CELL APPLICATIONS, INC.

# Rat KIM-1 ELISA Kit

Cat. No. CL0882
96-wells

| | |
|---|---|
| **Target Protein Species** | Rat |
| **Range** | 31.2pg/ml-2000pg/ml |
| **Specificity** | No detectable cross-reactivity with any other cytokine. |

## COMPONENTS

| Kit Component | Amount |
|---|---|
| 96-well plate precoated with anti-rat KIM-1 antibody | 1 Plate |
| Protein Standard: Lyophilized recombinant rat KIM-1 | 2 tubes, 10 ng/tube |
| Sample Diluent Buffer | 30 ml |
| Biotinylated Antibody (Anti-rat KIM-1) | 130 µl (100x) |
| Antibody Diluent Buffer | 12ml |
| Avidin-Biotin-Peroxidase Complex (ABC) Solution | 130 µl (100x) |
| ABC Diluent Buffer | 12 ml |
| Tetramethyllbenzidine (TMB) Color Developing Agent | 10 ml |
| TMB Stop Solution | 10 ml |

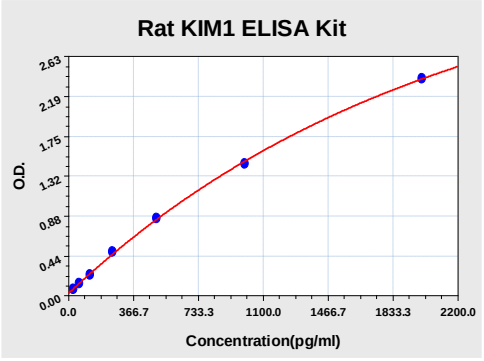
**Washing Buffer (not provided): TBS or PBS**

0.01M TBS: Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 900ml $H_2O$ and adjust pH to 7.2-7.6. Adjust the total volume to 1L.

0.01M PBS: Add 8.5g sodium chloride, 1.4g $Na_2HPO_4$ and 0.2g $NaH_2PO_4$ to 900ml distilled $H_2O$ and adjust pH to 7.2-7.6. Adjust the total volume to 1L.

**Storage**

Store at 4°C. Cell Applications, Inc. recommends using the kit within 6 months of order.




| X | pg/ml | 0.0 | 31.2 | 62.5 | 125 | 250 | 500 | 1000 | 2000 |
|---|---|---|---|---|---|---|---|---|---|
| Y | O.D.450 | 0.022 | 0.080 | 0.143 | 0.242 | 0.489 | 0.859 | 1.459 | 2.391 |

**Figure 1: KIM-1 Standard Curve.** Using the Rat KIM-1 ELISA Kit, O.D. data was graphed against KIM-1 protein concentration. The TMB reaction was incubated at 37°C for 20 min.

## BACKGROUND

The kidney injury molecule-1 (KIM-1) is a type I cell membrane glycoprotein which contains, in its extracellular portion, a novel six-cysteine immunoglobulin-like domain, two N-glycosylation sites and a T/SP rich domain characteristic of mucin-like O-glycosylated proteins. The structure of the protein showes adhesion molecule properties. KIM-1 is not normally present, but is expressed on the proximal tubule apical membrane with injury. KIM-1 is also expressed at high levels in patients with clear cell-type renal cell carcinoma (RCC). RCC, like renal tubular injury, is associated with proximal tubule cell dedifferentiation. It may participate in the progress of renal injury or repair. The ectodomain of KIM-1 is shed from cells. A soluble form of human KIM-1 can be detected in the urine of patients with acute tubular necrosis (ATN) and may serve as a useful biomarker for renal proximal tubule injury facilitating the early diagnosis of the disease and serving as a diagnostic discriminator.[1]

Many studies have illustrated the different functions of KIM-1 in various renal diseases including protective functions in acute kidney injury and damaging functions in chronic kidney disease. KIM-1 confers on epithelial cells the ability to recognize and phagocytose dead cells that are present in the post-ischaemic kidney and contribute to the obstruction of the tubule lumen that characterizes acute kidney injury (AKI).[2] KIM-1 is a phosphatidylserine receptor that recognizes apoptotic cells directing them to lysosomes. It also serves as a receptor for oxidized lipoproteins and hence is adept at recognizing apoptotic cell 'eat me' signals. Given these properties KIM-1 is unique in being the first non-myeloid phosphatidylserine receptor that transforms epithelial cells into semi-professional phagocytes. In addition to the facilitation of clearance of the apoptotic debris from the tubular lumen, KIM-1 may play an important role in limiting the autoimmune response to injury since it is known in many systems that phagocytosis of apoptotic bodies is one mechanism for limiting the proinflammatory response. Acute protective responses, however, may not necessarily translate to chronic effects of KIM-1 expression, a clinically relevant issue, since it has been shown that many individuals with chronic renal failure express the KIM-1 protein in their proximal tubules. KIM-1 is expected to be a therapeutic target for kidney injury.[3] In addition, KIM-1 is receptor for TIMD4. In case of human hepatitis A virus (HHAV) infection, it functions as a cell-surface receptor for the virus (HAVCR).

