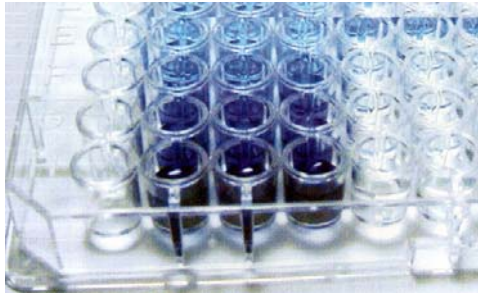


# Human RBP4 Competitive ELISA Kit

Cat. No. RC05H3EK



*Instruction Manual*  
**Version 1.3.0**

FOR RESEARCH USE ONLY  
NOT FOR USE IN DIAGNOSTIC PROCEDURES



## Table of Contents

Introduction .....	3
Assay Principles .....	3
Kit Components .....	4
Reagent Description .....	4
Storage of Reagents .....	4
Materials Required but not Supplied .....	5
Sample Collection and Storage .....	5
Flow Chart of Assay Procedure .....	6
Assay Procedure .....	7
Performance Characteristics .....	10
References .....	13
Plate Layout .....	14
Troubleshooting Guide .....	15

## **Introduction**

Retinol binding protein (RBP) 4 is the only specific transport protein for vitamin A in the circulation whose function is to deliver vitamin to target tissues (1). In obesity and type 2 diabetes, expression of Glut4 is significantly impaired in adipocytes. Glucose transport via Glut4 is the rate-limiting step for glucose use by muscle and adipose tissue (2). Yang et al. noted that adipocyte-specific deletion of Gluts led to notable elevation of RBP4 causing systemic insulin resistance, and that reduction of RBP4 improved insulin resistance (3). This identified a novel role of RBP4 in regulating insulin action and RBP4 is recorded as an adipocyte-derived hormone. Thus, measurement of serum or plasma RBP4 is a useful means for understanding of metabolic disorders.

## **Assay Principles**

This kit is a competitive enzyme-linked immunosorbent assay (ELISA) for quantitative determination of RBP4 in human serum, plasma or various tissue or cell culture supernatants. A polyclonal antibody recognizing native human RBP4 reacts with a series of predetermined recombinant human RBP4 standard proteins or serum or plasma under competition in the human RBP4-coated plate. Their relative reactivity is plotted with that of the standard proteins.

## Kit Components

- 1) Recombinant human RBP4 coated 96-well plate, 12X 8-well strips
- 2) 5X Wash concentrate, 100 ml
- 3) 5X Diluent, 50 ml
- 4) Antibody, 12 ml
- 5) 100X Detector, 150µl
- 6) Standard, recombinant human RBP4 expressed by HEK-293 cells, 1 vial, lyophilized
- 7) QC sample = a positive control of human serum RBP4, 1 vial, lyophilized (For actual concentration of QC sample, see the 'Certificate Of Analysis' enclosed)
- 8) Substrate I, 6 ml
- 9) Substrate II, 6 ml
- 10) Stop solution, 12 ml
- 11) Plate sealers, 3 sealers

## Reagents Description

**Antigen coated 96-well plate**, 12X 8-well strips, with absorbed recombinant human RBP4

**5X Wash concentrate**, buffered detergent solution, supplied as a 5X concentrate

**5X Diluent**, for sample and reagent dilution

**1X antibody**, polyclonal antibody against human RBP4

**100X detector**, HRP conjugated anti-rabbit IgG

**Substrate I and II**, chromogenic reagents

**Stop solution**, 1M H<sub>3</sub>PO<sub>4</sub>

**Standard**, 5µg, recombinant human RBP4

## Storage of Reagents

Reagent must be stored at 2-8°C when not in use. Reagents must be brought to room temperature before use. Do not expose reagents to temperature greater than 25°C. Diluted wash solution may be stored at room temperature for up to one month.

