Retinol-binding protein 4 (RBP4)

Retinol-binding protein 4 (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, a major part of circulating RBP4 forms complex with prealbumin (transthyretin) and according to Jaconi et al. only a small fraction of free RBP4 can be found in serum. (7)

RBP4 has been studied since the 1960s, mainly as a transporter of retinol. However, recent data suggests 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). Therefore, while on the one hand there is a growing body of evidence demonstrating that RBP4 is a promising marker of the risk of type 2 diabetes, on the other hand there is a conflicting situation in the literature regarding RBP4 clinical utility in terms of predicting insulin resistance and type 2 diabetes (3). Some authors show a strict correlation between circulating RBP4 and magnitude of insulin resistance in subjects with obesity and type 2 diabetes and non-obese subjects with a 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 at least partially be 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 the level of RBP4 determined by the assay, utilizing such an antibody, would be different from the results of measurements by the assays with antibodies that are not susceptible to such modifications.

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

PURIFIED ENDOGENOUS RBP4

Native RBP4 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 a 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. 1).

Both native free and native complexed RBP4 antigens are unaffected by multiple (at least 5 - 7) freeze-thaw cycles (Fig. 2).

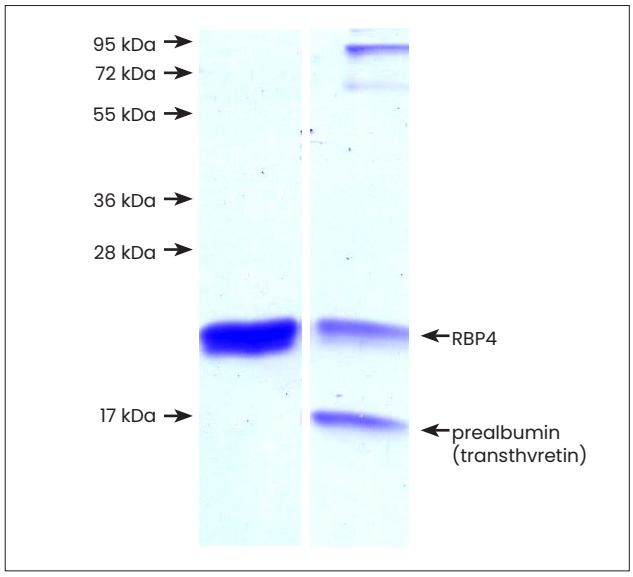

Figure 1.
RBP4 isolated from normal human serum (coomassie-stained gel after SDS-electrophoresis in reduced conditions).
Lanes:
1: free native RBP4, 3 mμ μg per track
2: native RBP4 complexed with prealbumin, 2 μg of total protein per track
Molecular weight marker positions are marked by arrows.

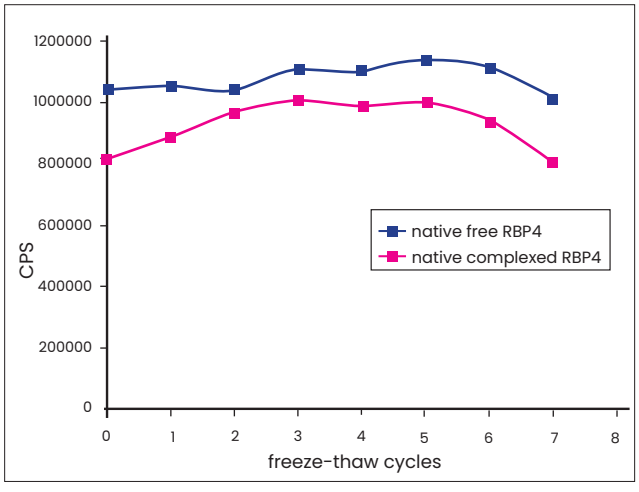

Figure 2.
Immunoreactivity of native free and native complexed RBP4 after several freeze-thaw cycles measured with the assay RB48 - RB42.
Capture antibody: RB48 (1 μg/well)
Detection antibody: RB42 labeled with stable Eu^{3+} chelate (0.2 μg/well)
Antigen: Native isolated RBP4.

