ab119597 – KIM-1 (TIM-1) Rat ELISA Kit

Instructions for Use

For quantitative detection of rat KIM-1 (TIM-1) in cell culture supernatants, serum, plasma (heparin, EDTA) and urine.

This product is for research use only and is not intended for diagnostic use.

Version 4 Last Updated 11 January 2019

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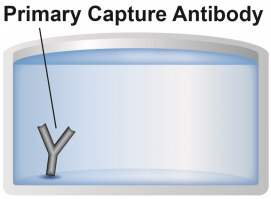
1. BACKGROUND

Abcam's rat KIM-1 (TIM-1) *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of rat KIM-1 in cell culture supernatants, serum, plasma (heparin, EDTA) and urine.

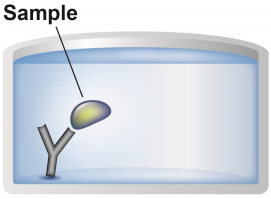
A KIM-1 specific mouse monoclonal antibody has been precoated onto 96-well plates. Standards and test samples are added to the wells and incubated. A biotinylated detection polyclonal antibody from goat specific for KIM-1 is then added followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex is added and unbound conjugates are washed away with PBS or TBS buffer. TMB is then used to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the rat KIM-1 amount of sample captured in plate.

KIM-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.

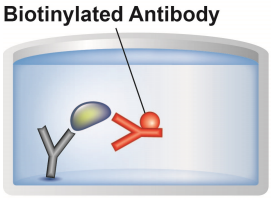
2. ASSAY SUMMARY



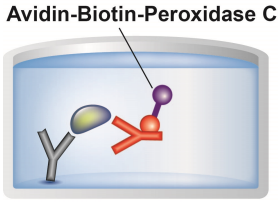
Prepare all reagents, samples and standards as instructed.



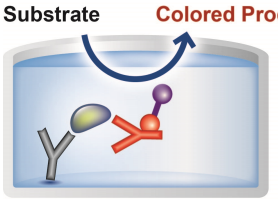
Add standard or sample to each well used. Incubate at room temperature or 37°C.



Add prepared biotin antibody to each well. Incubate at room temperature or 37°C.



Add prepared Avidin-Biotin-Peroxidase Complex (ABC). Incubate at room temperature or 37°C.



Add TMB to each well. Incubate at room temperature. Add Stop Solution to each well. Read

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20°C immediately upon receipt. Avoid multiple freeze-thaw cycles.

Refer to list of materials supplied for storage conditions of individual components.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Anti-rat KIM-1 antibody Microplate (12 x 8 wells)	96 Wells	-20ºC
Lyophilized recombinant rat KIM-1 standard	2 x 10 ng	-20ºC
Biotinylated anti-rat KIM-1 antibody	130 µL	-20ºC
Avidin-Biotin-Peroxidase Complex (ABC)	130 µL	-20ºC
Sample Diluent Buffer	30 mL	-20ºC
Antibody Diluent Buffer	12 mL	-20ºC
ABC Diluent Buffer	12 mL	-20ºC
TMB Color Developing Agent	10 mL	-20ºC
TMB Stop Solution	10 mL	-20ºC
Plate Seal	4 Units	20ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Standard microplate reader
- Automated plate washer (optional)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed
- Eppendorf tubes
- Washing buffer, either neutral PBS or TBS (see Section 9 for recipes)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted

8. TECHNICAL HINTS

- To determine the appropriate sample dilution to use in this ELISA a pilot experiment using standards and a small number of samples is recommended
- The TMB Color Developing agent is colorless and transparent before use
- Before using the kit, briefly centrifuge the tubes in case any of the contents are trapped in the lid
- It is recommended to assay all standards, controls and samples in duplicate
- Do not let the 96-well plate dry out as this will inactivate active components on plate
- To avoid cross contamination do not reuse tips and tubes
- In order to avoid marginal effects 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 be pre-warmed in 37°C for 30 minutes before using
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18 - 25°C) prior to use.

9.1 **1X Biotinylated anti-rat KIM-1**

Biotinylated anti-rat KIM-1 antibody must be diluted 1:100 with the Antibody Diluent Buffer and mixed thoroughly (i.e. add 1 µL Biotinylated anti-rat KIM-1 antibody to 99 µL Antibody Diluent Buffer.) The total volume required should be; 100 µL/well multiplied by the total number of wells (Allowing 100 – 200 µL more than total volume).

9.2 **1X Avidin-Biotin-Peroxidase Complex**

Avidin-Biotin-Peroxidase Complex (ABC) must be diluted 1:100 with ABC Diluent Buffer and mixed thoroughly (i.e. add 1 µL ABC to 99 µL ABC Diluent Buffer.) The total volume required should be; 100 µL/well multiplied by the total number of wells (allow 100 µL - 200 µL more than total volume).

9.3 **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 distilled water and adjust pH to 7.2 - 7.6. Finally, adjust the total volume to 1 L with distilled water.

9.4 **0.01 M PBS**

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

10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use. Reconstitution of the rat KIM-1 standard should be prepared no more than 2 hours prior to the experiment. Two tubes of rat KIM-1 standard (10 ng per tube) are included in each kit. Use one tube for each experiment.

10.1 Prepare a 10 ng/mL **Stock Standard** by reconstituting one vial of the KIM-1 standard with the addition of 1 mL Sample Diluent Buffer. Hold at room temperature for 10 minutes. The 10 ng/mL **Stock Standard** 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.

10.2 Label eight tubes with #1- 8.

10.3 Prepare a 2,000 pg/mL **Standard #1** by adding 200 µL of the 10 ng/mL Stock Standard into test tube #1 along with 800 µL Sample Diluent Buffer. Mix thoroughly and gently.

