

## CANINE KIDNEY INJURY MOLECULE -1 (KIM-1) ELISA KIT

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



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

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

### PRODUCT INFORMATION:

ELISA NAME	CANINE KIM-1 ELISA KIT
Catalog No.	SK00186-07
Lot No.:	20112137
Formulation	96 T
Standard range	125 - 8000 pg/ml
Sensitivity	20 pg/ml
Sample Volume	100 µl
Dilution	<b>Optimal dilutions should be determined by each laboratory for each application</b>
Sample Type	Urine
Specificity	Canine KIM-1 only
Calibration	Canine KIM-1 Recombinant
Intra-assay Precision	4 - 8%
Inter-assay Precision	6 - 8%
Storage	2 – 8° C
<b>This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.</b>	

### Order Contact:

AVISCERA BIOSCIENCE, INC.  
2348 Walsh Ave., Suite C  
Santa Clara, CA 95051  
USA

Tel: (408) 982 0300

Fax: (408) 982 0301

Email: [Sales@AvisceraBioscience.com](mailto:Sales@AvisceraBioscience.com)

[Info@AvisceraBioscience.com](mailto:Info@AvisceraBioscience.com)

[www.AvisceraBioscience.com](http://www.AvisceraBioscience.com)

## DESCRIPTION

This Canine KIM-1 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural Canine KIM-1 from urine in a sandwich ELISA format.

This immunoassay contains recombinant KIM-1 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural KIM-1 samples.

## ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for Canine KIM-1. The capture antibody can bind to the Canine KIM-1 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against KIM-1 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of Canine KIM-1 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

## PROCEDURAL LIMITATIONS

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

\_This ELISA kit should not be used beyond the expiration date on the kit label.

\_Do not mix 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.

\_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.

\_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

\_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

## COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
<b>KIM-1 Microplate</b> - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against KIM-1.	<b>186-07-01</b>	<b>1 plate</b>
<b>KIM-1 Standard</b> – 2000 pg/vial of recombinant KIM-1 in a buffered protein base with preservative; lyophilized.	<b>186-07-02</b>	<b>1 vial</b>
<b>Detection Antibody Concentrate</b> – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against KIM-1 with preservative; lyophilized.	<b>186-07-03</b>	<b>1 vial</b>
<b>Positive Control</b> - one vial of recombinant KIM-1; lyophilized.	<b>186-07-04</b>	<b>1 vial</b>
<b>Streptavidin-HRP Conjugate</b> - 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	<b>SAHRP</b>	<b>1 vial</b>
<b>Dilution Buffer</b> – 45 mL of buffered protein based solution with preservative.	<b>DB10</b>	<b>1 bottle</b>
<b>HRP Diluent Solution</b> – 12 mL of buffered protein based solution with preservative.	<b>DB08</b>	<b>1 bottle</b>
<b>Wash Buffer</b> - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	<b>WB01</b>	<b>1 bottle</b>
<b>TMB Substrate Solution</b> - 11 mL of TMB substrate solution.	<b>TMB01</b>	<b>1 bottle</b>
<b>Stop Solution</b> – 11 mL of 0.5M HCl.	<b>S-STOP</b>	<b>1 bottle</b>
<b>Plate Sealer</b>	<b>EAPS</b>	<b>1 piece</b>
<b>Plastic Pouch</b>	<b>P01</b>	<b>1 piece</b>

## 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.

Streptavidin-HRP Conjugate 100-fold concentrated solution (protect from light) and TMB Substrate Solution can be stored at 2 – 8° C for up to 8 months (**DO NOT FREEZE** and **PROTECT FROM LIGHT**). All 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.

### PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

### ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled 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.

**Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.**

### SAMPLE PREPARATION

Urine samples may require dilutions. **Optimal dilutions should be determined by each laboratory for each application.**

**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.

**KIM-1 Standard** - Reconstitute the KIM-1 Standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 8000 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 **8000 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	8000 pg/mL
# 1	250 µL of stock	250 µL	4000 pg/mL
# 2	250 µL of 1	250 µL	2000 pg/mL
# 3	250 µL of 2	250 µL	1000 pg/mL
# 4	250 µL of 3	250 µL	500 pg/mL
# 5	250 µL of 4	250 µL	250 pg/mL
# 6	250 µL of 5	250 µL	125 pg/mL

**Positive Control** - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. **Note:** Positive Control could be reused within a few days if stored at -20° C or -70° C.

**Detection Antibody** - Reconstitute the Detection Antibody Concentrate with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer 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 10.395 mL of HRP Diluent Solution (DB08) into a 15 mL centrifuge

tube and transfer 105  $\mu\text{L}$  of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days (**protect from light**).

### ELISA PROTOCOL

**Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.**

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  $\mu\text{L}$  per well of **Dilution Buffer** to blank wells.
4. Add 100  $\mu\text{L}$  of **Standard dilutions, samples, or positive control** per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
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  $\mu\text{L}$ ) 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  $\mu\text{L}$  of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 90 minutes on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100  $\mu\text{L}$  of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 40 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100  $\mu\text{L}$  of **Substrate Solution** to each well. Incubate for refer to lot on the plate shaker at room temperature. **Protect from light.**
11. Add 100  $\mu\text{L}$  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 3 minutes, using a microplate reader set to 450 nm.

### CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

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

### TYPICAL DATA

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

STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.082)
125	0.031
250	0.069
500	0.119
1000	0.239
2000	0.470
4000	0.993
8000	1.928








### SPECIFICITY

PROTEIN	CROSS-REACTIVITY
Canine KIM-1	100
Mouse KIM-1	0
Human KIM-1	0

### SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS



Add 100 µL of standard dilutions, samples, or 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 90 minutes on the plate shaker at RT.

Aspirate and wash 4 times.

Add 100 µL Streptavidin-HRP conjugate working solution to each well. Incubate 40 min on the plate shaker at RT. <b>Protect from light.</b>

Aspirate and wash 4 times.

Add 100 µL Substrate Solution to each well. Incubate refer to lot on the plate shaker at RT. <b>Protect from light.</b>

Add 100 µL Stop Solution to each well. Read 450nm within 3 min.