

## Human RBP4 Sandwich ELISA Kit Datasheet

For the quantitative detection of human RBP4 concentrations in serum, plasma, cell culture supernatants, urine and saliva.

### General Information

Catalogue Number	KE00056
Product Name	Human RBP4 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	31.25-1000 pg/mL
Tested applications	Quantification ELISA

### Database Links

Entrez Gene	5950
SwissProt	P02753

### Kit Components & Storage

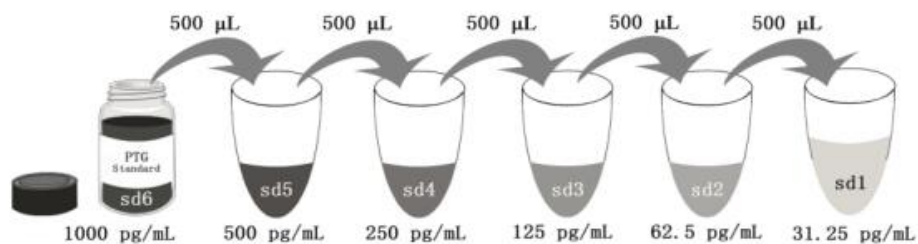
Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	<b>Unopened Kit:</b> Store at 2-8°C for 6 months or -20°C for 12 months.  <b>Opened Kit:</b> All reagents stored at 2-8°C for 7 days.  <b>Please use a new standard for each assay.</b>
Protein standard - 2000 pg/bottle; lyophilized*	2 bottles	
Detection Antibody, biotinylated (100X) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 1-ef - 30 mL/bottle	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

**NB: Do not use the kit after the expiration date.**

Sample Diluent PT 1-ef is for protein standard and samples.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

\*Add 2 mL Sample Diluent PT 1-ef in protein standard. This reconstitution gives a stock solution of 1000 pg/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1-ef	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

## Product Description

KE00056 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The RBP4 ELISA kit is to be used to detect and quantify protein levels of endogenous RBP4. The assay recognizes human RBP4. An antibody specific for RBP4 has been pre-coated onto the microwells. The RBP4 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for RBP4 is added to detect the captured RBP4 protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

## Background

RBP4 (retinol-binding protein 4) is a carrier protein that transports vitamin A (retinol) from the liver to the peripheral tissues. Synthesized primarily by hepatocytes and adipocytes as a 21 kDa non-glycosylated protein, RBP4 is secreted into the circulation as a retinol-RBP4 complex. In plasma the RBP4-retinol complex is bound to transthyretin (TRR), which prevents prevent kidney filtration. Two truncated forms of RBP4, RBP4-L (truncated at Leu-183) and RBP4-LL (truncated at Leu-182 and Leu-183), exist by proteolytic process. RBP4-L and RBP4-LL, which do not bind TTR, are normally excreted into the urine but accumulate in the serum during renal failure. Urinary RBP4 has been reported as marker for glomerular disease. RBP4 also was identified as an adipokine that elevated in some insulin-resistant states. Measurement of serum RBP4 could be used to assess the risk of insulin resistance, type 2 diabetes, obesity, and cardiovascular disease.

## Sample Preparation

Samples may require proper dilution to fall within the range of the assay. The serum or plasma is better to be diluted 1:200,000. 1:2 dilution is recommended for cell culture supernatants. 1:1,000 dilution is recommended for urine, 1:200 dilution is recommended for saliva.

## Safety Notes

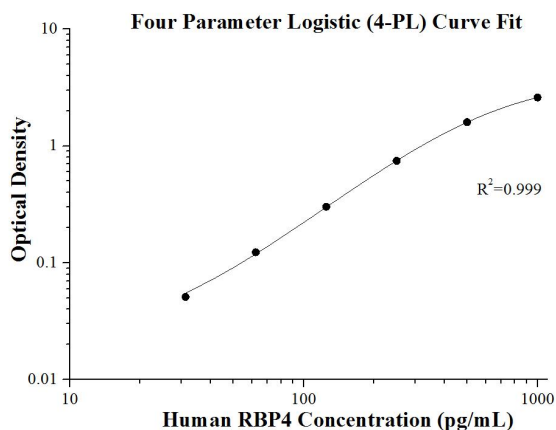
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## Assay Procedure Summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

## Example data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.091 0.088	0.090	-
31.25	0.142 0.139	0.141	0.051
62.5	0.21 0.215	0.213	0.123
125	0.388 0.394	0.391	0.302
250	0.833 0.835	0.834	0.745
500	1.68 1.694	1.687	1.598
1000	2.683 2.694	2.689	2.599

## Precision

**Intra-assay Precision** (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

**Inter-assay Precision** (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	500.0	13.9	2.8
2	20	115.0	3.1	2.7
3	20	22.9	2.2	9.4

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	572.0	42.3	7.4
2	20	115.6	8.1	7.0
3	20	33.0	3.2	9.9

## Recovery

The recovery of RBP4 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human plasma	1:200,000	100	92-110
	1:400,000	98	88-114
Cell culture supernatants	1:2	99	84-118
	1:4	100	94-111
Urine	1:1,000	105	99-111
	1:2,000	102	93-112
Saliva	1:200	118	117-121
	1:400	103	94-114

## Sample Values

Sample Type	Mean of Detectable (ng/mL)	Range (ng/mL)
Human serum (n=16)	43,250	9,560-150,130
Human plasma (n=16)	30,820	15,630-53,590
Urine (n=3)	151.4	139.4-240.5
Saliva (n=3)	32.3	28.8-36.5

## Sensitivity

The minimum detectable dose of human RBP4 is 2.3 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

## Linearity

To assess the linearity of the assay, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:25,000. The urine samples were initially diluted 1:100. The saliva samples were initially diluted 1:10.)

		Human plasma	Cell culture supernatants	Urine	Saliva
1:2	Average% of Expected	100	94	100	82
	Range (%)	99-101	82-100	99-101	79-85
1:4	Average% of Expected	100	104	91	100
	Range (%)	99-110	100-114	85-97	99-101
1:8	Average% of Expected	102	111	97	110
	Range (%)	95-109	104-120	93-101	100-119
1:16	Average% of Expected	105	104	101	102
	Range (%)	94-116	96-112	95-108	98-107

## References

1. Frey SK. et al. (2008). Lipids Health Dis. 7(29).
2. Yang Q. et al. (2005). Nature. 436(7049):356-62.