MONOCLONAL ANTIBODIES SPECIFIC TO RBP4

Western blotting

Hytest MAbs RB42, RB45, and RB48 could be used for RBP immunodetection in Western blotting (Fig. 3).

Immunoprecipitation

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

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. The best MAbs were further tested in sandwich immunoassay and several two-site combinations demonstrating the highest sensitivity for both recombinant and endogenous proteins were selected and recommended by our specialists for the development of RBP4 sandwich immunoassays.

Hytest offers MAbs RB42, RB45, RB48, and RB55, that are suitable for the 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
RB55 - RB45

Selected assays recognize endogenous antigen in highly diluted human plasma.

Immunoreactivity of native RBP4, being measured by recommended MAb combinations, is unchanged in the presence of EDTA in the tested sample (Fig. 4).

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

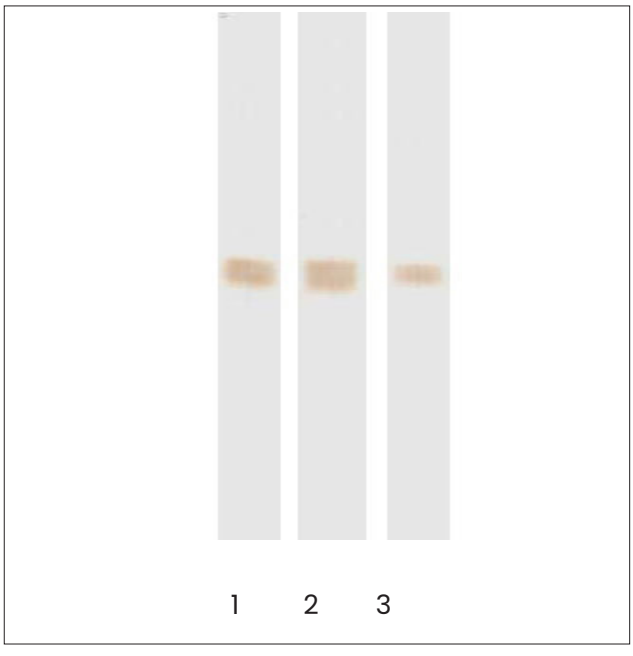

Figure 3.
Immunodetection of RBP4 in Western blotting after SDS-electrophoresis in reducing conditions by MAb RB42 (lane 1), RB45 (lane 2), and RB48 (lane 3).
1 µg per track of purified endogenous RBP4 was loaded onto gel.

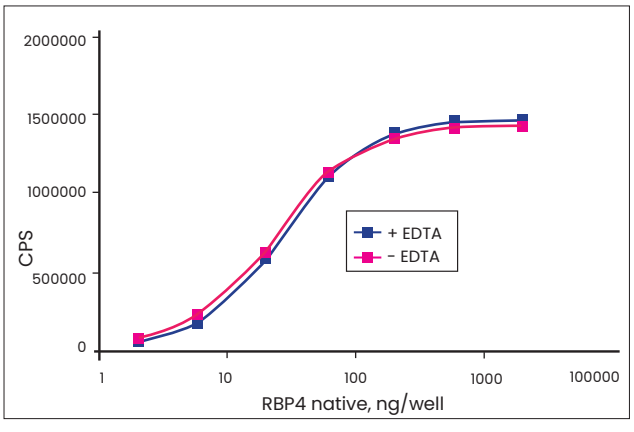

Figure 4.
Immunodetection of purified endogenous RBP4 in sandwich immunoassay by RB48-RB42 MAb assay in presence of 5 mM EDTA (blue line) or in absence of EDTA (pink line).
Capture antibody: RB48 (1 µg/well)
Detection antibody: RB42 labeled with stable Eu^{3+} chelate (0.2 µg/well)
Antigen: Native isolated RBP4

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ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Retinol-binding protein (RPB4)	4RB2	RB42	IgG1	EIA, WB
		RB45	IgG1	EIA, WB
		RB48	IgG1	EIA, WB
		RB55	IgG1	EIA, WB

ANTIGEN

Product name	Cat. #	Purity	Source
Retinol-binding protein 4 from human plasma, free form	8RF9	>95%	Pooled human plasma
Retinol-binding protein 4 from human plasma, complexed with prealbumin	8RP7	>70%	Pooled human plasma

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