

HyTest NEWS



Retinol-Binding Protein (RBP4)



Retinol-binding protein (RBP4) belongs to a lipocalin protein family and functions as a carrier protein for vitamin A in serum. Human retinol-binding protein circulating in blood consists of 183 amino acid residues. Several truncated isoforms of RBP4 lacking 1, 2, 4 or 6 of the very C-terminal residues were also described in literature (7). In blood RBP4 carries retinol (vitamin A) which is bound to RBP4 in equimolar ratio. Besides, major part of circulating RBP4 forms complex with prealbumin (transthyretin) and according to Jaconi et al. only small fraction of free RBP4 can be found in serum. (7)

RBP4 has been studied since 1960s mainly as a transporter of retinol, but recent data suggest that RBP4 may contribute to pathogenesis of type 2 diabetes. Yang et al. demonstrated that serum RBP4 levels are elevated in patients with obesity and type 2 diabetes. Studies in mice showed that serum RBP4 may cause insulin resistance (1). So, on the one hand, there is growing body of evidence demonstrating that RBP4 is a promising marker of the risk of type 2 diabetes, but on the other hand there is conflicting situation in the literature regarding RBP4 clinical utility

in predicting insulin resistance and type 2 diabetes (3). Some authors show strict correlation between circulating RBP4 and magnitude of insulin resistance in subjects with obesity and type 2 diabetes and non-obese subjects with family history of type 2 diabetes (2). On the contrary, others (4,5) had not found any correlation between those variables. This confusing situation could be at least partially explained by the heterogeneity of the RBP4 in serum and by methodological shortcomings in determining level of circulating RBP4 (6). If epitope of diagnostic antibody is influenced by RBP4 truncation or by complex formation with retinol or prealbumin, then level of RBP4 determined by the assay, utilizing such antibody, would be different from the results of measurements by the assays with antibodies not susceptible to such modifications.

HyTest offers set of mouse monoclonal anti-human RBP4 antibodies suitable for development of sandwich immunoassays for quantitative detection of circulating RBP4 in human plasma as well as for immunodetection of RBP4 in direct ELISA, Western blotting or to be used for immunoprecipitation of the antigen.

Antibody ordering information:

MAb	Cat.#	Specificity	Subclass	Application
RB42	4RB2	Retinol-binding protein 4 (RBP4)	IgG1	EIA, WB
RB45	4RB2	Retinol-binding protein 4 (RBP4)	IgG1	EIA, WB
RB48	4RB2	Retinol-binding protein 4 (RBP4)	IgG1	EIA, WB
RB49	4RB2	Retinol-binding protein 4 (RBP4)	IgG1	EIA, WB
RB51	4RB2	Retinol-binding protein 4 (RBP4)	IgG1	EIA, WB
RB55	4RB2	Retinol-binding protein 4 (RBP4)	IgG1	EIA, WB

Antigen ordering information:

Product	Cat.#	Purity	Source
Retinol-binding protein 4 (RBP4) from human plasma, complexed with prealbumin	8RP7	>70%	Human plasma
Retinol-binding protein 4 (RBP4) from human plasma, free	8RF9	>95%	Human plasma



Anti-human RBP4 monoclonal antibodies

Host animal:	Mice Balb/c
Cell line used for fusion:	Sp2/0
Antigen:	Human recombinant retinol-binding protein
Purification method:	Protein A affinity chromatography
Presentation:	MABs solution in PBS with 0.1 % sodium azide
Application:	ELISA, RBP4 sandwich immunoassay, Western blotting

Hybridoma clones have been derived from hybridization of Sp2/0 myeloma cells with spleen cells of Balb/c mice immunized with human recombinant RBP4.

Applications

1. Sandwich immunoassay for RBP4 detection in human plasma

Anti-human RBP4 MABs were obtained after mice immunization with human recombinant RBP4. All MABs were tested in direct ELISA with human recombinant and native (endogenous, purified from human blood) RBP4. Best MABs were further tested in sandwich immunoassay and several two-site combinations demonstrating highest sensitivity for both – recombinant and endogenous proteins, were selected (Fig. 1) and recommended by our specialists for the development of RBP4 sandwich immunoassays.

