ImmunoDiagnostics Limited

FOR RESEARCH USE ONLY

Human RBP4 ELISA Kit

(Catalog Number: 31060)

For the quantitative determination of human RBP4 concentrations in cell culture supernates, serum or plasma

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INTRODUCTION

Retinol binding protein 4 (RBP4), originally known as a specific transport of retinol in blood, is also a novel inflammatory and insulin resistance marker[1]. Serum RBP4 levels are elevated in insulin-resistant mice and humans with obesity and type 2 diabetes[2,3]. Animal experiments found that increased secretion of RBP4 might reduce insulin-dependent glucose uptake by muscle tissue by reducing the activity of phosphoinositide 3-kinase (PI(3)K), and increased hepatic glucose output by increasing the expression of the enzyme PEPCK[2]. Studies suggested that elevated serum RBP4 was associated with components of the metabolic syndrome, including increased body-mass index, waist-to-hip ratio, serum triglyceride levels, and systolic blood pressure and decreased high-density lipoprotein cholesterol levels[4]. Furthermore, circulating RBP4 concentrations were associated with subclinical cardiovascular disease, which imply that RBP4 could be involved in the development of atherosclerosis[5].

PRINCIPLE OF THE ASSAY

This assay is a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies against human RBP4. The microtiter plate is pre-coated with a monoclonal antibody specific for human RBP4 and the nonspecific binding sites are blocked. Standards and samples are pipetted into the wells and any human RBP4 present is bound by the immobilized antibody. After washing away any unbound substances, a horseradish peroxidase (HRP)-linked monoclonal antibody specific for human RBP4 is added to the wells. After wash step to remove any unbound reagents, an HRP substrate solution is added and color develops in proportion to the amount of human RBP4 bound initially. The assay is stopped, and the optical density of the wells is determined using a microplate reader. Since the increases in absorbance are directly proportional to the amount of captured human RBP4, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This Human RBP4 ELISA kit is designed for quantification of human RBP4 in serum, plasma, and adipocyte extracts or cell culture supernate samples.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Microtiter Strips (96 wells) coated with a mouse monoclonal antibody against human RBP4
2. 10×Wash Buffer, 40 mL
3. 5×Assay Buffer, 30 mL
4. 100×Detection Antibody, a mouse monoclonal antibody against human RBP4 conjugated with horseradish peroxidase, 0.12 mL
5. Human RBP4 Standard, 200 ng of recombinant human RBP4, lyophilized
6. Substrate solution, 12 mL, ready for use
7. Stop Solution, 12 mL, ready for use

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips
2. 96-well plate or manual strip washer
3. Buffer and reagent reservoirs
4. Paper towels or absorbent paper
5. Plate reader capable of reading absorbency at 450 nm
6. Distilled water or deionized water

STORAGE

The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antibody-coated strips from the human RBP4 microtiter plate, return them to the foil pouch and re-seal. Once opened, the strips may be stored for up to one month at 28°C.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1×Assay buffer

Prepare 1×Assay buffer by mixing all of the 5×Assay Buffer (30 mL) with 120 mL of distilled water or deionized water. If precipitates are observed in the 5×Assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. 1×Assay buffer may be stored at 2-8°C for up to one month.

B. 1×Wash buffer

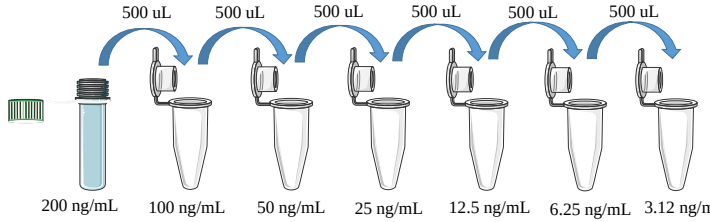
Prepare 1×Wash buffer by mixing all of the 10×Wash Buffer (40 mL) with 360 mL of distilled water or deionized water. If precipitates are observed in the 10×Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. 1×Wash buffer may be stored at 2-8°C for up to one month.

C. 1×Detection antibody solution

Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µL of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection Antibody to 2-8°C immediately after the necessary volume is removed.

PREPARATION OF STANDARDS AND SAMPLES

Human RBP4 Standards: Reconstitute the lyophilized standard with 1 mL of 1×Assay buffer to generate a standard stock solution of 200 ng/mL. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Pipette 500 µL of 1×Assay buffer to 100, 50, 25, 12.5, 6.25, 3.12 ng/mL tubes. Use the standard stock solution to produce a serial dilution as shown below.



