Rbp4 (Mouse) ELISA Kit

Catalog Number KA2008

96 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

For quantitative detection of mouse RBP4 in sera, plasma, body fluids, tissue lysates or cell culture supernates.

Background

Retinol binding protein 4, plasma, also known as RBP4, belongs to the lipocalin family and is the specific carrier for retinol (vitamin A alcohol) in the blood. It is protein that it is encoded by the *RBP4* gene. RBP4 gene resides just centromeric of the cluster of CYP2C genes on 10q24. The mouse Rbp4 locus is closely linked and just proximal to the locus for phenobarbital-inducible cytochrome P450-2c (Cyp-2c) at the distal end of chromosome 19. It delivers retinol from the liver stores to the peripheral tissues. In plasma, the RBP-retinol complex interacts with transthyretin, which prevents its loss by filtration through the kidney glomeruli. A deficiency of vitamin A blocks secretion of the binding protein posttranslationally and results in defective delivery and supply to the epidermal cells. The standard product used in this kit are recombinant mouse B7-1/CD80, D37-K245, consisting of dimer acids with two single straned. The standard used in this kit is recombinant protein, with E19-L201 aa sequence, the molecular weight is 22kda.

Principle of the Assay

Rbp4 (Mouse) ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Mouse RBP4 species-specific polyclonal antibodies were precoated onto 96-well plates. The mouse specific detection polyclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the mouse RBP4 amount of sample captured in plate.

General Information

Materials Supplied

List of component

Component	Amount
Lyophilized recombinant mouse RBP4 standard	20 ng/tube×2
One 96-well plate precoated with anti-mouse RBP4 antibody	1 plate
Sample diluent buffer	30 ml
Biotinylated anti-mouse RBP4 antibody	130μl, dilution 1:100
Antibody diluent buffer	12 ml
Avidin-Biotin-Peroxidase Complex (ABC)	130μl, dilution 1:100
ABC diluent buffer	12 ml
TMB color developing agent	10 ml
TMB stop solution	10 ml

Storage Instruction

Store at 4℃ for frequent use, at -20℃ for infrequent use. Avoid multiple freeze-thaw cycles. Expiration: Four months at 4℃ and eight months at -20℃.

Materials Required but Not Supplied

1. Microplate reader in standard size.
2. Automated plate washer.
3. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
4. Clean tubes and Eppendorf tubes.
5. Washing buffer (neutral PBS or TBS).

   Preparation of 0.01M TBS:

   Add 1.2g Tris, 8.5g NaCl; 450μl of purified acetic acid or 700μl of concentrated hydrochloric acid to 1000ml H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

   Preparation of 0.01 M PBS:

   Add 8.5g sodium chloride, 1.4g Na_2HPO_4 and 0.2g NaH_2PO_4 to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Precautions for Use

- ✓ To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
- ✓ The TMB Color Developing agent is colorless and transparent before using, contact us freely if it is not the case.
- ✓ Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
- ✓ Duplicate well assay is recommended for both standard and sample testing.
- ✓ Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
- ✓ Don't reuse tips and tubes to avoid cross contamination.
- ✓ To avoid to use the reagents from different batches together.
- ✓ In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37℃ for 30 min before using.

Assay Protocol

Reagent Preparation

- Reconstitution of the mouse RBP4 standard: RBP4 standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of RBP4 standard (20 ng per tube) are included in each kit. Use one tube for each experiment.
  - ✓ 20 ng/ml of mouse RBP4 standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
  - ✓ 10 ng/ml → 0.312 ng/ml of mouse RBP4 standard solutions: Label 6 Eppendorf tubes with 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.312 ng/ml respectively. Aliquot 0.3 ml of the sample diluents buffer into each tube. Add 0.3 ml of the above 20 ng/ml RBP4 standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.
    Note: The diluted standard solution (20,000 pg/ml) is best used within 12 hours and may be stored at -20℃ for up to 48 hours. Avoid repeated freeze-thaw cycles.

- Preparation of biotinylated anti-mouse RBP4 antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.
  - ✓ The total volume should be: 0.1 ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
  - ✓ Biotinylated anti-mouse RBP4 antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly.

- Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.
  - ✓ The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
  - ✓ Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly.

Sample Preparation

Store samples to be assayed within 24 hours at 2-8℃. For long-term storage, aliquot and freeze samples at -20℃. Avoid repeated freeze-thaw cycles.

- ✓ Cell culture supernate, tissue lysate or body fluids: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20℃
- ✓ Serum: Allow the serum to clot in a serum separator tube (about 30 min) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20℃.

- ✓ Plasma: Collect plasma using heparin, EDTA, citrate as an anticoagulant. Centrifuge for 15 min at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20℃.

- Sample Dilution Guideline

  The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. The sample must be well mixed with the diluents buffer.

- ✓ High target protein concentration (200-2000 ng/ml). The working dilution is 1:100. i.e. Add 3 µl sample into 297 µl sample diluent buffer.
- ✓ Medium target protein concentration (20-200 ng/ml). The working dilution is 1:10. i.e. Add 25 µl sample into 225 µl sample diluent buffer.
- ✓ Low target protein concentration (0.132-20 ng/ml). The working dilution is 1:2. i.e. Add 100 µl sample to 100 µl sample diluent buffer.
- ✓ Very Low target protein concentration (≤ 0.132 ng/ml). No dilution necessary, or the working dilution is 1:2.

Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37℃ for 30 min before use at least. When diluting samples and reagents, they must be mixed completely and evenly. Standard RBP4 detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of RBP4 amount in samples.

1. Aliquot 0.1 ml per well of the 20 ng/ml, 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.312 ng/ml mouse RBP4 standard solutions into the precoated 96-well plate. Add 0.1 ml of the sample diluent buffer into the control well (Zero well). Add 0.1 ml of each properly diluted sample of mouse sera, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. See “Sample Dilution Guideline” above for details. We recommend that each mouse RBP4 standard solution and each sample is measured in duplicate.
2. Seal the plate with the cover and incubate at 37℃ for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1 ml of biotinylated anti-mouse RBP4 antibody working solution into each well and incubate the plate at 37℃ for 60 min.
5. Wash plate 3 times with 0.01 M TBS or 0.01 M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.（Plate Washing Method: Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2

minutes. Repeat this process two additional times for a total of THREE washes. Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.）

6. Add 0.1 ml of prepared ABC working solution into each well and incubate the plate at 37℃ for 30 min.
7. Wash plate 5 times with 0.01 M TBS or 0.01 M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).
8. Add 90 µl of prepared TMB color developing agent into each well and incubate plate at 37℃ in dark for 25-30 min (Note: For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated mouse RBP4 standard solutions; the other wells show no obvious color).
9. Add 0.1 ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450 nm in a microplate reader within 30 min after adding the stop solution.

- Summary

1. Add samples and standards and incubate the plate at 37℃ for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37℃ for 60 min. Wash plate 3 times with 0.01 M TBS.
3. Add ABC working solution and incubate the plate at 37℃ for 30 min. Wash plate 5 times with 0.01 M TBS.
4. Add TMB color developing agent and incubate the plate at 37℃ in dark for 15-20 min.
5. Add TMB stop solution and read.

Data Analysis

Calculation of Results

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The mouse RBP4 concentration of the samples can be interpolated from the standard curve.

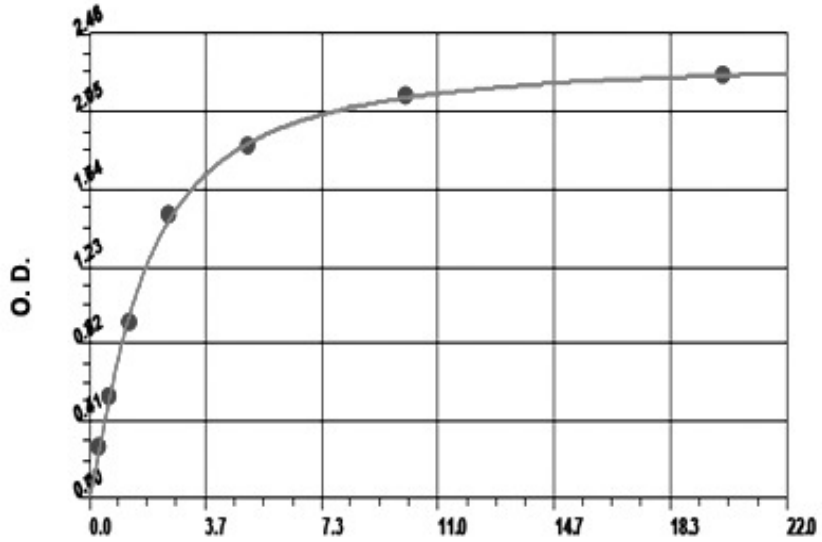
Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

- **Typical result**

  Typical Data Obtained from Mouse RBP4

  (TMB reaction incubate at 37℃ for 25 min)

Concentration	0.0ng/ml	0.312ng/ml	0.625ng/ml	1.25ng/ml	2.5ng/ml	5ng/ml	10ng/ml	20ng/ml
O.D.	0	0.342	0.612	0.875	1.196	1.385	1.717	1.834



Concentration (pg/mL)

This standard curve was demonstration purpose only. A standard curve must be run with each assay.

Performance Characteristics

- **Range**

  0.132 ng/ml-20 ng/ml

- **Sensitivity**

  < 10 pg/ml

- **Specificity**

  No detectable cross-reactivity with any other cytokine.

Resources

References

1. Rask L, Anundi H, Fohlman J, Peterson PA (1987). "The complete amino acid sequence of human serum retinol-binding protein". *Upsala Journal of Medical Sciences* 92 (2): 115–46.
2. Rocchi M, Covone A, Romeo G, Faraonio R, Colantuoni V (March 1989). "Regional mapping of RBP4 to 10q23----q24 and RBP1 to 3q21----q22 in man ". *Somatic Cell and Molecular Genetics* 15 (2): 185–90.
3. "Entrez Gene: RBP4 retinol binding protein 4, plasma". M., Covone, A., Romeo, G., Faraonio, R., Colantuoni, V. Regional mapping of RBP4 to 10q23-q24 and RBP1 to 3q21-q22 in man. Somat. Cell Molec. Genet. 15: 185-190, 1989.

Plate Layout

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