HyTest offers MABs RB42, RB48, RB45, RB49, RB51 and RB55, which are suitable for immunodetection of native RBP4 in direct ELISA and sandwich immunoassay. Recommended combinations of antibodies for the development of sandwich immunoassay are (capture -detection):

- RB48 - RB42
- RB48 - RB49
- RB48 - RB51
- RB55 - RB45

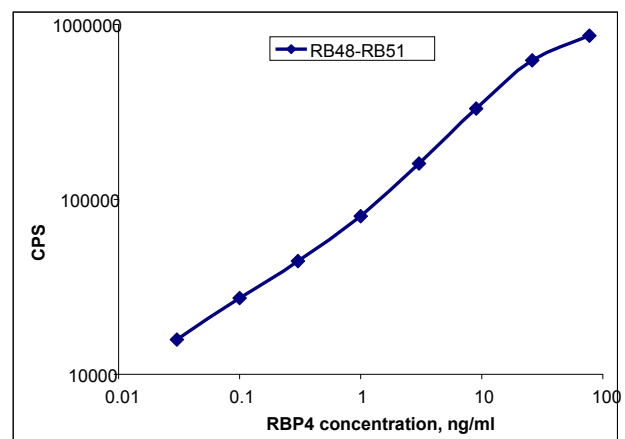


Figure 1. Calibration curve of RBP4 sandwich immunoassay.
Capture antibody: RB48 (1 µg/well)
Detection antibody: RB51 labeled with stable Eu³⁺ chelate (0.2µg/well)
Antigen: Purified endogenous RBP4



Selected assays recognize endogenous antigen in highly diluted human plasma (Fig. 2)

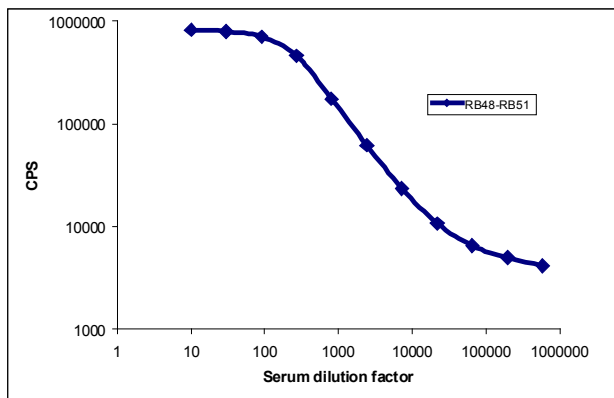


Figure 2. Titration curve of human plasma sample.
 Capture antibody: RB48 (1 µg/well)
 Detection antibody: RB51 labeled with stable Eu^{3+} chelate (0.2 µg/well)
 Antigen: Normal human serum diluted with PBS containing 0.1% Tween-20

Immunoreactivity of native RBP4, being measured by recommended MAb combinations, is not changed in the presence of EDTA in the tested sample (Fig. 3)

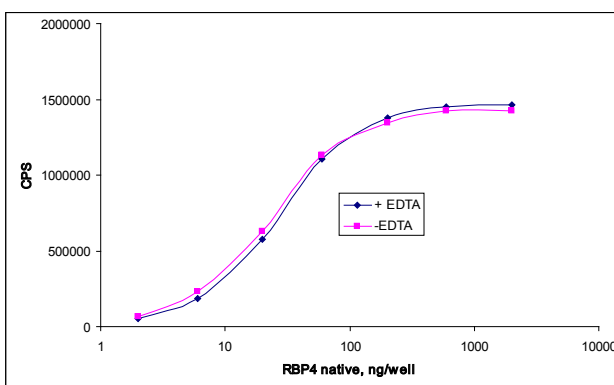


Figure 3. Immunodetection of purified endogenous RBP4 in sandwich immunoassay by RB48-RB42 MAb assay in presence of 5 mM EDTA (blue line) or in absence of EDTA (pink line).
 Capture antibody: RB48 (1 µg/well)
 Detection antibody: RB42 labeled with stable Eu^{3+} chelate (0.2 µg/well)
 Antigen: Native isolated RBP4

All HyTest anti-RBP4 MAbs recognize both free RBP4 and RBP4 complexed with prealbumin.

2. Immunoprecipitation

HyTest anti-RBP4 MAbs being immobilized onto BrCN-activated Sepharose could be used as an affinity matrix for immunoprecipitation of RBP4.

3. Western blotting

HyTest MAbs RB42, RB45, RB48, RB51 could be used for RBP immunodetection in Western blotting (Fig. 4)

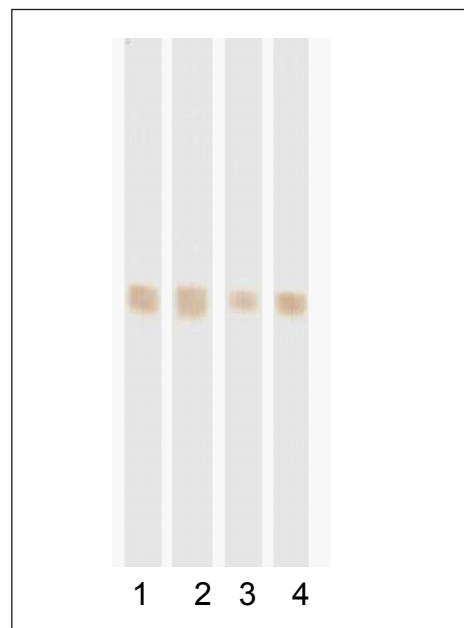


Figure 4. Immunodetection of RBP4 in Western blotting after SDS-electrophoresis in reducing conditions by MAb RB42 (lane 1), RB45 (lane 2), RB48 (lane 3) and RB51 (lane 4).
 1 µg per track of purified endogenous RBP4 was loaded onto gel.

