Canine Kidney Injury Molecule-1 ELISA

For the quantitative determination of KIM-1 in canine serum, plasma, and urine.

For Research Use Only. Not For Use in Diagnostic Procedures.

Catalog Number: 41-KIMCA-E01
Size: 96 wells
Version: 4.1 – ALPCO 2.0

INTENDED USE

The Canine Kidney injury molecule (KIM-1) ELISA is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for the determination of KIM-1 in canine serum and plasma. For research use only. Not for use in diagnostic procedures.

PRINCIPLE OF THE ASSAY

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay, the KIM-1 present in samples reacts with the anti-KIM-1 antibodies which have been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound proteins by washing, the detection antibody, biotin conjugated anti-KIM-1, is added and complexes are formed. Following washing step, the horseradish peroxidase (HRP) conjugated streptavidin is added and complexes are formed. After another washing step, the complexes are assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of KIM-1 in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of KIM-1 in the test sample. The quantity of KIM-1 in the test sample can be interpolated from the standard curve constructed from the standards and corrected for sample dilution.

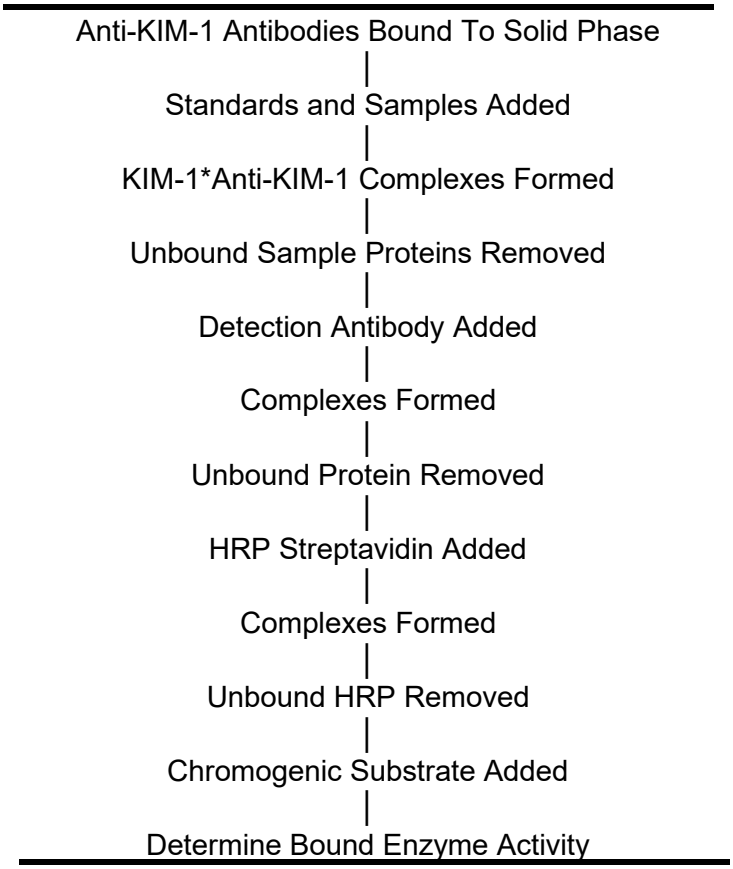

Figure 1.

LIMITATION OF THE PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is carried out with a full understanding of the information contained in the kit insert instructions and with adherence to good laboratory practice. Factors that might affect the performance of the assay include instrument function, cleanliness of glassware, quality of distilled/deionized water, accuracy of reagent and sample pipetting, washing technique, incubation time or temperature. Do not mix or substitute reagents with those from other lots or sources.

KIT COMPONENTS

The expiration date for the kit and its components is stated on the box label. All components should be stable up to the expiration date if stored and used per these instructions.

Component	Description	Preparation	Storage	Stability
ELISA Microplate, antibody coated	One plate of 12 removable 8 well strips, antibody coated	Ready to use as supplied.	2-8°C, In sealed foil bag with desiccant	With proper storage the plate strips are stable until the expiration date.
Detection Antibody 100X	One vial of 150µL of 100X affinity purified antibody conjugated with biotin in a stabilizing buffer	Dilute 1:100 immediately prior to use.	2-8°C in the dark	The 1X working antibody should be diluted immediately prior to use and is stable up to one hour if stored in the dark. The 100X antibody is stable until the expiry date.
HRP-Streptavidin 100X	One vial of 150µL of 100X horseradish peroxidase conjugated streptavidin in a stabilizing buffer	Dilute 1:100 immediately prior to use.	2-8°C in the dark	The 1X working SA-HRP should be diluted immediately prior to use and is stable up to one hour if stored in the dark. The 100X SA-HRP is stable until the expiry date.
Calibrator	One vial of calibrator. The calibrator is of human origin.	Refer to the Certificate of Analysis (CoA).	2-8°C for lyophilized calibrator. Aliquoted and frozen if reconstituted. Avoid multiple freeze-thaw cycles.	The working calibrators should be prepared immediately prior to use.
Diluent Solution	One 60 mL bottle of diluent solution	Ready to use as supplied.	2-8°C	The diluent solution is stable until the expiration date.
Wash Solution Concentrate	One 50 mL bottle of 20X wash solution	Dilute 1:20 to make 1X working wash buffer	2-8°C for both 1X working solution and 20X concentrate	The 1X working solution is stable for at least one week from the date of preparation. The 20X concentrate is stable until the expiry date.
Chromogen-Substrate Solution	One bottle of 12 mL 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.	Ready to use as supplied	2-8°C in the dark	Protect from light. The Substrate Solution is stable until the expiration date.
STOP Solution WARNING: Avoid Contact with Skin	One 12 mL bottle of 0.3 M sulfuric acid.	Ready to use as supplied	2-8°C	The Stop Solution is stable until the expiration date.

