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RBP4 (Human) Competitive ELISA Kit

(Catalog # K4911-100; 100 assays; Store at 4°C)

I. **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. In obesity and type 2 diabetes, the 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. Adipocyte-specific deletion of Gluts leads to notable elevation of mouse RBP4 causing systemic insulin resistance, and that reduction of RBP4 improves insulin resistance. This identified a novel role of RBP4 in regulating insulin action and RBP4 is recorded as an adipocyte-derived hormone. The RBP4 (human) ELISA Kit is to be used for the in vitro quantitative determination of human RBP4 in serum, urine and cell culture supernatant. This assay is a competitive ELISA which utilizies a 96-well microtiter plate which was pre-coated with a human RBP4. A purified polyclonal which recognizing native human RBP4 reacts with a series of predetermined recombinant human RBP4 standard proteins or the test samples under competition in the human RBP4-coated plate. Their relative reactivity is plotted with that of the standard proteins. This ELISA is specific for the measurement of natural and recombinant human RBP4. It does not cross-react with mouse RBP4, rat RBP4, human adiponectin, rat adiponectin, human resistin, human vaspin, human clusterin, human leptin, human IL-23, human IL-33, human GPX3, human Nampt, human ANG1, human ANG2, human ANGPTL3, human ANGPTL4, human ANGPTL6, human FABP4, human RELM-β, rat RELM-α, mouse Nampt, human PAI-1. The assay range is 0.001 – 5 µg RBP4/ml and a detection limit of 1 ng/ml (based on adding two standard deviations to the mean value of the (50) zero standards).

II. **Sample Type:** Serum, Plasma, Urine or Cell Culture Supernatant

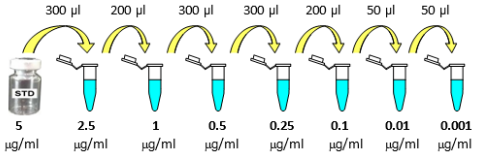
III. **Kit Contents:**

Components	K4911-100	Part No.
Plate coated with human RBP4 Recombinant protein	6 x 16-wells strips	K4911-100-1
Wash Buffer (10x)	30 ml x 2	K4911-100-2
ELISA Buffer (10X)	30 ml x 2	K4911-100-3
Detection Antibody (DET)	20 µl	K4911-100-4
HRP 100X (HRP Conjugated anti-rabbit IgG)	150 µl	K4911-100-5
Human RBP4 Standard (lyophilized)	5 µg	K4911-100-6
TMB Substrate Solution	12 ml	K4911-100-7
Stop Solution	12 ml	K4911-100-8
Plate sealers (plastic film)	2	K4911-100-9
Silica Gel Minibags	2	K4911-100-10

IV. **User Supplied Reagents and Equipment:**
- Microtiterplate reader at 450 nm, with the correction wavelength set at 540 nm or 570 nm
- Calibrated precision single and multi-channel pipettes. Disposable pipette tips
- Deionized water
- Microtubes or equivalent for preparing dilutions
- Disposable plastic containers for preparing working buffers
- Plate washer: automated or manual
- Glass or plastic tubes for diluting and aliquoting standard

V. **Storage and Handling:** Reagent must be stored at 2-8°C when not in use. Plate and reagents should be at room temperature before use. Do not expose reagents to temperatures greater than 25°C.

VI. **Reagent and Sample Preparation and Storage Conditions:**
- Bring all reagents and samples to room temperature (18 - 25°C) before use.
- Wash Buffer 10X has to be diluted with deionized water 1:10 before use (e.g. 50 ml Wash Buffer 10X + 450 ml water) to obtain Wash Buffer 1X.
- ELISA Buffer 10X has to be diluted with deionized water 1:10 before use (e.g. 20 ml ELISA Buffer 10X + 180 ml water) to obtain ELISA Buffer 1X.
- Detection Antibody (DET) has to be diluted to 1:1000 in ELISA Buffer 1X (10 µl DET + 10 ml ELISA Buffer 1X).
  NOTE: The diluted Detection Antibody is not stable and cannot be stored.
- HRP 100X (HRP Conjugated anti-rabbit IgG) has to be diluted to the working concentration by adding 100 µl in 10 ml of ELISA Buffer 1X (1:100).
  - The diluted HRP is used within one hour of preparation
- Human Progranulin Standard (STD) has to be reconstituted with 1 ml of deionized water. This reconstitution produces a stock solution of 5 µg/ml. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes. Mix well prior to making dilutions. NOTE: The reconstituted standard is aliquoted and stored at -20°C
  - Dilute the standard protein concentrate (STD) (5 µg/ml) in Diluent 1X. A seven-point standard curve in Diluent 1X is recommended.
  - Suggested standard points are: 5, 2.5 , 1 , 0.5 , 0.25 , 0.1 , 0.01 and 0.001 µg/ml.