Purified endogenous RBP

Native RBP represents the most natural form of RBP4 and is therefore the antigen of choice for assay calibration. It is known that in serum RBP4 exist mostly as a 1:1 complex with prealbumin (transthyretin) and only small part of RBP4 in the blood is presented as a free form. (7)

HyTest offers two types of purified native RBP4 antigen: free and complexed with prealbumin. Both forms of endogenous RBP4 (free and complexed) were purified from normal human serum in mild conditions using several chromatographic steps. (Fig. 5)

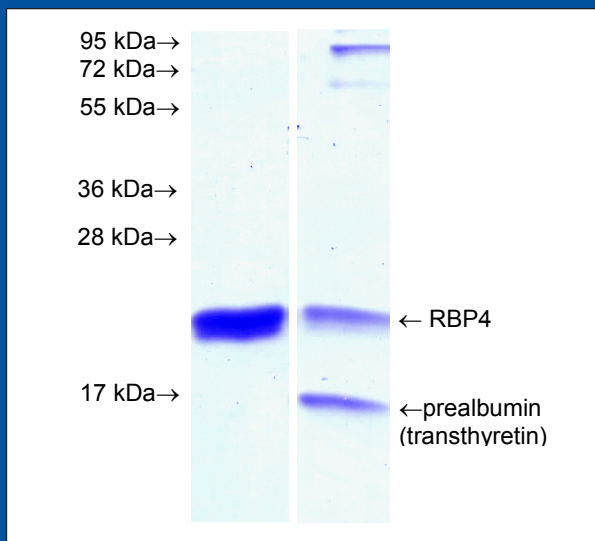


Figure 5: RBP4 isolated from normal human serum (coomassie-stained gel after SDS-electrophoresis in reduced conditions).

Lanes:

1: free native RBP4, 3 µg per track

2: native RBP4 complexed with prealbumin, 2 µg of total protein per track
Molecular weight marker positions are marked by arrows.

Both native free and native complexed RBP4 antigens are not affected by multiple (at least 5 - 7) freeze-thaw cycles (Fig. 6).

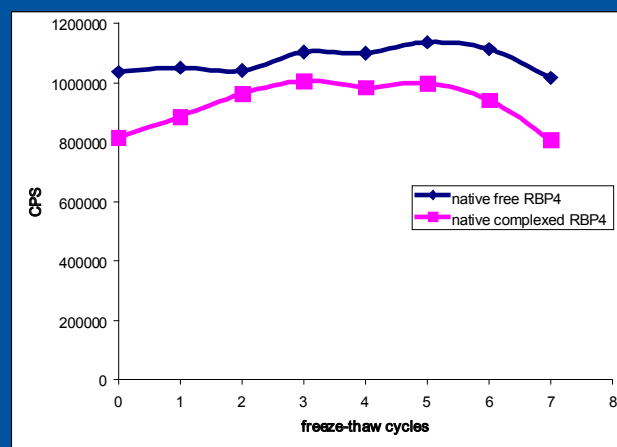


Figure 6. Immunoreactivity of native free and native complexed RBP4 after several freeze-thaw cycles measured with the assay RB48 - RB42. Capture antibody: RB48 (1 µg/well) Detection antibody: RB42 labeled with stable Eu³⁺ chelate (0.2µg/well) Antigen: Native isolated RBP4.

References:

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2. Graham, T., et al., Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *New Engl. J. Med.*, 2006, 354, 24, 2552-2563.
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4. Lewis, J., et al., Plasma retinol-binding protein is unlikely to be a useful marker of insulin resistance. *Diabetes Res. Clin. Pract.* 2008, 80, 13-15.
5. Promintzer, M., et al., Insulin resistance is unrelated to circulating retinol binding protein and protein C inhibitor. *J. Clin. Endocrinol. Metab.* 2007, 92, 4306-4312.
6. Graham, T., et al., Shortcomings in methodology complicate measurements of serum retinol binding protein (RBP4) in insulin resistant human subjects. *Diabetologia*, 2007, 50, 814-824.
7. Jaconi, S., Characterization of two post-translationally processed forms of human serum retinol-binding protein: altered ratios in chronic renal failure. *J. Lip. Res.*, 1995, 36, 1247-1253.



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