DRG® Retinol Binding Protein (RBP) ELISA (EIA-4391)

CE

Revised 29 July 2008 (Vers. 3.0) **RUO in the USA**

1 INTENDED USE

The DRG RBP ELISA 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 use only.

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. β-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

Kit Components	Quantity
Microtiter plate, one holder with precoated strips	12 x 8 wells
Wash Buffer concentrate 10x	2 x 100 mL
Enzyme Conjugate, (rabbit anti RBP/RBP4, peroxidase-labled)	200 µL
Control, lyophilized	2 vials
Sample Dilution Buffer, ready-to-use	100 mL
Standards, lyophilized (0; 1.1; 3.3; 11; 33 µg/L)	2 x 5 vials
TMB Substrate Solution (Tetramethylbenzidin), ready-to-use	15 mL
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 **Wash Buffer concentrate** must be diluted with aqua bidest. **1:10** before use (100 mL Wash Buffer + 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 **Standards 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 Standards and Control could be stored for two weeks at 2 - 8°C.
- The **Enzyme Conjugate** (POD-Antibody) must be diluted 1:100 in wash buffer (100 µL POD antibody and 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 could 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* use only.
- The standards 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 or serum
Samples can be stored for two weeks at 4°C. For longer storage, freeze at or below -20°C.
The samples should be **diluted 1:5000** in Dilution Buffer before use.
For Example
Dilution I:
20 µL sample + 980 µL Dilution Buffer = 1:50
Dilution II:
10 µL Dilution I + 990 µL Dilution Buffer = 1:100.
It results a final dilution of 1:5000.

Urine
Adjust the urine to a pH of 6 to 8 with 1 N NaOH. Samples are stable at 2-8°C for 2 weeks. For longer storage, freeze at or below -20°C.
Urine has to be **diluted 1:10** (e.g. 100 µL Urine + 900 µL Dilution Buffer).
Urine with a RBP4 **concentration > 330 µg/L** must be diluted **1:100**, e.g. 10 µL urine + 990 µL Dilution Buffer

9 ASSAY PROCEDURE

9.1 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. DRG can therefore not be held reliable for any damage resulting from this.
Carry out the assay with the actual manual delivered with the kit.

9.2 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*, *Control* 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** 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 cavities **5 x with 250 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 with an ELISA reader at 450 nm against 620 nm (or 690 nm) as reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the measurement range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as reference.

10 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 algorhythm 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 in place of data generations at the time of the assay

Serum or Plasma
Multiply the result **with 5000** to get the real concentration.

Urine
Multiply the result **with the dilution factor** to get the real concentration.

11 LIMITATIONS

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

12 QUALITY CONTROL

DRG recommends commercial control samples for 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.

12.1 Expected values

Plasma or Serum:	Adults	30 – 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

13.1 Precision and reproducibility

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

Intra-Assay, n= 16

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

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

Inter-Assay, n= 25

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

13.2 Sensitivity

The detection limit was defined as S0 + 2 SD and determined to be 0.9 µg/lL.

13.3 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 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. DRG 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 DRG along with a written complaint.

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SYMBOLS USED WITH DRG ASSAYS

Symbol	English	Deutsch	Francais	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Distributeur	Distributeur	Distribuidor	Distributtore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..

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