10.4 Add 300 µL Sample Diluent Buffer into tubes #2 - 8.

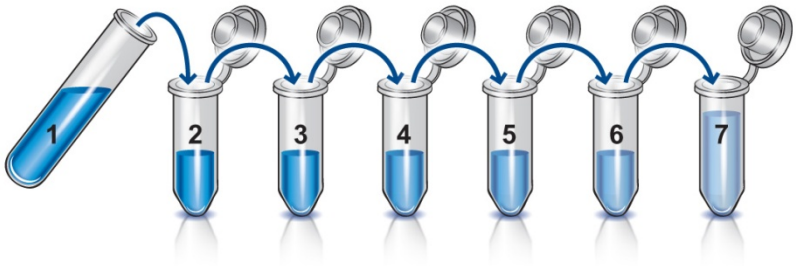
10.5 Prepare **Standard #2** by transferring 300 µL from Standard #1 to tube #2. Mix thoroughly and gently.

10.6 Prepare **Standard #3** by transferring 300 µL from Standard #2 to tube #3. Mix thoroughly and gently.

10.7 Using the table below as a guide, repeat for tubes #4 through #7.

10.8 **Standard #8** contains no protein and is the Blank control.

Standard #	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Stock	200	800	10,000	2,000
2	Standard #1	300	300	2,000	1,000
3	Standard #2	300	300	1,000	500
4	Standard #3	300	300	500	250
5	Standard #4	300	300	250	125
6	Standard #5	300	300	125	62.5
7	Standard #6	300	300	62.5	31.2
8	None	-	300	-	-



11. SAMPLE COLLECTION 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.

11.1 **Cell Culture Supernatants**

Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.

11.2 **Serum**

Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1,000 x *g* for 15 minutes. Analyze the serum immediately or aliquot and store samples at -20°C.

11.3 **Plasma**

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x *g* within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C.

11.4 **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 samples at -20°C.

12. SAMPLE PREPARATION

General Sample information:

The user needs to estimate the concentration of the target protein in the sample and select the correct dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve.

Dilute the samples using the provided Sample Diluent Buffer. The following is a guideline for sample dilution. Several trials may be

necessary to determine the optimal dilution factor. The sample must be thoroughly mixed with the Sample Diluent Buffer before assaying.

- 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 - 2,000 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 (≤ 31.2 pg/mL). No dilution necessary, or the working dilution is 1:2.

13. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section

14. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

14.1 Prepare all reagents, working standards, and samples as directed in the previous sections

14.2 Add 100 µL of prepared standards and diluted samples to appropriate wells.

14.3 Seal the plate with a new plate seal and incubate at RT for 120 minutes or at 37°C for 90 minutes.

14.4 Remove the seal, discard contents of each well, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.

14.5 Add 100 µL of 1X Biotinylated anti-rat KIM-1 antibody into each well, seal the plate with a new plate seal and incubate the plate at RT for 90 minutes or at 37°C for 60 minutes.

14.6 Wash the plate three times with 300 µL 0.01 M TBS or 0.01 M PBS, and each time let the washing buffer stay in the wells for one minute. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.

Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with each wash. Blot the plate onto paper towels or other absorbent material.

14.7 Add 100 µL of 1X Avidin-Biotin-Peroxidase Complex working solution into each well, seal the plate with a new plate seal and incubate the plate at RT for 40 minutes or at 37°C for 30 minutes.

14.8 Wash plate five times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 - 2 minutes. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 14.6 for plate washing method).

14.9 Add 90 µL of prepared TMB color developing agent into each well, seal the plate with a new plate seal and incubate in dark for 30 minutes at RT or at 37°C in dark for 25 - 30 minutes.

Note: The optimal incubation time should be determined by end user. The shades of blue should be seen in the wells with the four most concentrated rat KIM-1 standard solutions; the other wells show no obvious color.

14.10 Add 100 µL of prepared TMB Stop Solution into each well. The color changes into yellow immediately.

14.11 Read the O.D. absorbance at 450 nm in a microplate reader within 30 minutes after adding the stop solution.

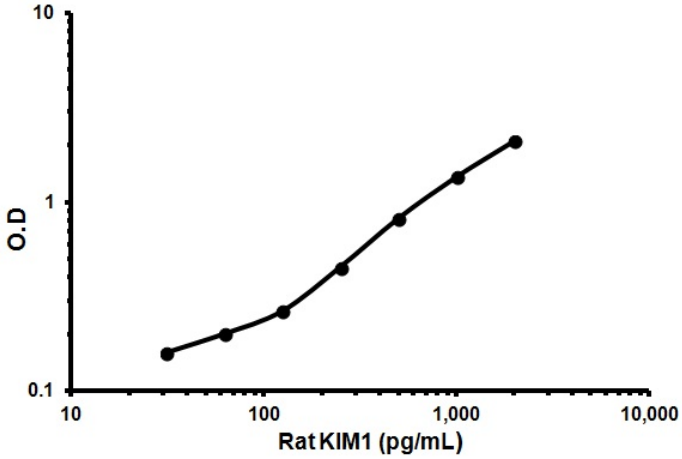
15. CALCULATIONS

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, make sure to account for this in your calculations.

16. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Conc. (pg/mL)	O.D. 450nm
0.0	0.118
31.2	0.159
62.5	0.200
125	0.264
250	0.452
500	0.817
1,000	1.354
2,000	2.099

17. TYPICAL SAMPLE VALUES

RANGE – 31.2 – 2,000 pg/mL

SENSITIVITY – < 2 pg/mL

18. ASSAY SPECIFICITY

This kit detects both endogenous and recombinant rat KIM-1.

No detectable cross-reactivity with other relevant proteins.

19. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

20. NOTES

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