

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

FOR THE QUANTITATIVE DETERMINATION
OF KIM-1 CONCENTRATIONS IN MOUSE
URINE



PURCHASE INFORMATION:

ELISA NAME	MOUSE KIM-1 ELISA
Catalog No.	SK00186-03
Lot No.:	
Formulation	96 T
Standard range	31-2000 pg/mL
Sensitivity	15 pg/mL
Sample Volume	100 μ L
Dilution	8 (Optimal dilutions should be determined by each laboratory for each application)
Sample Type	Urine
Specificity	Mouse KIM-1 only
Intra-assay Precision	4 - 8%
Inter-assay Precision	6 - 8%
Storage	2°C - 8°C

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DIAGNOSTIC PROCEDURES.

INTRODUCTION

Mouse KIM-1 ultrasensitive immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure KIM-1 in mouse urine. 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. A monoclonal 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 polyclonal 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
KIM-1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against KIM-1.	186-03-01	1 plate
KIM-1 Standard – 2000 pg/vial of recombinant KIM-1 in a buffered protein base with preservatives; lyophilized.	186-03-02	1 vial
Detection Antibody Concentrate – 105 µL/vial, 100-fold concentrate of biotinylated polyclonal antibody against KIM-1 with preservatives; lyophilized.	186-03-03	1 vial
Positive Control - one vial of recombinant KIM-1, lyophilized	186-03-04	1 vial
Streptavidin-HRP Conjugate - 60 uL/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP	SAHRP	1 vial
Dilution Buffer – 60 mL of buffered protein based solution with preservatives	DB05	1 bottle
HRP Diluent Solution – 12 mL of buffered protein based solution with preservatives	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 12 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard and Detection Antibody Concentrate Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrate and other components may be stored at 2 - 8°C for up to 12 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack and seal along entire edge of zip-seal. 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, with the correction wavelength set at 540 nm or 570 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.

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.

Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Urine samples may require an 8- fold dilution. A suggested 8-fold dilution is 40 µL sample + 280 µL Dilution Buffer.

Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

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.

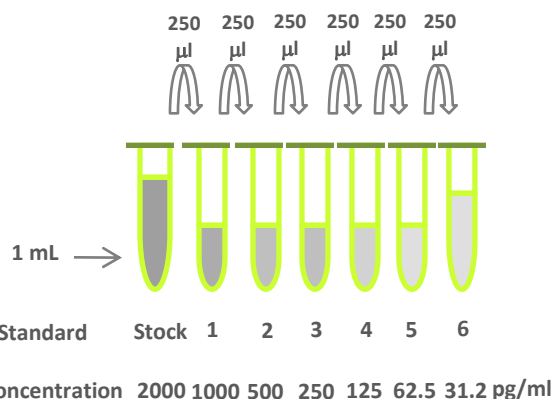
REAGENT PREPARATION

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 Wash Buffer.

KIM-1 Standard - Refer to vial label for reconstitution volume. Reconstitute the KIM-1 Standard with 1.0 mL of Dilution Buffer. 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 #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 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



Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with 105 μL of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 105 μL of 100-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.94 mL of 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.

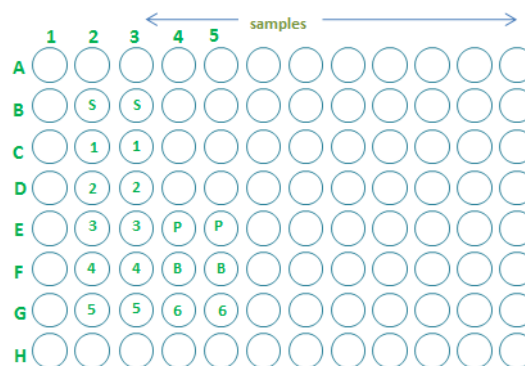
Positive Control - Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. **Note:** Positive control should be used prepared and used immediately.

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 duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate 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 (F4, F5).
4. Add 100 μL of **Standard** (B2, B3 to G2, G3 and G4, G5), **sample**, or **positive control** (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate 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 micro-plate 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 micro-plate 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 5-10 minutes 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 micro-plate 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 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(μ g)/Urine_{creatinine}(g)].

CALIBRATION

This immunoassay is calibrated against a highly purified NSO-expressed recombinant mouse KIM-1.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of KIM-1 was 15 pg/mL.

TYPICAL DATA

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

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.076)
31.25	0.012
62.5	0.024
125	0.057
250	0.136
500	0.352
1000	0.766
2000	1.541

- Lot No.:
- Positive Control: 150 – 400 pg/mL

LINEARITY

To assess the linearity of the assay, research mouse urine samples were diluted with Dilution Buffer (DB05) and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
8 X	179.706	1437.648	100
16 X	99.351	1589.616	110

SPECIFICITY

This assay recognizes both natural and recombinant Mouse 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 rh KIM-1 control

were assayed for interference. No significant cross-reactivity or interference was observed.

Mouse Recombinant Proteins:

KIM-3, NGAL, sRAGE, RBP-4

Human Recombinant Proteins:

KIM-1

Rat Recombinant Proteins:

KIM-1

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

Add 100 μ L of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.

Aspirate and wash 4 times.

Add 100 μ L Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.

Aspirate and wash 4 times.

Add 100 μ L 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 μ L Substrate Solution to each well. Incubate 5-10 min on the plate shaker. **Protect from light.**

Add 100 μ L Stop Solution to each well. Read 450nm within 15 min