Product information

User´s Manual

Rat KIM-1 ELISA

For detection of KIM-1 in rat serum, plasma, urine, cell culture supernatant or tissue samples.

	BE69041
Σ	**96**
Storage:	**2-8°C**

RUO **For Research Use Only – Not for Use in Diagnostic Procedures.**

Manufactured for:
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1 INTRODUCTION

1.1 Intended Use

For detection of KIM-1 in rat serum, plasma, urine, cell culture supernatant or tissue samples. For research use only, not for use in diagnostic procedures.

1.2 Background

KIM1, also known as Hepatitis A virus cellular receptor 1, is a protein that in rats is encoded by the HAVCR1 gene, which maps to 5q33.2. It is a major cause of orally transmitted acute hepatitis, infects primate cells, but not dog or rat cells, after binding to the HAV cellular receptor (HAVCR). Infection of canine osteogenic sarcoma cells expressing HAVCR1 with HAV led Feigelstock et al. (1998) to conclude that the protein is indeed a receptor for the virus. Khademi et al. (2004) found that differential expression of TIMs by Th1 and Th2 cells may be implicated in different phases of an autoimmune disease.

2 PRINCIPLE OF THE TEST

This kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. The purified anti-KIM-1 antibody was pre-coated onto 96-well plates. And the HRP conjugated anti-KIM-1 antibody was used as detection antibodies. The standards, test samples and HRP conjugated detection antibody were added to the wells subsequently, mixed and incubated, then, unbound conjugates were washed away with wash buffer. TMB substrates (A & B) were 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 KIM-1 amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of KIM-1 can be calculated.

3 WARNINGS AND PRECAUTIONS

1. This kit is for research use only, not for use in diagnostic procedures.
2. Before the experiment, centrifuge each kit component for several minutes to bring down all reagents to the bottom of tubes.
3. It is recommend to measure each standard and sample in duplicate.
4. Do NOT let the plate completely dry at any time! Since the dry condition can inactivate the biological material on the plate.
5. Do not reuse pipette tips and tubes to avoid cross contamination.
6. Do not use the expired components or the components from different lot numbers.
7. Store the TMB substrate B (Kit Component 9) in dark.
8. Prolong the incubation time if the hypochromasia obtained. Heat the water in the water bath during diluting if the crystalloid appeared in Wash buffer (Kit Component 4).
9. Do not remove microplate from the storage bag until needed, and the unused strips should be stored at 2-8℃ in their pouch or the provided Hermetic bag (Kit Component 11).

4 REAGENTS

4.1 Reagents provided

1. One 96-well plate pre-coated with anti-rat KIM-1 antibody
2. Standard: 0.5ml (2250pg /mL)
3. Standard diluent buffer: 1.5 ml
4. Wash buffer (30×): 20 ml. Dilution: 1:30
5. Sample diluent buffer: 6 ml
6. HRP conjugated anti-rat KIM-1 antibody (RTU): 6ml
7. Stop solution: 6 ml
8. TMB substrate A: 6ml
9. TMB substrate B: 6ml
10. Plate sealer: 2
11. Hermetic bag: 1

4.2 Materials required but not provided

1. 37° incubator
2. Microplate reader (wavelength: 450nm)
3. Precise pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5ml of Eppendorf tubes
7. Absorbent filter papers
8. Plastic or glass container with volume of above 1L

4.3 Storage Conditions / Expiration

Store at 2-8℃ for 6 months.

4.4 Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20℃for long term. Avoid multiple freeze-thaw cycles.

- ✧ **Serum:** Coagulate at room temperature for 10-20 min, then, centrifuge at the speed of 2000-3000 r.p.m. for 20 min to collect supernatant. If precipitation appeared, centrifuge again.
- ✧ **Plasma:** Collect plasma using EDTA or citrate plasma as an anticoagulant, and mix for 10-20 min, centrifuge at the speed of 2000-3000 r.p.m. for 20 min of collection. If precipitation appeared, centrifuge again.
- ✧ **Urine:** Collect urine using a sterile container, centrifuge at the speed of 2000-3000 r.p.m. for 20 min to collect supernatant. If precipitation appeared, centrifuge again. For collection of hydrothorax and cerebrospinal fluid, take reference to this operation.
- ✧ **Cell culture supernatant:** For secretory components: use a sterile container to collect. Centrifuge at the speed of 2000-3000 r.p.m. for 20 min to collect supernatant. For intracellular components: Dilute cell suspension with PBS （pH7.2-7.4) to make the cell concentration reached 1 million / ml. Damage cells and release of intracellular components through repeated freeze-thaw cycles. Centrifuge at the speed of 2000-3000 r.p.m. For 20 min to collect supernatant. If precipitation appeared, centrifuge again.
- ✧ **Tissue samples:** Cut samples and weight, add certain volume of PBS (pH7.4), rapidly frozen with liquid nitrogen. After melting, store samples at 2-8℃. Add certain volume of PBS (pH7.4), homogenize thoroughly, centrifuge at the speed of 2000-3000 r.p.m. for 20 min to collect supernatant.

