



[Go to Product page](#)

Datasheet for ABIN1979266

RBP4 ELISA Kit

7 Publications

Overview

Quantity:	96 tests
Target:	RBP4
Reactivity:	Human, Mouse, Rat
Method Type:	Competition ELISA
Detection Range:	0.1-1.000 ng/mL
Minimum Detection Limit:	0.1 ng/mL
Application:	ELISA

Product Details

Purpose:	Human/Mouse/Rat RBP-4 EIA Kit optimized for serum and cell culture medium. Competition-based ELISA on a 96-well strip plate.
Sample Type:	Cell Culture Supernatant, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This kit detects RBP4 (183aa) and all other active forms including RBP4-182,181,179,176. Cross Reactivity: This EIA kit shows no cross-reactivity with any of the cytokines tested: Ghrelin, Nesfatin, Angiotensin II, NPY and APC.
Cross-Reactivity (Details):	This ELISA kit shows no cross-reactivity with any of the cytokines tested: Ghrelin, Nesfatin, Angiotensin II, NPY and APC. This kit detects RBP4 (183aa) and all other active forms including RBP4-182,181,179,176.

Product Details

Sensitivity: 460 pg/mL

Characteristics:

- Strip plates and additional reagents allow for use in multiple experiments
- Quantitative protein detection
- Establishes normal range
- The best products for confirmation of antibody array data

Components:

- Pre-Coated 96-well Strip Microplate
- Wash Buffer
- Standard Peptide
- Assay Diluent(s)
- Biotinylated Peptide
- HRP-Streptavidin
- TMB One-Step Substrate
- Stop Solution
- Assay Diagram
- Positive Control Sample
- Capture Antibody
- User Manual

Material not included:

- Distilled or deionized water
- Precision pipettes to deliver 2 μ L to 1 mL volumes
- Adjustable 1-25 mL pipettes for reagent preparation
- 100 mL and 1 liter graduated cylinders
- Tubes to prepare standard and sample dilutions
- Orbital shaker
- Aluminum foil
- Saran Wrap
- Absorbent paper
- Microplate reader capable of measuring absorbance at 450nm
- SigmaPlot software (or other software that can perform four-parameter logistic regression models)

Target Details

Target: RBP4

Alternative Name: RBP4 ([RBP4 Products](#))

Background: Retinol Binding Protein 4 (RBP4)

Gene ID: 5950

UniProt: [P02753](#)

Target Details

Pathways: [Regulatory RNA Pathways](#), [Positive Regulation of Peptide Hormone Secretion](#), [Carbohydrate Homeostasis](#), [Production of Molecular Mediator of Immune Response](#)

Application Details

Application Notes: Recommended Dilution for serum and plasma samples Human: 4X / Mouse: 2X / Rat:

Sample Volume: 100 μ L

Assay Time: 5 h

Plate: Pre-coated

Protocol:

1. Prepare all reagents, samples and standards as instructed.
2. Add 100 μ L detection antibody to each well.
3. Incubate 1.5 h at RT or O/N at 4 $^{\circ}$ C.
4. Add 100 μ L standard or sample to each well.
5. Incubate 2.5 h at RT.
6. Add 100 μ L prepared streptavidin solution.
7. Incubate 45 min at RT.
8. Add 100 μ L TMB One-Step Substrate Reagent to each well.
9. Incubate 30 min at RT.
10. Add 50 μ L Stop Solution to each well.
11. Read plate at 450 nm immediately.

Reagent Preparation:

1. Keep kit reagents on ice during steps. Equilibrate plate to room temperature before opening the sealed pouch.
2. Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water.
3. Briefly centrifuge the Anti-RBP4 Antibody vial (Item N) before use. Add 50 μ L of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently.
4. The antibody concentrate should then be diluted 100-fold with 1x Assay Diluent B. This is your anti-RBP4 antibody working solution, which will be used in step 2 of the Assay Procedure. NOTE: the following steps may be done during the antibody incubation procedure (step 2 of Assay Procedure).
5. Briefly centrifuge the vial of Biotinylated RBP4 (Item F) before use. Add 5 μ L of Item F to 5 mL of the appropriate Assay Diluent. Pipette up and down to mix gently. The final concentration of biotinylated RBP4 will be 10 ng/mL. This solution will only be used as the diluent in step 6 of Reagent Preparation.
6. Preparation of Standards: Label 6 microtubes with the following concentrations: 1000 ng/mL, 100 ng/mL, 10 ng/mL, 1 ng/mL, 100 pg/mL and 0 pg/mL. Pipette 450 μ L of biotinylated RBP4

solution into each tube, except for the 1000 ng/mL (leave this one empty). It is very important to make sure the concentration of biotinylated RBP4 is 10 ng/mL in all standards. a. Briefly centrifuge the vial of RBP4 (Item C). In the tube labeled 1000 ng/mL, pipette 8 μ L of Item C and 792 μ L of 10 ng/mL biotinylated RBP4 solution (prepared in step 5 above). This is your RBP4 stock solution (1000 ng/mL RBP4, 10 ng/mL biotinylated RBP4). Mix thoroughly. This solution serves as the first standard. b. To make the 100 ng/mL standard, pipette 50 μ L of RBP4 stock solution the tube labeled 100 ng/mL. Mix thoroughly. c. Repeat this step with each successive concentration, preparing a dilution series as shown in the illustration below. Each time, use 450 mL of biotinylated RBP4 and 50 mL of the prior concentration until 100 pg/mL is reached. Mix each tube thoroughly before the next transfer. d. The final tube (0 pg/mL RBP4, 10 ng/mL biotinylated RBP4) serves as the zero standard (or total binding).