FOR RESEARCH USE ONLY! Not to be used on humans.

- Sample Preparation:
  - Serum Samples: 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 ≤ -20°C for later use. Avoid repeated freeze/thaw cycles.
  - Plasma: Collect plasma using heparin, EDTA, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma sample in aliquot at ≤ -20°C for later use. Avoid repeated freeze/ thaw cycles.
  - Urine: Aseptically collect the urine of the day, voided directly into a sterile container. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze/thaw cycles.
  - Serum, Plasma, Urine, Cell Culture Supernatant or CSF have to be diluted in ELISA Buffer 1X. Samples containing visible precipitates must be clarified before use.
    NOTE: As a starting point, 1/100 dilution of serum or plasma is recommended! If sample values fall outside the detection range of the assay, a lower or higher dilution may be required!

To obtain	Add	Into
5 µg/ml	---	---
2.5 µg/ml	300 µl of RBP4 (5 µg/ml)	300 µl of 1X Diluent
1 µg/ml	200 µl of RBP4 (2.5 µg/ml)	300 µl of 1X Diluent
0.5 µg/ml	300 µl of RBP4 (1 µg/ml)	300 µl of 1X Diluent
0.25 µg/ml	300 µl of RBP4 (0.5 µg/ml)	300 µl of 1X Diluent
0.1 µg/ml	200 µl of RBP4 (0.25 µg/ml)	300 µl of 1X Diluent
0.01 µg/ml	50 µl of RBP4 (0.1 µg/ml)	450 µl of 1X Diluent
0.001 µg/ml	50 µl of RBP4 (0.01 µg/ml)	450 µl of 1X Diluent

VII. Assay Protocol:

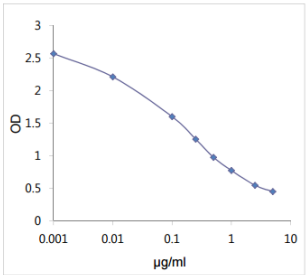
- Determine the number of 16-well strips needed for the assay and insert them in the frame for current use. The extra strips should be resealed in the foil pouch bag and stored at 4°C.
  NOTE: Remaining 16-well strips coated with RBP4 protein when opened can be stored at 4°C for up to 1 month.
- Add 50 µl of the different **standards** into the appropriate wells in duplicate! At the same time, add 50 µl of **diluted serum, plasma, urine or cell culture supernatant** samples in duplicate to the wells.
- Add 50 µl to each well of the **Detection Antibody** and tap gently on the side of the plate to mix. Cover the plate with plate sealer and incubate for 1 hour at 37°C.
- Aspirate the coated wells and add 300 µl of **Wash Buffer 1X** using a multichannel pipette or auto-washer. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.
- Add 100 µl to each well of the diluted **HRP Conjugated anti-rabbit IgG.** Cover the plate with plate sealer and incubate for 1 hour at 37°C.
- Aspirate the coated wells and add 300 µl of **Wash Buffer 1X** using a multichannel pipette or auto-washer. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.
- Add 100 µl to each well of **TMB Substrate Solution**. Allow the color reaction to develop at room temperature in the dark for 20 minutes.
- Stop the reaction by adding 100 µl of **Stop Solution.** Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
- Measure the OD at 450 nm in an ELISA reader within 30 minutes.

VIII. Calculation of Results:

- Average the duplicate readings for each standard, control and sample.
- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the vertical (Y) axis vs. the corresponding progranulin concentration (ng/ml) on the horizontal (X) axis (see Typical Data).
- Calculate the RBP4 concentrations of samples by interpolation of the regression curve formula as shown above in a form of a 4-parameter logistic equation.
- If the test samples were diluted, multiply the interpolated values by the dilution factor to calculate the concentration of human RBP4 in the samples.

IX. Typical Data:

The following data are obtained using the different concentrations of standard as described in this protocol:



Standard hRBP4 (µg/ml)	Optical Density (mean)
5	0.368
2.5	0.519
1	0.739
0.5	0.975
0.25	1.182
0.1	1.434
0.01	1.862
0.001	2.059

Figure: Standard curve

X. RELATED PRODUCTS:

Progranulin, Human recombinant
Progranulin, Mouse recombinant
ELISA kits
Apoptosis Assay Kits and Reagents
Recombinant Growth Factors and Cytokines
Progranulin, Rat recombinant
Recombinant proteins and enzymes
Metabolism Assay kits and Reagents
Cellular Fractionation Kits
Polyclonal and monoclonal antibodies

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