Note: 1. Coagulate blood samples completely, then, centrifuge, and avoid hemolysis and particle.

2. NaN_3 can not be used as test sample preservative, since it is the inhibitor for HRP.
3. After collecting samples, analyze immediately or aliquot and store frozen at -20°C. Avoid repeated freeze-thaw cycles.

2. Wash buffer

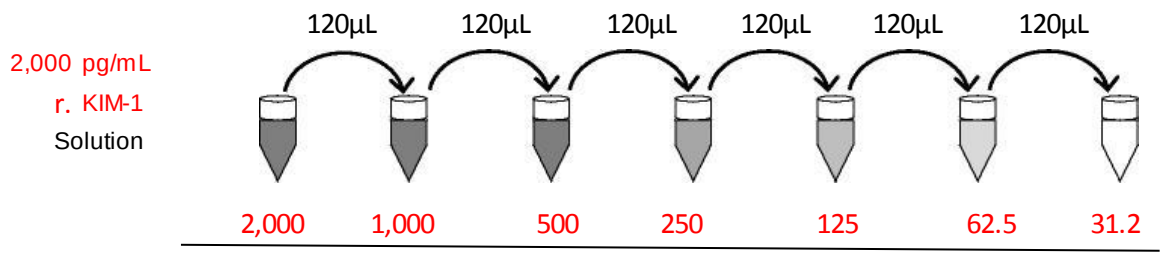
Dilute concentrated Wash buffer (Kit Component 4) 30-fold (1:30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water).

3. Standard

Dilution of the rat KIM-1 standard (Kit Component 2): standard solution should be prepared no more than 2 hours prior to the experiment. **(Note: Do not dilute the standard directly in the plate)**

a. 2000pg/ml of standard solution: Add 240 µL of the 2250pg/ml standard (Kit Component 2) into 30 µL Standard diluent buffer (Kit Component 3) and mix thoroughly.

b. 1000 pg/ml → 31.2 pg/ml of 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 120 µL of the Standard diluent buffer (Kit Component 3) into each tube. Add 120 µL of the above 2000 pg/ml standard solution into 1st tube and mix thoroughly. Transfer 120 µL from 1st tube to 2nd tube and mix thoroughly. Transfer 120 µL from 2nd tube to 3rd tube and mix thoroughly, and so on.



KIM-1 Protein Standards (pg/mL)

Note: The standard solutions are best used within 2 hours. Avoid repeated freeze-thaw cycles.

5 ASSAY PROCEDURE

5.1 General Remarks

Before adding to wells, equilibrate the kit components for at least 30 minutes at 37°. It is recommended to plot a standard curve for each test.

5.2 Test Procedure

1. Equilibrate kit components for at least 30 min at 37°.
2. Set test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. Add 50µl of diluted standards (2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.2 pg/ml) into the standard wells. Add 50µl of Standard diluent buffer (Kit Component 3) into the control (zero) well. Do not add sample and HRP conjugated antibody to control (zero) well.
3. For test sample wells, add 40µl of Sample diluent buffer first, then, add 10µl of sample. Add the solution at the bottom of each well without touching the side wall. Shake the plate mildly to mix thoroughly.
4. Cover the plate with Plate sealer (Kit Component 10) and incubate at 37° for 30 min.
5. Remove the sealer, and wash plate using one of the following methods:

   Manual Washing: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers. Fill each well completely with Wash Buffer (1×) and vortex mildly on ELISA shaker for 2 min, then aspirate contents from the plate, and clap the plate on absorbent filter papers. Repeat this procedure four more times for a **total of FIVE washes**.

