

# **Retinol-binding protein 4 (RBP4) Turbidimetric Immunoassay Kit**

Catalogue number: 51060

For the quantitative determination of RBP4  
in human serum and plasma

This package insert must be read in its entirety before using this product  
Use only the current version of product data sheet enclosed with the kit

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**FOR RESEARCH USE ONLY  
NOT FOR USE IN DIAGNOSTIC PROCEDURES**

Version: 5.22



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**PACKING SPECIFICATION**

<b>Cat. No.</b>	<b>Size</b>	<b>Approximately tests</b>
51060-05	R1: 15ml, R2: 5ml	100
51060-10	R1: 30ml, R2: 10ml	200
51060-20	R1: 60ml, R2: 20ml	400
51060-50	R1: 150ml, R2: 50ml	1000
51060-100	R1: 300ml, R2: 100ml	2000

**INTRODUCTION**

Retinol-binding protein 4 (RBP4) is a 21 KDa protein mainly synthesized by hepatic cells, and its serum levels decreased significantly under the condition of liver injury or malnutrition. Since RBP4 is mainly absorbed and degraded by the proximal renal tubular epithelial cells, urine RBP4 is currently the most sensitive functional biomarker of proximal tubule. Its serum and urine levels increased 50- to 100-fold in several renal disorders. The normal range of RBP4 is 17- 61 mg/L in serum, and is 0-1.4 mg/L in urine respectively. Lower serum levels of RBP4 are suggestive of chronic liver disease, cirrhosis or Vitamin A deficiency, whereas urine concentration of RBP4 higher than the upper limit is indicative of renal Fanconi syndrome.

RBP4 PETIA kit developed by IMD can accurately measure RBP4 concentrations in serum, plasma and urine samples

**PRINCIPLE OF THE ASSAY**

This assay is a turbidimetric immunoassay for the quantitative measurement of RBP4 in human serum and urine. A standard or sample is added into a cuvette and mixed with the reaction buffer R1. After a short incubation, the test reagent R2, which is a suspension of microparticles coated with RBP4 antibodies, is added into the cuvette and mixed. The presence of RBP4 in the standard or sample causes the immune-particles to aggregate. The extent to which the microparticles aggregate is quantified by the amount of light scattering measured as absorbance by a chemistry analyzer. The concentration of RBP4 in unknown samples can be interpolated from a reference curve using the standards provided.

## **REAGENTS SUPPLIED**

R1 – Reaction buffer, a ready-to-use buffer solution containing salt, polyether compound and preservative

R2 – Test reagent, a ready-to-use suspension of polymer microparticles coated with rabbit anti-RBP4 polyclonal antibodies in storage buffer

## **OTHER MATERIALS REQUIRED, BUT NOT PROVIDED**

1. Clinical chemistry analyzer
2. RBP4 calibrator, provided separately (Cat No: 51060-S1)
3. RBP4 quality controls , optional, provided separately (51060-C1)
4. Deionized water
5. Analyzer-specific reagent containers for R1 and R2

## **STORAGE**

The kit should be stored at 2-8°C upon receipt. Once opened, the reagents may be stored at 2-8°C for up to 4 weeks.

## **SAMPLE HANDLING**

This kit can be used to determine RBP4 in human serum and plasma samples. Blood specimens should be collected aseptically into appropriate tubes. Plasma should be prepared by standard techniques for laboratory testing. The prepared specimens should be stored in closed vessels. If the assay cannot be performed within 24 hours or specimens are to be shipped, the specimens should be frozen at -20°C or below. For long-term storage of specimens, -70°C or below is recommended. To avoid freeze-thaw cycles, specimens should be aliquoted. Do not use hemolyzed, hyperlipemic, heat-treated or contaminated specimens. No dilution of the sample is required in this assay.

### ASSAY PROCEDURE

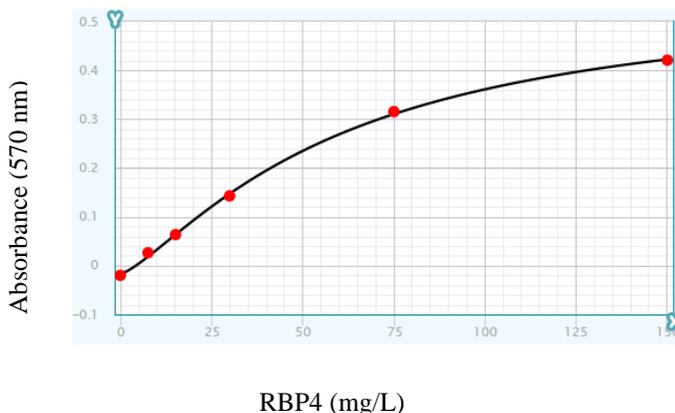
Assay procedures may vary depending on the automated chemistry analyzer to be used. A general example of assay procedures is stated as follow:

1. Dispense 150µl of R1 into a clean cuvette
2. Add 1.5µl of sample and incubate at 37°C for 5 minutes
3. Further add 50µl of R2
4. Read change of absorbance at 570 nm for 5 - 8 minutes after the addition of R2
5. Calculate the concentration of RBP4 in unknown sample by interpolation from a reference curve using the standards provided

### TYPICAL STANDARD CURVE

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

RBP4 (mg/L)	Absorbance (570 nm)
0	-0.0020
7.5	0.0263
15	0.0635
30	0.1429
75	0.3154
150	0.4208



## 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. 4-parameter curve fitting can be used for calculation.
3. Determine RBP4 concentration of samples from standard curve.

## ASSAY CHARACTERISTICS

### A. Sensitivity

The sensitivity is defined as the lower limit of detection and is estimated as the mean of the blank sample plus three times the SD obtained from the blank sample. The sensitivity of RBP4 assay is 2 mg/L.

### B. Precision

The precision of the RBP4 assay is  $CV < 6\%$ . Four samples consisting of two RBP4 controls and two serum samples were assayed 20 times separately.

Sample	Mean RBP4 (mg/L)	SD (mg/L)	CV
Control	6.8	0.2	3.40%
Control 2	55.5	0.1	1.90%
Serum1	19.7	0.4	2.20%
Serum 2	18.5	0.3	1.62 %

### C. Linearity

The RBP4 assay is linear between 2 mg/L to 150 mg/L.

### D. Interference

No interference was detected with hemoglobin up to 5 g/L, conjugated bilirubin up to 300 mg/L, free bilirubin up to 300 mg/L, and up to 5g/L lipid emulsion.

**References**

1. Tacke, F., Weiskirchen, R. et al. Liver function critically determines serum retinol-binding protein 4 (RBP4) levels in patients with chronic liver disease and cirrhosis. *Hepatology* 2008 Nov; 48(5), 1724–1725
2. Chaves GV, Peres WA, et al. Vitamin A and retinol-binding protein deficiency among chronic liver disease patients. *Nutrition*. 2015 May; 31(5): 664-8.
3. Norden AG, Lapsley M, et al. Urine retinol-binding protein 4: a functional biomarker of the proximal renal tubule. *Adv Clin Chem*. 2014;63:85-122.



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