RAT KIDNEY INJURY MOLECULE -1 (KIM-1) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF KIM-1 CONCENTRATIONS IN RAT URINE, EDTA PLASMA AND SERUM

PURCHASE INFORMATION:

ELISA NAME	RAT KIM-1 ELISA
Catalog No.	SK00186-02
Lot No.	
Formulation	96 T
Standard range	15.6-1000 pg/mL
Sensitivity	5 pg/mL
Sample Volume	100 µL
Dilution factor	**Optimal dilutions should be determined by each laboratory for each application**
Sample Type	Urine, EDTA Plasma and Serum
Specificity	Rat KIM-1 only
Intra-assay Precision	4 - 8%
Inter-assay Precision	6 - 8%
Storage	2 – 8 °C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTRODUCTION

Rat KIM-1 ultrasensitive immunoassay is a solid phase ELISA designed to measure KIM-1 in rat urine, EDTA plasma and serum. It contains recombinant KIM-1 and antibodies raised against this protein. It has been shown to accurately quantify recombinant KIM-1. Results obtained with naturally occurring KIM-1 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural KIM-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for KIM-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any KIM-1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for KIM-1 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of KIM-1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute reagents with those from other lots or sources.
_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Rat KIM-1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against rat KIM-1.	**186-02-01**	**1 plate**
Rat KIM-1 Standard – 2000 pg/vial of recombinant KIM-1 in a buffered protein base with preservative; lyophilized.	**186-02-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against KIM-1 with preservative; lyophilized.	**186-02-03**	**1 vial**
Positive Control – one vial of recombinant KIM-1; lyophilized.	**186-02-04**	**1 vial**
Streptavidin-HRP Conjugate - 60 µl/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	**DB05**	**1 bottle**
Antibody & HRP Diluent Solution – 30 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of **TMB** substrate solution.	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8 °C for up to 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 °C or -70 °C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted

Standard (stock) and Detection Antibody concentrated solution SHOULD BE STORED at -20 °C or -70 °C for up to one month.
Streptavidin-HRP Conjugate 200-fold concentrated solution and other components may be stored at 2 – 8 °C for up to 8 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8 °C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 ml and 500 ml graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution.
We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

SAMPLE COLLECTION AND STORAGE

Urine - Freshly collected urine samples were allowed to sit at room temperature for 30 minutes to sediment, and the supernatant was aliquoted and stored at -70 °C until analysis. Centrifuge again before assaying to remove any additional precipitates that may appear after storage.
Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20 °C. Avoid repeated freeze-thaw cycles.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

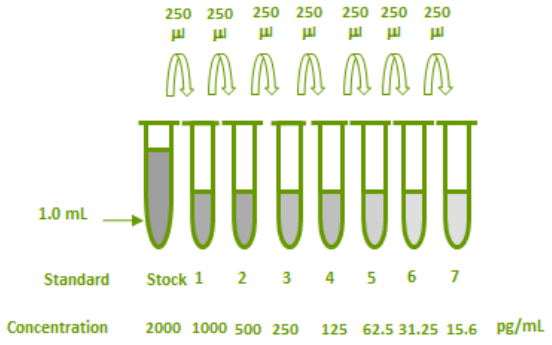
Optimal dilutions should be determined by each laboratory for each application with a sample pretest.
Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 ml of Wash Buffer Concentrate into deionized or distilled water (450 ml) to prepare 500 ml of 1x Wash Buffer.

Rat KIM-1 Standard - Refer to vial label for reconstitution volume. Reconstitute the KIM-1 standard with 1.0 mL of **Dilution Buffer (DB05)**. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **1000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

Tube	Standard	Dilution Buffer	Concentration
stock	Powder	1000 µl	2000 pg/ml
# 1	250 µl of stock	250 µl	1000 pg/ml
# 2	250 µl of 1	250 µl	500 pg/ml
# 3	250 µl of 2	250 µl	250 pg/ml
# 4	250 µl of 3	250 µl	125 pg/ml
# 5	250 µl of 4	250 µl	62.5 pg/ml
# 6	250 µl of 5	250 µl	31.25 pg/ml
# 7	250 µl of 6	250 µl	15.625 pg/ml



Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.05 mL of **Antibody & HRP Diluent Solution (DB01)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Antibody & HRP Diluent Solution into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

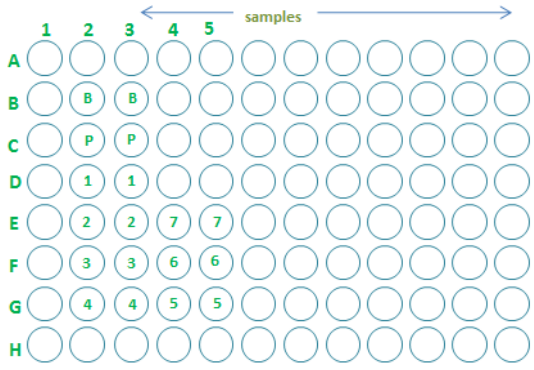
Streptavidin-HRP Conjugate - Pipette 11.94 mL of **Antibody & HRP Diluent Solution (DB01)** into a 15 mL centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the positive control with 1.0 mL of **Dilution Buffer (DB05)** to make positive control solution. **Note:** Positive Control could be reused within a few days if stored at -20 °C or -70 °C.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL of Dilution Buffer to Blank wells (B2, B3).
4. Add 100 µL of standard solutions from #7 to #1 in reverse order of serial dilution (E4, E5 to G4, G5 and G2, G3 to D2, D3), sample, or positive control (C2, C3) per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 8-12 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As

an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the standard concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

The amount of KIM-1 in each urine sample is normalized to that of the creatinine level. The normalized data is expressed as microgram of KIM-1 per gram of creatinine [KIM-1(µg)/$Urine_{creatinine}$(g)].

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

KIM-1 (PG/ML)	CORRECTED (450NM)
Blank	0 (0.097)
15.6	0.051
31.25	0.102
62.5	0.218
125	0.408
250	0.816
500	1.598
1000	2.757

- **Lot No.:**
- **Positive Control:**

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant rat KIM-1.

SENSITIVITY

The minimum detectable dose (MDD) of KIM-1 was 5 pg/mL.

SPECIFICITY

This assay recognizes both natural and recombinant rat KIM-1. The factors listed below were prepared at 50 ng/ml in Dilution Buffer, and assayed for cross reactivity. Preparations of the following factors at 50 ng/ml in a mid-range rr KIM-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Proteins	Cross-reactivity
Rat KIM-1	100%
Rat sRAGE	0
Rat NGAL	0
Mouse KIM-1	0
Mouse TIM-3	0
Mouse TIM-4	0
Human KIM-1	0
Human NGAL	0
Human sRAGE	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. **Protect from light**.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate solution to each well. Incubate 8-12 min on the plate shaker at RT. **Protect from light**.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.