invitron
high sensitivity diagnostics

IV3-101E

English

Retinol Binding Protein (RBP4) ELISA Kit

For in-vitro diagnostic use only

Definitions


Instructions for use

REF — Catalogue number


Use by

LOT — Lot/Batch Code


Storage temperature limitations

IVD — In vitro diagnostic medical device

Contains sufficient for <N> tests

CE

Invitron Ltd.
Wyastone Business Park, Wyastone Leys
Monmouth NP25 3SR, UK
www.invitron.com

Invitron Retinol Binding Protein (RBP4) ELISA Kit

Intended Use

The Invitron Retinol Binding Protein (RBP4) Assay is intended for the quantitative determination of free retinol binding protein as well as that complexed with transthyretin in human serum, plasma or urine. This assay is for *in vitro* diagnostic use only.

Summary and Explanation

Retinol binding protein (RBP4) is a 21Kd transport protein for vitamin A, which forms a complex with prealbumin in the circulation but which loses its affinity for prealbumin once the vitamin A has been delivered to target cells. The protein is produced by adipocytes. The free RBP4 molecule is rapidly filtered by the glomerulus and is broken down in the renal tubules after reabsorption by the proximal tubular cells (as with 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.
RBP4 seems to play a key role in the development of insulin resistance (Yang *et al* 2005). An elevated level of RBP4 is generally associated with systemic insulin resistance. A reduction in RBP4 is accompanied by improved insulin sensitivity. As a consequence, it has been suggested that RBP4 alters insulin sensitivity, at least in part, by affecting insulin signalling in muscle through alterations in the degree of tyrosine-phosphorylated ILS-1 and PI(3)K activation. Thus RBP4 may contribute to the pathogenesis of type 2 diabetes and a reduction in circulating RBP4 may have therapeutic opportunities.

Indications

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

Principle

This ELISA is designed to be used for the quantitative determination of RBP4 in serum, plasma and urine. In a first incubation RBP4 is bound to a rabbit antibody immobilised in the wells of a microtitre plate. After a wash step to remove unreacted substances, antibody labelled with peroxidise is added and a second incubation is carried out. After a further wash, the bound enzyme is quantified to provide a measure of bound RBP4, which is proportional to its concentration in the sample. Quantification is achieved by comparison with RBP4 calibrators.

Materials Provided

- Coated Microtitre Plate (a)
  (8 x 12) stripwells coated with a rabbit antibody.
- Labelled Antibody Concentrate (b)
  (200 µl) Peroxidase labelled rabbit anti-RBP4 antibody.
- Sample Diluent (c)
  (100 ml) sample dilution buffer, ready to use.
- Standards (d-h)
  (2 x 5 vials) lyophilised calibrators: 0, 1.1, 3.3, 11.0 and 33 µg/l concentrations.
- Controls (i,j)
  (2 x 2 vials) lyophilised low and high controls.
- Substrate (k)
  Tetramethylbenzidine (TMB) substrate, ready to use.
- Stop Solution (l)
  Dilute sulphuric acid, ready to use.
- Wash Buffer Concentrate (m)
  (2 x 100 ml) 10x concentration phosphate buffered saline containing detergent and preservative.

Materials Required but not Supplied

- Deionised water
- Calibrated pipettes capable of delivering 5 to 1000 µl
- Plate sealers
- Microtitre plate shaker
- Multi-channel or repeating dispenser
- Vortex mixer
- Microtitre plate reader

Warnings and Precautions

- For *in vitro* diagnostic use only.
- Human materials used in the kit components have tested negative for HIV, hepatitis B and hepatitis C. Nevertheless, for safety reasons, all components should be treated as potentially infective.
- Stop solution is diluted sulphuric acid and therefore must be handled with care. It can cause burns and so gloves, safety glasses and appropriate protective clothing should be worn when handling it. Any spills should be wiped up immediately using large volumes of water.
- Reagents should not be used beyond the expiry date shown on the kit label.

Preparation and Storage of Reagents

- To run the assay more than once, refer to the kit storage conditions indicated on the label. Prepare only the required quantity of reagents for each run. Up to 4 runs may be performed with each kit.
- Reagent vials containing a volume of less than 100 µl should be centrifuged before use.
- Wash buffer concentrate should be diluted 1:10 in deionised water before use. If any crystals have formed during storage, these should be re-dissolved by warming the wash buffer to 37º prior to dilution. Wash buffer concentrate is stable when stored at 2-8º up to the expiry date on the label. Diluted wash buffer can be stored in a closed vessel for up to 4 weeks at 2-8ºC.
- Lyophilised calibrators and controls must be reconstituted with 0.5 ml deionised water before use. Allow the vials to stand for 10 minutes and then mix thoroughly by gentle inversion. Reconstituted calibrators and controls are stable for 2 weeks when stored at 2-8º.
- Labelled antibody concentrate should be diluted 1:100 in wash buffer before use. The concentrate is stable when stored at 2-8ºC up to the expiry date**. Dilute labelled antibody is not stable and cannot be stored for further use.**
- All other test reagents are ready to use. These reagents are stable until the expiry date given on the kit label when stored at 2-8ºC.