   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1×). After the final wash, invert plate, and clap the plate on absorbent filter papers until no moisture remained. It is recommended that the washer be set for a soaking time of 10 seconds or shaking.
6. Add 50µl of HRP conjugated anti-KIM-1 antibody (Kit Component 6) into each well (except control well).
7. Cover the plate with Plate sealer (Kit Component 10) and incubate at 37° for 30 min.
8. Remove the sealer, and wash the plate. (See Step 5)
9. Add 50µl of TMB chromogenic reagent A (Kit Component 8) into each well, and then, add 50µl of TMB chromogenic reagent B (Kit Component 9), vortex gently the plate on ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds), and incubate in dark at 37° for 15 min. The shades of blue can be seen in the wells.
10. Add 50µl of Stop solution (Kit Component 7) into each well and mix thoroughly. The color changes into yellow immediately.
11. Read the O.D. absorbance at 450nm in a microplate reader within 15 min after adding the stop solution.

5.3 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 KIM-1 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.

6 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. It is recommended to use controls according to state and federal regulations. The use of controls is advised to assure the day to day validity of results. It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results. Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials, results of unknowns should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor or IBL-America directly.

7 PERFORMANCE CHARACTERISTICS

7.1 Range

31.2pg/ml-2000pg/ml

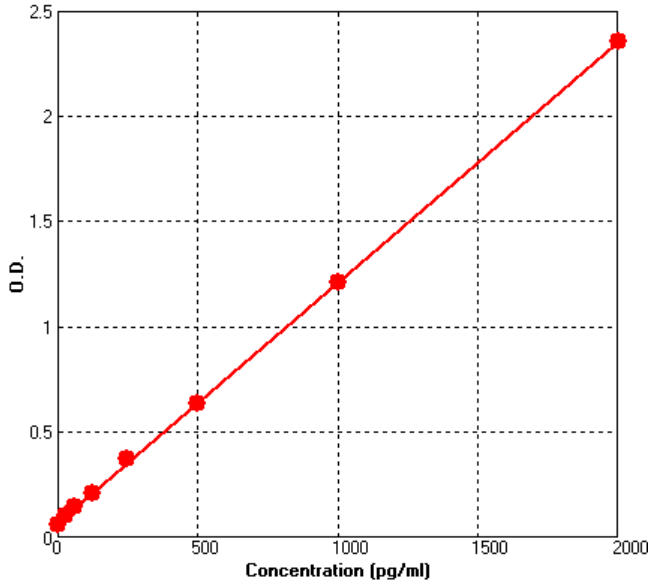
7.2 Sensitivity

<10pg/ml

7.3 Typical Data & Standard Curve

Results of a typical standard run of a rat KIM-1 ELISA Kit are shown below**. This standard curve was generated at our lab for demonstration purpose only.** Each user should obtain their own standard curve as per experiment. (N/A=not applicable)

X	Pg/ml	0	31.2	62.5	125	250	500	1000	2000
Y	OD450	0.063	0.101	0.145	0.209	0.371	0.633	1.209	2.353



7.4 References:

1. Feigelstock D, Thompson P, Mattoo P, Zhang Y, Kaplan GG (Aug 1998). "The rat homolog of HAVcr-1 codes for a hepatitis A virus cellular receptor". J Virol 72 (8): 6621–8.
2. McIntire, J. J., Umetsu, S. E., Akbari, O., Potter, M., Kuchroo, V. K., Barsh, G. S., Freeman, G. J., Umetsu, D. T., DeKruyff, R. H. Identification of Tapr (an airway hyperreactivity regulatory locus) and the linked Tim gene family. Nature Immun. 2: 1109-1116, 2001.
3. Khademi, M., Illes, Z., Gielen, A. W., Marta, M., Takazawa, N., Baecher-Allan, C., Brundin, L., Hannerz, J., Martin, C., Harris, R. A., Hafler, D. A., Kuchroo, V. K., Olsson, T., Piehl, F., Wallstrom, E. T cell Ig- and mucin-domain-containing molecule-3 (TIM-3) and TIM-1 molecules are differentially expressed on mouse Th1 and Th2 cells and in cerebrospinal fluid-derived mononuclear cells in multiple sclerosis. J. Immun. 172: 7169-7176, 2004

8 ORDERING INFORMATION

This kit is manufactured for Immuno-Biological Laboratories, Inc. (IBL-America). For ordering information, please contact:

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