## Materials Required but not Supplied

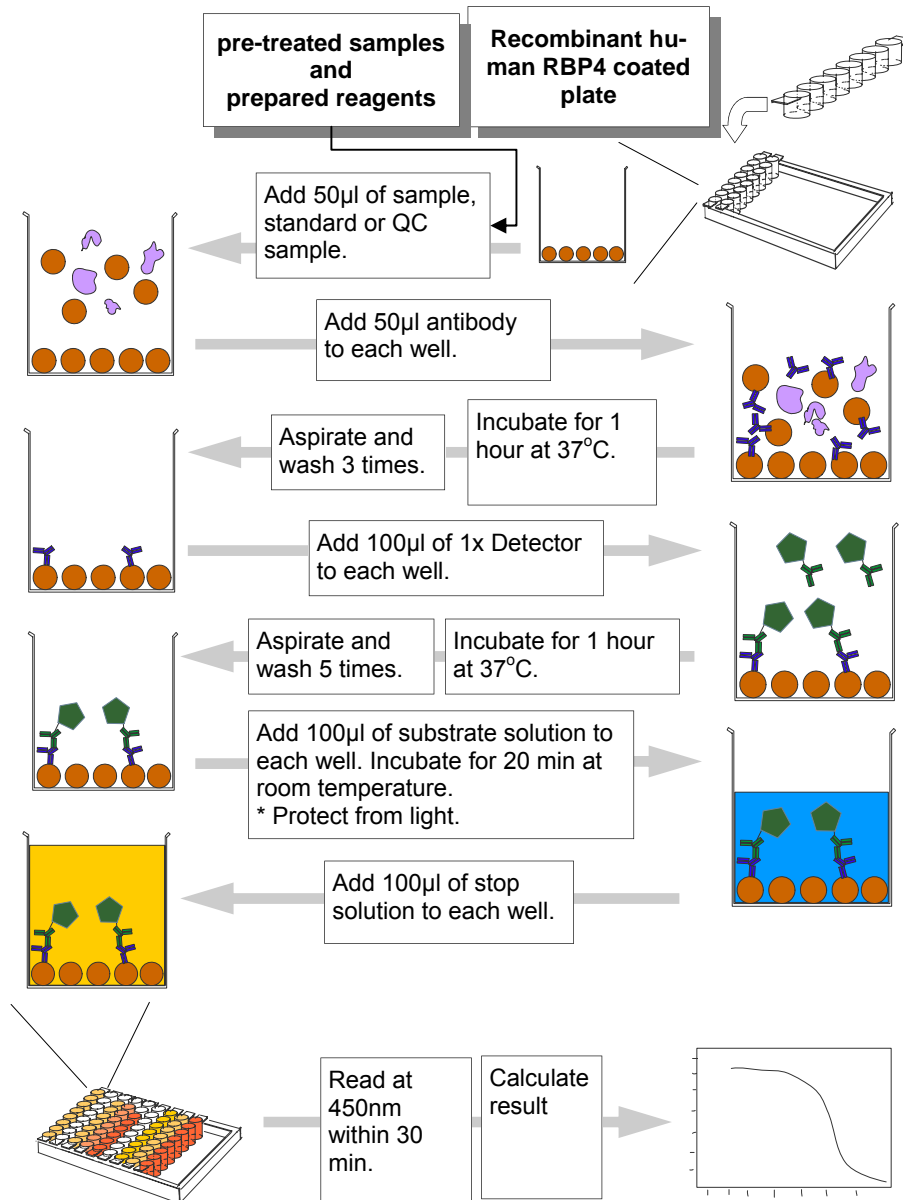
Precision single and multi-channel pipettes.  
Disposable pipette tips.  
Microtubes or equivalent for preparing dilutions.  
Disposable plastic containers for preparing working reagents.  
Reagent reservoirs.  
Microwell or microstrip plate reader 450 nm  
Deionized water

## Sample Collection and Storage

**Serum** Use a serum separator tube. Let samples clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1,000xg. Assay freshly prepared serum or store serum in aliquot at  $\leq -20^{\circ}\text{C}$  for later use. Avoid repeated freeze/thaw cycles.

**Plasma** Collect plasma using heparin, EDTA, or citrate as an anticoagulant. Centrifuge for 15minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma sample in aliquot at  $\leq -20^{\circ}\text{C}$  for later use. Avoid repeated freeze/thaw cycles.