Specimen Collection and Preparation

Plasma and Serum

Plasma and serum samples may be stored at 4ºC for up to two weeks prior to assay. For longer storage, samples should be frozen and kept at -20ºC.

Samples should be diluted 1:5000 with Sample Diluent before use. This can be carried out effectively by two sequential dilutions, namely 20µl sample + 980 µl buffer (1:50), followed by 10 µl of diluted sample + 990 µl diluent (1:100).

Urine

Adjust the pH of the urine to 7.0 (± 0.5) with 1.0 M NaOH. Samples are then stable for up to two weeks when stored at 4ºC. For longer storage, urine specimens should be frozen and stored at -20ºC.

Before use, dilute the urine 1:10 in Sample Diluent or 1:100 in the event that the urine concentration of RBP4 exceeds 330 µg/l.

Assay Procedure

1. Bring all components to room temperature before use.
2. Wash the required number of wells 5 times with working strength Wash Buffer (250 µl/well).
3. Add 100 µl of standard, control or diluted sample to the appropriate well. It is recommended that all determinations are carried out in duplicate.
4. Cover with a plate sealer and incubate for 1 hour at room temperature on a plate shaker.
5. Discard the contents of the wells by inversion and wash the plate 5 times with working strength Wash Buffer (250 µl/well).
6. Add 100µl working strength Labelled Antibody solution to each well.
7. Incubate for 1 hour at room temperature on a plate shaker.
8. Discard the contents of the wells by inversion and wash the plate 5 times with working strength Wash Buffer (250 µl/well).
9. Add 100 µl TMB Substrate to each well
10. Incubate for 10-20 minutes at room temperature in the dark to allow sufficient colour development.
11. Add 50µl of Stop Solution to each well.
12. Read the absorption in an ELISA reader at a wavelength of 450 nm.

Results

The results may be calculated automatically using a cubic spline or 4-parameter curve fit. The concentration of the samples can be read directly from this standard curve. Results of serum or plasma samples should be multiplied by 5000 to derive the original concentration. Results of urine measurements should be multiplied by the appropriate dilution factor.

Limitations

- Only if test instructions are rigidly followed will optimum results be achieved.
- Reproducible results depend on careful pipetting, observation of incubation periods and temperature, as well as thorough mixing of all prepared solutions.
- While rinsing, check that all wells are filled evenly with wash buffer, and that there are no residues in the wells.

Quality Control

The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels. It is also recommended to make use of national or international Quality Assessment programs where possible in order to ensure the accuracy of the results. Employ appropriate statistical methods for analyzing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. If, after checking the above mentioned items, there is no error, contact Invitron directly.

Expected Values

Normal Ranges in Serum or Plasma

Adults	20-75 mg/l
Newborns	11-34 mg/l
Age 6 months	18-50 mg/l

Urine

0.01-0.54 mg/l

It is recommended that each laboratory establishes its own reference range.

Performance

Precision

Intra-assay precision of the Invitron RBP4 assay was estimated at two dose levels based on 16 measurements as shown in the following table.

Sample	Mean Concentration (µg/l)	CV (%)
A	24.1	5.0
B	11.1	5.0

Linearity

A single patient sample was diluted in Sample Diluent. The results obtained in the RBP4 assay are shown in the table below.

Sample Dilution	Expected Concentration (µg/l)	Observed Concentration (µg/l)
1:7000	4.8	4.8
1:14000	2.4	2.8
1:28000	1.2	1.2
1:56000	0.6	0.8

References

Yang Q *et al*. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 2005; 436: 356-362.

Graham TE *et al*. Retinol-binding protein 4 and insulin resistance in lean, obese and diabetic subjects. New Eng J Med 2006; 354: 2552-2563.

For additional information and product support please contact:

Invitron Limited
Wyastone Business Park, Wyastone Leys, Monmouth NP25 3SR, UK
Tel: +44 (0)1600 891536
Fax: +44 (0)1600 891537
info@invitron.com
www.invitron.com