Human RBP4
ELISA Kit

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Hinweis/Note:
Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

The datasheet is only a first information.
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

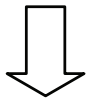
Thank you for choosing Assaypro.

Symbol Key

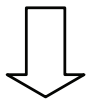
Consult instructions for use.

Assay Summary

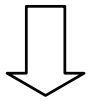
Add 50 µl of Standard/ Sample per well.
Incubate 2 hours.



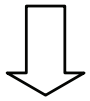
Wash, then add
50 µl of Biotinylated Antibody per well.
Incubate 1 hour.



Wash, then add
50 µl of SP Conjugate per well.
Incubate 30 minutes.



Wash, then add
50 µl of Chromogen Substrate per well.
Incubate 10 minutes.



Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Assay Template

	A	B	C	D	E	F	G	H
1								
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AssayMax Retinol Binding Protein 4 (RBP4) ELISA Kit

Catalog No. ER3005-1
Sample Insert/Reference Only

Introduction

Serum retinol binding protein (RBP4), secreted by the liver and adipocytes, is implicated in systemic insulin resistance. RBP4 transports retinol and circulates in the plasma by binding to the larger transthyretin (TTR) homotetramer, forming a protein complex that reduces renal clearance of RBP4. In insulin-resistant ob/ob mice, urinary fractional excretion of RBP4 was reduced, which is consistent with increased retention, while TTR level is elevated (1). RBP4 is encoded by the *RBP4* gene which maps to chromosome 10q23-q24 and is linked to increased risk for type 2 diabetes in different populations (2, 3). Transgenic overexpression of human RBP4 or injection of recombinant RBP4 in normal mice causes insulin resistance. Conversely, genetic deletion of RBP4 enhances insulin sensitivity. Increasing serum RBP4 induces hepatic expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase and impairs insulin signaling in muscle tissue (4). Expression of RBP4 is induced in adipose tissue as a consequence of decreased glucose transporter GLUT4 expression. Increased human serum RBP4 is associated with insulin resistance, Type II diabetes, and metabolic syndrome such as obesity, glucose intolerance, dyslipidemia, and hypertension (5, 6). Human plasma RBP4 concentration might be a biomarker of nephropathy and cardiovascular disease in type 2 diabetic subjects (7).

Principle of the Assay

The AssayMax Human Retinol Binding Protein 4 (RBP4) ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human RBP4 in plasma, serum, urine, milk, saliva, CSF, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures RBP4 in less than 4 hours. A polyclonal antibody specific for RBP4 has been pre-coated onto a 96-well microplate with removable strips. RBP4 in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for RBP4, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- **Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.**
- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- **Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acidic solution.

Reagents

- **Human RBP4 Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human RBP4.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes, which can be cut to fit the format of the individual assay.
- **Human RBP4 Standard:** Human RBP4 in a buffered protein base (1000 ng, lyophilized).
- **Biotinylated Human RBP4 Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against RBP4 (140 µl).
- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.

- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation, and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:1000 into EIA Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes, and remove serum. Dilute samples 1:1000 into EIA Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes and assay. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Saliva:** Collect saliva using sample tube. Centrifuge samples at 800 x *g* for 10 minutes and assay. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:2 into EIA Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **CSF:** Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:2 into EIA Diluent and assay. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **EIA Diluent Concentrate (10x)**: If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Human RBP4 Standard:** Reconstitute the 1000 ng of Human RBP4 Standard with 2.5 ml of EIA Diluent to generate a 400 ng/ml standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (400 ng/ml) 1:2 with EIA Diluent to produce 200, 100, 50, 25, 12.5, and 6.25 ng/ml solutions. EIA Diluent serves as the zero standard (0 ng /ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[RBP4] (ng/ml)
P1	Standard (400 ng/ml)	400.0
P2	1 part P1 + 1 part EIA Diluent	200.0
P3	1 part P2 + 1 part EIA Diluent	100.0
P4	1 part P3 + 1 part EIA Diluent	50.00
P5	1 part P4 + 1 part EIA Diluent	25.00
P6	1 part P5 + 1 part EIA Diluent	12.50
P7	1 part P6 + 1 part EIA Diluent	6.250
P8	EIA Diluent	0.000

- **Biotinylated Human RBP4 Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with EIA Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch

securely to minimize exposure to water vapor and store in a vacuum desiccator.