1×Assay buffer serves as the zero standard (0 ng/mL). The reconstituted standard stock should be aliquoted and frozen at -20ºC for one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample Preparation:

Serum sample generally requires a **500-fold** dilution in this assay. A twostep dilution is suggested. Step1-Add 10 µL of sample to 490 µL of 1×Assay buffer to give a 50-fold diluted sample solution. Step 2-Add 50 µL of the 50-fold diluted sample solution to 450 µL of 1×Assay buffer to give a final 500-fold diluted sample solution. Cellular extract and culture media dilutions will vary and need to be optimized by the user, also use 1×Assay buffer to prepare these samples. It is recommended that the users establish their own dilution factors based on the concentration range of their samples.

ASSAY PROCEDURE

It is recommended that all standards and samples be assayed in duplicate.

1. Add 100 µL of standards and samples to each well, incubate at room temperature for 1 hour.
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µL of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
3. Add 100 µL of 1×Detection antibody solution to each well, incubate at room temperature for 1 hour.
4. Wash each well 4 times as described in step 2.
5. Add 100 µL of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
6. Add 100 µl of Stop solution to each well, mix well by gently tapping the plate.
7. Measure absorbance of each well at 450 nm immediately.

CALCULATION

1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against RBP4 concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4parameter or log-log curve fitting can be used for calculation.

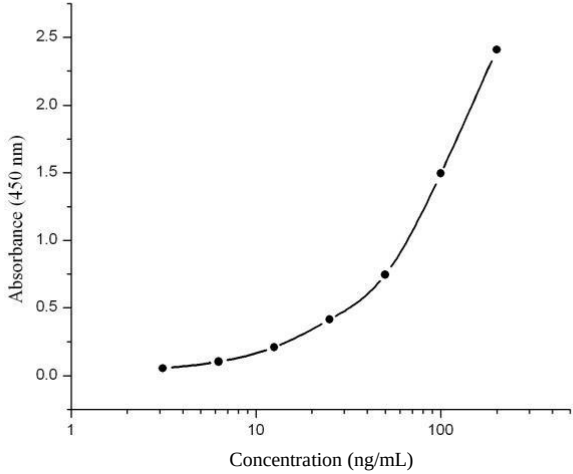
3. Determine RBP4 concentration of samples from standard curve and multiply the value by the dilution factor.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each set of sample assay.

Human RBP4 (ng/mL)	Absorbance (450 nm)	Blanked Absorbance
0	0.091	0
3.12	0.146	0.055
6.25	0.194	0.103
12.5	0.30	0.209
25	0.504	0.413
50	0.836	0.745
100	1.586	1.495
200	2.499	2.408

Human RBP4 standard curve (4-parameter)



ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of RBP4 that can be detected by this assay is 3.12 ng/mL.

B. Specificity

The antibody pair used in this assay is specific to human RBP4 and does not cross-react with mouse and rat RBP4, and other cytokine or hormone molecules.

C. Precision

Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested 16 times on one plate.

Sample	Mean (µg/mL)	SD (µg/mL)	CV (%)
1	18.03	0.654	3.63
2	49.07	1.158	2.36
3	3.91	0.117	2.99

Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in 10 separate assays.

Sample	Mean (µg/mL)	SD (µg/mL)	CV (%)
1	6.53	0.284	4.35
2	17.11	0.524	3.06
3	44.21	1.154	2.61

D. Recovery

Serum samples were spiked with different amounts of human RBP4 and assayed.

Sample	Average % Recovery	Range %
Serum (n=4)	102	92-115

REFERENCES

1. Shepherd PR, et al. (1999). *N Engl J Med* 341:248-257.
2. Yang Q, et al. (2005). *Nature.* 436:356–362.
3. Cho YM, et al. (2006). *Diabetes Care* 29: 2457–2461.
4. Graham TE, et al. (2006). *N Engl J Med* 354: 2552–2563.
5. Ingelsson, E, et al. (2009). *Diabetes Care* 32: 733-735.

SUMMARY OF ASSAY PROCEDURE

Add 100 µL of Standard or sample to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well three times.
↓
Add 100 µL of 1×Detection antibody solution to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well four times.
↓
Add 100 µL of Substrate solution to each well.
↓
Incubate at room temperature for 15 minutes.
↓
Add 100 µL of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
↓
Calculation