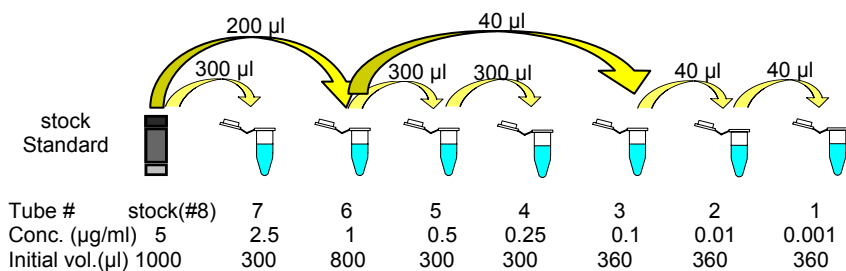
## Flow Chart of Assay Procedure



## Assay Procedure

### 1. Preparation of Reagents

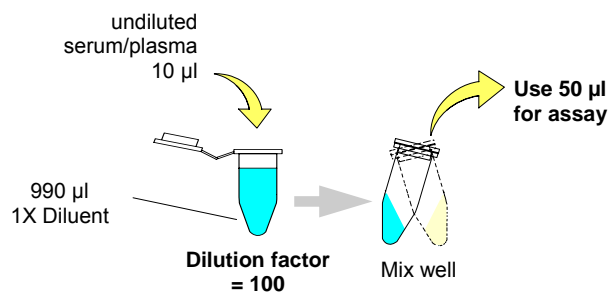
- 1) Allow all samples and kit components to equilibrate to room temperature (20-25°C).
- 2) Plan the plate configuration and create a plate map. Calculate the amount of working reagents to use.  
It is recommended that Standards and samples be run in duplicate.
- 3) Prepare **1X Wash Solution**. Dilute 5X Wash Concentrate 1:5 with deionized water (1 part 5X Wash Concentrate with 4 parts deionized water). The diluted 1X Wash Solution is stable for one month at room temperature.
- 4) Prepare **1X Diluent**. Dilute 5X Diluent 1:5 with deionized water (1 part 5X Diluent with 4 parts deionized water).
- 5) Prepare **1X Detector**. Dilute 100X Detector 1:100 with 1X Diluent (1 part 100X Detector with 99 parts 1X Diluent). Use the 1X Detector within one hour of preparation.
- 6) Freshly prepare just before use the **Substrate Solution** by adding one part Substrate I to one part Substrate II.
- 7) Prepare working aliquots of the Standard as follows:  
When opening the lyophilized Standard, remove cap gently as the lyophilizate may have become dislodged during shipping. Add 1 ml of deionized water to the Standard vial to make a stock Standard concentration of 5µg/ml(#8). Mix well. A recommended dilution scheme is as follows:



- a. Label 7 microcentrifuge tubes #1-7. Add 1X Diluent to the microcentrifuge tubes #1-7 as shown on page 7, respectively.
  - b. Add 300  $\mu$ l of the stock Standard solution(#8) to tube #7 and mix well. This is Standard tube #7 with a concentration of 2.5 $\mu$ g/ml.
  - c. Standard #6(1 $\mu$ g/ml) is then prepared by performing a 1:5 dilution of the stock Standard(#8). Standards #5 to #4 are prepared by performing a 1:2 dilution of the preceding Standard solution #6 and #5, respectively and mix well.
  - d. Standards #3 are then prepared by performing a 1:10 dilution of #6 Standard solution.
  - e. Standard #2 to #1 are prepared by performing a 1:10 dilution of the preceding solution #3 and #2, respectively and mix well.
  - f. Use 50 $\mu$ l of the final diluted Standards(#8 to #1) for ELISA.
- 8) Reconstitute QC sample in 1 ml of deionized water.

## 2. Sample dilution

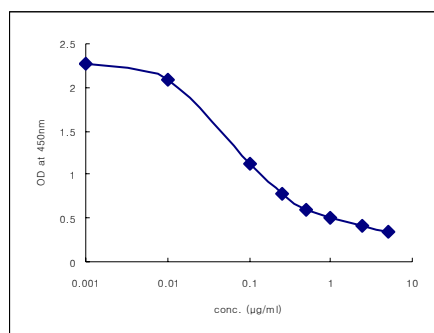
- 1) Dilute samples 1:100 with 1X Diluent (for example, 10  $\mu$ l sample plus 990  $\mu$ l 1X Diluent, final 1:100)
  - \* If samples fall the outside range of assay, a lower or higher dilution may be required.
- 2) Use 50 $\mu$ l of the final diluted sample for ELISA.



### 3. Experiment procedure

- 1) Remove the appropriate number of microwell strips from the sealed foil pouch.
- 2) Pipette 50  $\mu$ l of Standards #1 to #8, the reconstituted QC sample and pre-treated sample into the recombinant protein-coated plate according to the plate configuration. Use a new pipette tip for each Standard or sample.
- 3) Add 50  $\mu$ l antibody to each well and tap gently on the side of the plate to mix.
- 4) Cover the plate with plate sealer and incubate at 37°C for 1 hour.
- 5) Remove the solution and wash 3 times with 250  $\mu$ l of 1X Wash Solution per well.
- 6) Add 100 $\mu$ l 1X Detector to each well.
- 7) Cover the plate with sealer and incubate at 37°C for 1 hour.
- 8) Remove the solution and wash 5 times with 250  $\mu$ l of 1X Wash Solution per well.
- 9) Using the multi-channel pipette, add 100  $\mu$ l of the Substrate Solution to each well.
- 10) Cover the plate with sealer and incubate at room temperature for 20 min.  
\* Protect from light.
- 11) Using the multi-channel pipette, add 100  $\mu$ l Stop Solution to each well.
- 12) Read at 450 nm.
- 13) The dose-response curve of this assay fits best to a sigmoidal 4-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4-parameter logistic function.
- 14) A measurable range is typically shown between 0.001  $\mu$ g/ml and 5  $\mu$ g/ml.