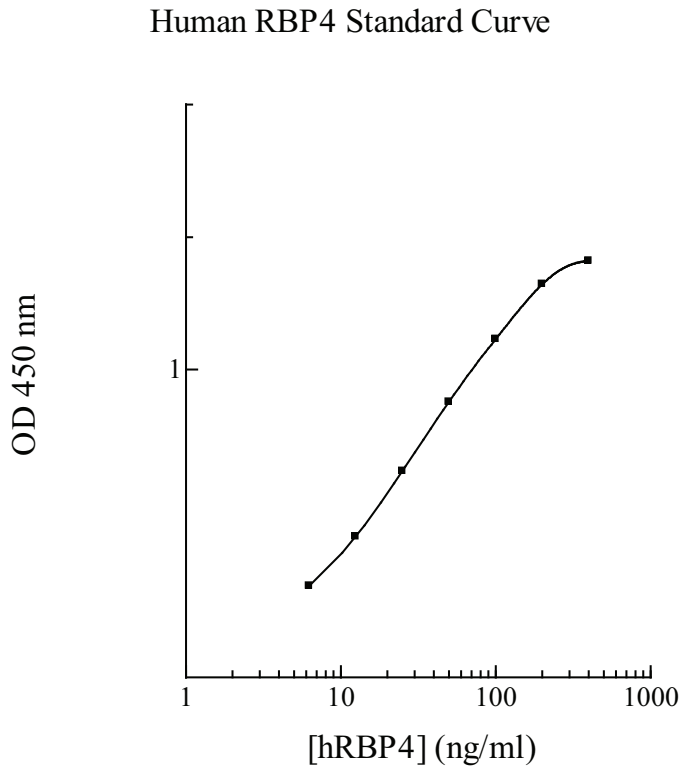
- Add 50 μl of Human RBP4 Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 μl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 μl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 μl of Biotinylated Human RBP4 Antibody to each well and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 μl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 μl of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 μl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of RBP4 is typically ~ 6 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.8% and 7.1% respectively.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:500	90%	89%
1:1000	98%	97%
1:2000	103%	104%

	Average Percentage of Expected Value		
Sample Dilution	**Urine**	**Saliva**	**Milk**
No dilution	92%	88%	91%
1:2	97%	94%	97%

Recovery

Standard Added Value	12.5 – 200 ng/ml
Recovery %	85 – 111%
Average Recovery %	98%

Cross-Reactivity

Species	% Cross Reactivity
Beagle	None
Bovine	None
Monkey	None
Mouse	None
Rat	None
Swine	None
Rabbit	None
Human	100%

- 10% FBS in culture media will not affect the assay.

References

(1) Mody N *et al.* (2008) *Am. J. Physiol Endocrinol Metab*. 294(4): E785-793
(2) Meigs JB *et al.* (2002) *Diabetes* 51:833–840
(3) Duggirala R *et al*. (1998) *Diabetes* 47 (Suppl. 1): A170
(4) Yang Q *et al.* (2005) Nature 436 (7049): 356-362
(5) Graham T.E. *et al.* (2006) *N. Engl. J. Med.* 354:2552-2563
(6) McTernan PG *et al.* (2007*) J. Clin. Endocrinol. Metab*. 92:2430 –2432
(7) Cabre A *et al.* (2007) J. Intern Med. 262(4): 496-503

Version 3.2

Related Products

- ER1005-1 AssayMax Human RBP ELISA Kit (Plasma, Serum samples)
- ER2005-1 AssayMax Human RBP ELISA Kit (Urine, Milk, and Cell Culture samples)
- EMR3005-1 AssayMax Mouse RBP4 ELISA Kit (Plasma, Serum, Urine, and Cell Culture samples)