Havcr1 (Rat) ELISA Kit

Catalog Number KA1410

96 assays

Version: 06

Intended for research use only

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Introduction

Intended Use

Sandwich ELISA kit for quantitative detection of rat KIM1 in cell culture supernates, tissue homogenates, serum, plasma (heparin, EDTA) and urine.

Background

KIM1 (TIM-1), also known as Hepatitis A virus cellular receptor 1, is a protein that in Rat is encoded by the *HAVCR1* gene. Infection of canine osteogenic sarcoma cells expressing HAVCR1 with HAV led to conclude that the protein is indeed a receptor for the virus. Immunofluorescence microscopy demonstrated internalization of HAV by dog cells expressing HAVCR1. Using a monoclonal antibody to rat Tim1, Tim1 was expressed after activation of naive T cells and on T cells differentiated in Th2-polarizing conditions. By homology of synteny with the rat Tim1 gene and database analysis, was mapped the HAVCR1 gene to 5q33.2.

Principle of the Assay

Havcr1 (Rat) ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for KIM1 has been precoated onto 96-well plates. Standards (Expression system for standard: NSO, Immunogen sequence: S18-V238) and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for KIM1 is added 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 rat KIM1 amount of sample captured in plate.

General Information

Materials Supplied

List of component

Component	Amount
96-well plate precoated with anti- Rat KIM1 antibody	96 (8x12) wells
Lyophilized recombinant Rat KIM1 standard	10 ng/tube×2
Biotinylated anti- Rat KIM1 antibody, dilution 1:100	130 µL
Avidin-Biotin-Peroxidase Complex (ABC), dilution 1:100	130 µL
Sample diluent buffer	30 mL
Antibody diluent buffer	12 mL
ABC diluent buffer	12 mL
TMB color developing agent	10 mL
TMB stop solution	10 mL
Adhesive cover	4 slides

Storage Instruction

Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles.

Materials Required but Not Supplied

- ✓ Microplate reader in standard size.
- ✓ Automated plate washer.
- ✓ Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
- ✓ Clean tubes and Eppendorf tubes.
- ✓ Washing buffer (neutral PBS or TBS).
    - Preparation of 0.01 M TBS:
      Add 1.2 g Tris, 8.5 g NaCl; 450 µL of purified acetic acid or 700 µL of concentrated hydrochloric acid to 1000 mL H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1 L.
    - Preparation of 0.01 M PBS:
      Add 8.5 g sodium chloride, 1.4 g Na_2HPO_4 and 0.2 g NaH_2PO_4 to 1000 mL distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1 L.

Precautions for Use

Please read the following instructions before starting the experiment.

- ✓ 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.
- ✓ Avoid using 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°C for 30 min before using.

Assay Protocol

Reagent Preparation

- ✓ Reconstitution of the Rat KIM1 standard: KIM1 standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of KIM1 standard (10 ng per tube) are included in each kit. Use one tube for each experiment.
  - 10,000 pg/mL of rat KIM1 standard solution: Add 1 mL sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
  - 2000 pg/mL of rat KIM1 standard solution: Add 0.2 mL of the above 10 ng/mL KIM1 standard solution into 0.8 mL sample diluent buffer and mix thoroughly.
  - 1000 pg/mL→31.2 pg/mL of rat KIM1 standard solutions: Label 6 Eppendorf tubes with 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.2 pg/mL, respectively. Aliquot 0.3 mL of the sample diluent buffer into each tube. Add 0.3 mL of the above 2000 pg/mL KIM1 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 standard solutions are best used within 2 hours. The 10 ng/mL standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

- ✓ Preparation of biotinylated anti-Rat KIM1 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-Rat KIM1 antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly. (i.e. Add 1 µL Biotinylated anti-Rat KIM1 antibody to 99 µL antibody diluent buffer.)

- ✓ 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: 0.1 mL/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. (i.e. Add 1 µL ABC to 99 µL ABC diluent buffer.)

Sample Preparation

✓ Sample Preparation and Storage

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

- Cell culture supernate: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C.
- Tissue Homogenates: Rinse tissue with PBS to remove excess blood, chopped into 1-2 mm pieces, and homogenize with a tissue homogenizer in PBS or in lysate solution, lysate solution: tissue net weight = 10 mL : 1 g (i.e. Add 10 mL lysate solution to 1 g tissue). Centrifuge at approximately 5000 X g for 5 min. Assay immediately or aliquot and store homogenates at -20°C. Avoid repeated freeze-thaw cycles.
- Serum: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 x g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.
- Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 min at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store samples at -20°C.
- Urine: Aseptically collect the first urine of the day, micturate directly into a sterile container. Remove particular impurities by centrifugation, assay immediately or aliquot and store sample at -20°C.

✓ 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 (20-200 ng/mL). The working dilution is 1:100. i.e. Add 1 µL sample into 99 µL sample diluent buffer.
- Medium target protein concentration (2-20 ng/mL). The working dilution is 1:10. i.e. Add 10 µL sample into 90 µL sample diluent buffer.
- Low target protein concentration (31.2-2000 pg/mL). The working dilution is 1:2. i.e. Add 50 µL sample to 50 µL sample diluent buffer.
- Very Low target protein concentration (0-31.2 pg/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°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard KIM1 detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of KIM1 amount in samples.

1. Aliquot 0.1 mL per well of the 2000 pg/mL, 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.2 pg/mL Rat KIM1 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 rat cell culture supernates, tissue homogenates, serum, plasma (heparin, EDTA) or urine to each empty well. See "Sample Dilution Guideline" above for details. It is recommended that each Rat KIM1 standard solution and each sample be measured in duplicate.
2. Seal the plate with a new adhesive cover provided and incubate at 37°C 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-Rat KIM1 antibody working solution into each well, seal the plate with a new adhesive cover provided and incubate at 37°C 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: 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, seal the plate with a new adhesive cover provided and incubate at 37°C 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, seal the plate with a new adhesive cover provided and incubate at 37°C in dark for 15-20 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 rat KIM1 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°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01 M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C 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 rat KIM1 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 Data Obtained from Rat KIM1

(TMB reaction incubate at 37°C for 15-20 min)

Concentration (pg/mL)	0.0	31.2	62.5	125	250	500	1000	2000
O.D.	0.016	0.080	0.137	0.252	0.485	0.920	1.589	2.314

Performance Characteristics

- ✓ Range: 31.2 pg/mL-2000 pg/mL
- ✓ Sensitivity: < 2 pg/mL
- ✓ Specificity: Natural and recombinant rat KIM1
- ✓ Cross-reactivity: There is no detectable cross-reactivity with other relevant proteins.
- ✓ Precision
  - Intra-Assay Precision (Precision within an assay)
    Three samples of known concentration were tested on one plate to assess intra-assay precision.
  - Inter-Assay Precision (Precision between assays)
    Three samples of known concentration were tested in separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean (pg/mL)	276	662	1186	359	710	1332
Standard deviation	11.6	35.1	41.51	22.26	48.28	75.9
CV (%)	4.2	5.3	3.5	6.2	6.8	5.7

Resources

References

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2. McIntire JJ, Umetsu SE, Akbari O, Potter M, Kuchroo VK, Barsh GS, Freeman GJ, Umetsu DT, DeKruyff RH (Nov 2001). "Identification of Tapr (an airway hyperreactivity regulatory locus) and the linked Tim gene family". *Nat Immunol* 2 (12): 1109–16. doi:10.1038/ni739.
3. "Entrez Gene: HAVCR1 hepatitis A virus cellular receptor 1".

Plate Layout

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