

Contents

Intended Use

The PromoKine Assay is intended for the quantitative determination of Retinol-binding protein (RBP) in plasma, serum and urine. For *in vitro* research use only.

Introduction

Retinol-binding protein (RBP) 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 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 4 (RBP4) seems to play a key role in the of insulin resistance. The fat cell derived peptide RBP also modulates the glucose homeostasis and impairs the insulin sensitivity as well as insulin resistance. The elevation of serum RBP4 causes systemic insulin resistance, whereas its reduction improves the insulin action. As a conclusion from the results, the authors suggest that 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, RBP4 may contribute to the pathogenesis of type 2 diabetes, and lowering 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

Principle of the Test

This Enzyme-Linked Immunosorbent Assay (ELISA) can be used for quantitative determination of Retinol-binding protein (RBP) in plasma, serum and urine. In a first incubation step, RBP in the samples is bound to polyclonal rabbit anti RBP antibodies, immobilized on the microtitre plate. A peroxidase-conjugated anti RBP 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 present in the patient samples is determined directly from this curve.

Material Supplied

Kit Components	Quantity
One holder with precoated strips	12 x 8 wells
ELISA wash buffer concentrate 10x	2 x 100 ml
Conjugate, (rabbit anti RBP, peroxidase-labeled)	200 µl
Control, lyophilized	2 vials
Sample dilution buffer, ready-to-use	100 ml
Calibrators, lyophilized (0; 1.1; 3.3; 11; 33 µg/l)	2 x 5 vials
TMB Substrate (Tetramethylbenzidin), ready-to-use	15 ml
ELISA Stop solution, ready-to-use	7 ml

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)

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 wash buffer concentrate (WASHBUF) should be diluted with aqua bidest. 1:10 before use (100 ml concentrate + 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.

Calibrators and Control 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 Control can be stored for two weeks at 2 - 8 °C.

Conjugate (POD-Antibody) must be diluted 1:100 in wash buffer (100 µl POD antibody + 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.

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.

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 dilution buffer before use.

Dilution I: 20 µl sample + 980 µl dilution buffer = 1:50

Dilution II: 10 µl Dilution I + 990 µl dilution buffer = 1:100

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, e.g.

100 µl urine + 900 µl dilution buffer

Urine with a RBP concentration > 330 µg/l must be diluted 1:100, e.g. 10 µl urine + 990 µl dilution buffer.

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. PromoCell 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 microtiter plate 5 x with 250 µl ELISA wash buffer.

Carry out the tests in duplicate.

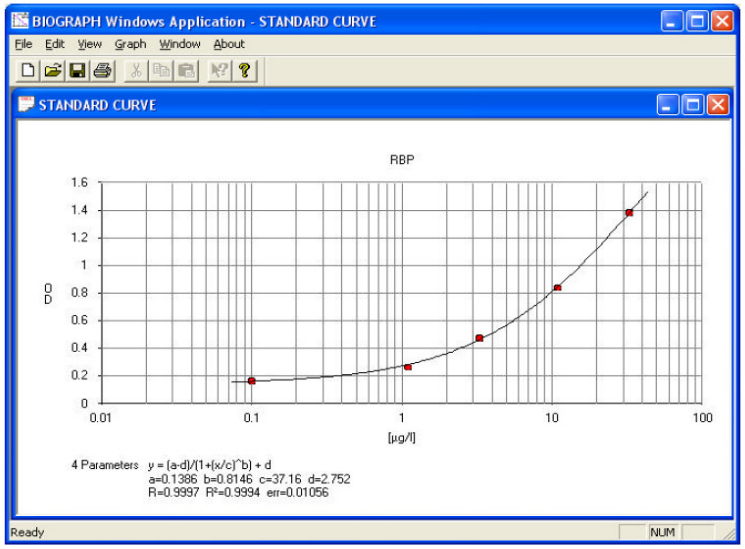
1. Add 100 µl of standard and prediluted patient samples into the wells.
2. Incubate for 1 hour at room temperature shaking on a horizontal mixer.
3. Decant the content of the plate and wash the walls 5 x with 200 µl of washing buffer.
4. Add 100 µl diluted Conjugate into each well.
5. Incubate for 1 hour at room temperature, shaking on a horizontal mixer.
6. Decant the content of the plate and wash the walls 5 x with 200 µl of washing buffer.
7. Add 100 µl of 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 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.

Results

A calibration curve is constructed from the standards. Commercially available software can be used as well as graph paper. Results of the samples are read from this calibration curve. THE CALIBRATION CURVE IS NOT LINEAR, therefore a spline- or 4PL algorithm is recommended.

Typical calibration curve



Concentration [µg/l]	33	11	3.3	1.1	0
OD mean values	1.864	1.151	0.548	0.287	0.163

The data is for demonstration only and cannot be used for the evaluation of test results.

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.

Limitations

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

Quality Control

PromoCell 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: 30 – 75 mg/l

Urine: 0.01 – 0.54 mg/l

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

Performance Characteristics

Precision and Reproducibility

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

Intra-Assay CV n= 16

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

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

Inter-Assay CV n= 25

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

Sensitivity

The detection limit was defined as B0 + 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

References

Yang et al., Nature 2005 Jul 21;436(7049):356-62

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 supplier. Any variation of the test procedure, which is not coordinated with the supplier, may influence the results of the test. PromoCell 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 PromoCell along with a written complaint.

Ordering Information

Product Name	Size	Catalog Number
RBP ELISA Kit, human	96 Tests	PK-EL-K6110

For in vitro research use only.
Not for diagnostic or therapeutic procedures.

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