## ELISA OVERVIEW

Cell Applications ELISA Kits are based on standard sandwich enzyme-linked immunosorbent assay technology. Freshly prepared standards, samples, and solutions are recommended for best results.

1. Prepare test samples.
2. Prepare a protein standard of the target protein.
3. Add test samples and standards to the pre-coated 96-well plate. Do not wash.
4. Add biotinylated detection antibodies. Wash.
5. Add Avidin-Biotin-Peroxidase Complex (ABC) Solution. Wash.
6. Add Tetramethyllbenzidine (TMB) Color Developing Agent, containing HRP substrate.
7. Add TMB Stop Solution
8. Subject the plate to analysis.

**NOTES:**

- Before using the kit, quick spin tubes to bring down all solution to the bottom of tube.
- Duplicate assay wells are recommended for both standard and sample testing.
- Do not let the 96-well plate dry, this will lead to inactivation of plate components.
- When diluting samples and reagents, ensure that they are mixed completely and evenly.
- Pre-warm diluted ABC and TMB solutions at 37℃ for 30 min before use to avoid variable temperature effects.
- For washes, use TBS or PBS. Do not touch well walls.
- A protein standard is included in the kit. A protein standard detection curve should be generated with each experiment, no more than 2 hours prior to the experiment.
- The user will determine sample dilution fold by estimation of target protein amount in samples.

**References**


1. Han, W.K. et al: Kidney Int. 62:237-44, 2002
2. Bonventre, J.V.: Scand. J. Clin. Lab. Invest. Suppl. 241:78-83, 2008
3. Huo, W. et al: Transplant. Rev. (Orlando) 24:143-6, 2010

CELL APPLICATIONS, INC.

# Rat KIM-1 ELISA Kit

Cat. No. CL0882
96-wells

| | |
|---|---|
| **Target Protein Species** | Rat |
| **Range** | 31.2pg/ml-2000pg/ml |
| **Specificity** | No detectable cross-reactivity with any other cytokine. |

## PROTOCOL

### I. 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.

### II. Preparation of Test Samples

**Test Sample Processing**

- **Cell culture supernate, tissue lysate or body fluids**: Remove particulates by centrifugation.
- **Serum**: Allow the serum to clot in a serum separator tube (about 2 hours) at room temperature. Centrifuge at approximately 1000 X g for 10 min.
- **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.

**Sample Dilution Guideline**

Estimate the concentration of the target protein in the sample and select a proper dilution factor such that the diluted target protein concentration falls within the standard curve range. Depending on the sample, several trial dilutions may be necessary. Dilute the sample using the provided diluent buffer, mixing well. Suggested working dilutions of samples are as follows:

| Target Protein Concentration Range | Sample Working Dilution | Sample Vol. | Diluent Buffer Vol. |
|---|---|---|---|
| 20-200 ng/ml | 1:100 | 1 µl | 99 µl |
| 2-20 ng/ml | 1:10 | 10 µl | 90 µl |
| 31.2-2,000 pg/ml | 1:2 | 50 µl | 50 µl |
| ≤31.2 pg/ml | n/a | 100µl | n/a |

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

### III. Preparation of Reagents

**Reconstitution of the Standard**

The standard solutions should be prepared no more than 2 hours prior to the experiment. Two tubes of the standard are included in each kit. Use one tube for each experiment.

1. 10,000pg/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.
2. 2000pg/ml of Rat KIM1 standard solution: Add 0.2 ml of the above 10ng/ml KIM1 standard solution into 0.8 ml sample diluent buffer and mix thoroughly.
3. 1000pg/ml→31.2pg/ml of Rat KIM1 standard solutions: Label 6 Eppendorf tubes with 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 2000pg/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.

**Preparation of Biotinylated Antibody Working Solution**

The solution should be prepared no more than 2 hours prior to the experiment.

1. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
2. Biotinylated antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly.

**Preparation of the Avidin-Biotin-Peroxidase Complex (ABC) Working Solution**

The solution should be prepared no more than 1 hour prior to the experiment.

1. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
2. Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly.

### IV. ELISA

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. A standard detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of target protein amount in samples.

1. Aliquot 0.1ml per well of the 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml Rat KIM1 standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Blank well). Add 0.1ml of each properly diluted sample of Rat sera, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. **See "Sample Dilution Guideline"** for details. We recommend that each Rat KIM1 standard solution and each sample is measured in duplicate.
2. Seal the plate with the cover 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 0.1ml of biotinylated anti-Rat KIM1 antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash plate 3 times with 0.01M TBS or 0.01M 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 0.1ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01M TBS or 0.01M 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°C in dark for 20-25 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 KIM-1 standard solutions; the other wells show no obvious color).
9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.

### V. Calculating Protein Concentration

- For all wells, determine O.D.450(Relative):
  O.D.450(Relative) = O.D.450(Reading) – O.D.450(Blank)

- Plot the standard curve:
  Plot O.D.450(Relative) of each standard solution (Y) vs. the respective concentration of the standard solution (X). See **Figure 1** for a typical standard curve.

- The target protein concentration in samples can be interpolated from the standard curve. Multiply the interpolated concentration by the dilution factor to obtain the target protein concentration in the sample.