The RBP4 concentrations calculated must be multiplied by dilution factor to obtain the concentrations of the undiluted samples (Dilution factor of lyophilized QC sample is 100).



## Performance Characteristics

### 1) Sensitivity

The limit of detection: 1 ng/ml

### 2) Precision

#### a. Intra-Assay (precision within an assay)

5 samples were tested 4 times to assess intra-assay precision.

Sample	Mean ( $\mu\text{g/ml}$ )	SD ( $\mu\text{g/ml}$ )	CV(%)
1	16.423	0.434	2.643
2	19.141	0.695	3.629
3	22.290	1.006	4.511
4	25.312	1.343	5.306
5	45.755	4.219	9.220

#### b. Inter-Assay (precision between assays)

5 samples were tested 4 times to assess inter-assay precision.

Sample	Mean ( $\mu\text{g/ml}$ )	SD ( $\mu\text{g/ml}$ )	CV(%)
1	15.394	0.535	3.478
2	21.478	1.536	7.152
3	24.977	1.622	6.495
4	26.886	2.308	8.583
5	31.880	3.275	10.274

### 3) Specificity

- a. No cross reaction with mouse and rat sera
- b. Cross Reactivity

Analyte	Max. Conc. ( $\mu\text{g/ml}$ )	Cross Reactivity (%)
Human RBP4	0.1	100
Human Adiponectin	1	N. R.
Rat Adiponectin	1	N. R.
Human Resistin	1	N. R.
Rat RELM $\alpha$	1	N. R.
Human RELM $\beta$	1	N. R.
Human AGF	1	N. R.
Human FABP4	1	N. R.
Human Ang 1	1	N. R.
Human Ang 2	1	N. R.
Human GPX3	1	N. R.
Human visfatin	1	N. R.
Mouse visfatin	1	N. R.
Human PAI-1	1	N. R.
Human leptin	1	N. R.
Human IL23	1	N. R.

**N. R. : No Cross-reactivity**

#### 4) Linearity - Effect of Serum Dilution

Three serum samples were pre-treated as described in the protocol, resulting in the final dilution of x100(labeled in the table below as dilution: 1)

Sample No.	Serum Dilution	Expected (µg/ml)	Observed (µg/ml)	% Of Expected
1	1	14.587	14.587	100
	1/2	7.947	7.294	109
	1/4	4.014	3.647	110
	1/8	1.953	1.823	107
2	1	23.091	23.091	100
	1/2	9.811	11.546	85
	1/4	4.707	5.773	82
	1/8	2.334	2.886	81
3	1	31.032	31.032	100
	1/2	13.551	15.516	87
	1/4	6.693	7.758	86
	1/8	3.264	3.879	84

$$\% \text{ of expected} = \text{observed} / \text{expected} \times 100\%$$

#### 5) Comparison of serum samples with plasma samples

Sample No.	Serum (µg/ml)	Plasma (µg/ml)		
		Sodium citrate	EDTA	Heparin
1	19.941	15.047	22.040	19.808
2	25.909	18.949	22.296	28.416

## References

1. Quadro L, Blaner WS, Salchow DJ, Vogel S, Piantedosi R, Gouras P, Freeman S, Cosma MP, Colantuoni V, Gottesman ME. 1999 Impaired retinol function and vitamin A availability in mice lacking retinol - binding protein. *EMBO J.* 18:4633-44.
2. Shepherd PR, Kahn BB. 1999 Glucose transporters and insulin action - implications for insulin resistance and diabetes mellitus. *N Engl J Med.* 341:248-57.
3. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, Kotani K, Quadro L, Kahn BB. 2005 Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* 436:356-62.



## Troubleshooting Guide

Problem	Possible Cause	Solution
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be appropriate for the system.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of detector too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.
	Technique problem	Proper mixing of reagents and wash steps are critical.

AdipoGen Inc.  
641-B, Graduate School of Life Science  
and Biotechnology, Korea Univ.,  
1, 5-ka, Anam-dong, Sungbuk-ku,  
Seoul, Korea

© 2005 AdipoGen Inc. All right reserved.



TECHNICAL INFORMATION

Web [www.adipogen.com](http://www.adipogen.com)

E-mail [bsyoun@adipogen.com](mailto:bsyoun@adipogen.com)

Phone +82+2-927-1470

Fax +82-2-926-1670