7. Prepare a 10-fold dilution of Item F. To do this, add 2 mL of Item F to 18 mL of the appropriate Assay Diluent. This solution will be used in steps 8 and 10.

8. Positive Control Preparation: briefly centrifuge the positive control vial (Item M). To the tube of Item M, add 101 μ L 1x Assay Diluent B. Also add 2 μ L of 10-fold diluted Item F (prepared in step 7) to the tube. This is a 2-fold dilution of the positive control. Mix thoroughly. The positive control is a cell culture medium sample that is meant to be a system control (to verify that the detection & kit components are working). It may be diluted further if desired, but be sure the final concentration of biotinylated RBP4 is 10 ng/mL.

9. If Item B (20X Wash Concentrate) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

10. Sample Preparation: Use Assay Diluent A + biotinylated RBP4 to dilute serum/plasma samples. For cell culture medium and other sample types, use 1X Assay Diluent B + biotinylated RBP4 as the diluent. It is very important to make sure the final concentration of the biotinylated RBP4 is 10 ng/mL in every sample.

Example: to make a 4-fold dilution of sample, mix together 2.5 μ L of 10-fold diluted Item F (prepared in step 7), 185 mL of appropriate Assay Diluent, and 62.5 μ L of your sample, mix gently. The total volume is 250 μ L, enough for duplicate wells on the microplate. Do not use Item F diluent from Step 5 for sample preparation. If you plan to use undiluted samples, you must still add biotinylated RBP4 to a final concentration of 10 ng/mL.

Example: Add 2.5 mL of 10-fold diluted Item F to 247.5 mL of sample.

11. Briefly centrifuge the HRP-Streptavidin vial (Item G) before use. The HRP-Streptavidin concentrate should be diluted 400- fold with 1X Assay Diluent B. Note: Do not use Assay Diluent A for HRP-Streptavidin preparation in Step 11.

Application Details

Sample Preparation: Use Assay Diluent A + biotinylated RBP4 to dilute serum/plasma samples. For cell culture medium and other sample types, use 1X Assay Diluent B + biotinylated RBP4 as the diluent. It is very important to make sure the final concentration of the biotinylated RBP4 is 10 ng/mL in every sample. EXAMPLE: to make a 4-fold dilution of sample, mix together 2.5 μ L of 10-fold diluted Item F (prepared in step 7), 185 μ L of appropriate Assay Diluent, and 62.5 μ L of your sample, mix gently. The total volume is 250 μ L, enough for duplicate wells on the microplate. Do not use Item F diluent from Step 5 for sample preparation. If you plan to use undiluted samples, you must still add biotinylated RBP4 to a final concentration of 10 ng/mL. EXAMPLE: Add 2.5 mL of 10-fold diluted Item F to 247.5 mL of sample.

Assay Procedure:

1. Keep kit reagents on ice during reagent preparation steps. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 μ L anti-RBP4 antibody (see Reagent Preparation step 4) to each well. Incubate for 1.5 hours at room temperature with gentle shaking (1-2 cycles/sec). You may also incubate overnight at 4 degrees C. 0
3. Discard the solution and wash wells 4 times with 1x Wash Buffer (200-300 μ L each), Washing may be done with a multichannel pipette or an automated plate washer. Complete removal of liquid at each step is essential to good assay performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 μ L of each standard (see Reagent Preparation step 6), positive control (see Reagent Preparation step 8) and sample (see Reagent Preparation step 10) into appropriate wells. Be sure to include a blank well (Assay Diluent only). Cover wells and incubate for 2.5 hours at room temperature with gentle shaking (1-2 cycles/sec) or overnight at 4 °C.
5. Discard the solution and wash 4 times as directed in Step 3.
6. Add 100 μ L of prepared HRP-Streptavidin solution (see Reagent Preparation step 11) to each well. Incubate with gentle shaking for 45 minutes at room temperature. It is recommended that incubation time should not be shorter or longer than 45 minutes.
7. Discard the solution and wash 4 times as directed in Step 3.
8. Add 100 μ L of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec).
9. Add 50 μ L of Stop Solution (Item I) to each well. Read absorbances at 450 nm immediately.

Calculation of Results: Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the blank optical density. Plot the standard curve using SigmaPlot software (or other

Application Details

software which can perform four-parameter logistic regression models), with standard concentration on the x-axis and percentage of absorbance on the y-axis. Draw the best-fit curve through the standard points.

Assay Precision: Intra-Assay: CV < 10 %
Inter-Assay: CV < 15 %

Restrictions: For Research Use only

Handling

Handling Advice: Avoid repeated freeze/thaw cycles.

Storage: -20 °C

Storage Comment: Standard, Biotinylated Retinol Binding Protein 4 peptide, and Positive Control should be stored at -20°C after arrival. Avoid multiple freeze-thaws. The remaining kit components may be stored at 4°C. Opened Microplate Wells and antibody (Item N) may be stored for up to 1 month at 2° to 8°C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

Expiry Date: 6 months

Publications

Product cited in: Wu, Lin, Tseng, Cheng, Lee, Lin, Chou, Cheng: "Comparison of two stroke volume variation-based goal-directed fluid therapies for supratentorial brain tumour resection: a randomized controlled trial." in: **British journal of anaesthesia**, Vol. 119, Issue 5, pp. 934-942, (2019) ([PubMed](#)).

Ragab, Fathy, El-Aziz, Helal et al.: "The Diagnostic Value of Pulsed Wave Tissue Doppler Imaging in Asymptomatic Beta- Thalassemia Major Children and Young Adults; Relation to Chemical Biomarkers of Left Ventricular Function and Iron ..." in: **Mediterranean journal of hematology and infectious diseases**, Vol. 7, Issue 1, pp. e2015051, (2015) ([PubMed](#)).

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