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipette (2 µL to 1000µL) for making and dispensing dilutions
- Test tubes
- Squirt bottle or Microtiter washer/aspirator
- Distilled or Deionized H2O
- Microtiter Plate reader
- Microplate shaker
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Centrifuge
- Anticoagulant (for plasma sample collection)

SAMPLE COLLECTION AND HANDLING

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing.

If blood samples are clotted, grossly hemolyzed, lipemic, or the integrity of the sample is of concern, make a note and interpret results with caution.

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

- Serum samples: Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. Remove serum and assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.
- Plasma samples: Blood should be collected into a container with an anticoagulant and then centrifuged. Assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles
- Urine samples: Collect mid-stream using sterile or clean urine collector. Centrifuge to remove cellular debris. Assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.
- Known interfering substances: Azide and thimerosal at concentrations higher than 0.1% inhibit the enzyme reaction.

DILUTION OF SAMPLES

The assay requires that each test sample be diluted before use. All samples should be assayed in duplicate each time the assay is performed. The recommended dilutions are only suggestions. Dilutions should be based on the expected concentration of the unknown sample such that the diluted sample falls within the dynamic range of the standard curve. If unsure of sample values, assay of a serial dilution with one or two representative samples prior to running the entire plate is highly recommended.

- Serum and Plasma samples: Recommended starting dilution is 1:5. To prepare a 1:5 dilution of a sample, transfer 60 µL of sample to 240 µL of diluent. This gives a 1:5 dilution. Mix thoroughly.

- Urine samples: Recommended starting dilution is 1:40. To prepare a 1:40 dilution of a sample, transfer 10 µL of sample to 390 µL of diluent. This gives a 1:40 dilution. Mix thoroughly.

REAGENT PREPARATION

Bring all reagents to room temperature (16°C to 25°C) before use.

Diluent Solution – Ready to use as supplied.

Wash Solution Concentrate - The Wash Solution supplied is a 20X Concentrate and must be diluted 1:20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH2O). Crystal formation in the concentrate may occur when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

Detection Antibody – Calculate the required amount of 1X working detecting antibody for each microtiter plate test strip by adding 10 µL Detection Antibody to 990 µL of Diluent for each test strip to be used for testing. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.

HRP-Streptavidin – Calculate the required amount of working HRP-streptavidin solution for each microtiter plate test strip by adding 10 µL HRP-Streptavidin to 990 µL of Diluent for each test strip to be used for testing. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.

Pre-coated ELISA Microplate - Ready to use as supplied. Unseal foil pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and reseal along with desiccant.

Canine kidney injury molecule (KIM-1) Calibrator – Prepare according to the lot specific Certificate of Analysis.

ASSAY PROCEDURE

Bring all reagents to room temperature (16°C to 25°C) before use.

1. **All samples and standards should be assayed in duplicate.**
2. The Standards and the sample(s) should be loaded into the ELISA wells as quickly as possible to avoid a shift in OD readings. Using a multichannel pipette would reduce this occurrence.

   Pipette 100 µL of

   Standard 0 (0.0 pg/mL) in duplicate
   Standard 1 (31.25 pg/mL) in duplicate
   Standard 2 (62.50 pg/mL) in duplicate
   Standard 3 (125 pg/mL) in duplicate
   Standard 4 (250 pg/mL) in duplicate
   Standard 5 (500 pg/mL) in duplicate
   Standard 6 (1000 pg/mL) in duplicate
3. Pipette 100 µL of prepared sample (in duplicate) into pre-designated wells.
4. Incubate the microtiter plate while shaking on a microplate shaker at 400 rpm at room temperature for 2 hours (± 2 minutes). Keep plate covered and level during incubation.

5. Following incubation, aspirate the contents of the wells.
6. Completely fill each well with 1X working Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with 1X working wash buffer, invert the plate then pour/shake out the contents into a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.
7. Pipette 100 µL of 1X working Detection Antibody to each well. Incubate the microtiter plate while shaking on a microplate shaker at 400 rpm at room temperature for 20 minutes (± 2 minutes). Keep plate covered and level during incubation.
8. Wash and blot the wells as described in Steps 5 and 6.
9. Pipette 100 µL of 1X working HRP-streptavidin to each well. Incubate the microtiter plate while shaking on a microplate shaker at 400 rpm at room temperature for 20 minutes (± 2 minutes). Keep plate covered and level during incubation.
10. Wash and blot the wells as described in Steps 5 and 6.
11. Pipette 100 µL of TMB Substrate Solution into each well.
12. Incubate in the dark at room temperature for precisely ten (10) minutes, while shaking on a microplate shaker at 400 rpm. Keep plate covered and level during incubation.
13. After ten minutes, add 100 µL of Stop Solution to each well.
14. Determine the absorbance (450 nm) of the contents of each well within 30 minutes using a plate reader calibrated to manufacturer's specifications.

CALCULATION OF RESULTS

1. Subtract the average background value (average absorbance reading of Standard Zero) from the test values for all wells.

2. Average the duplicate readings for each standard and use the results to construct a Standard Curve. Construct the standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) curve fit. A second order polynomial (quadratic) or other curve fits may also be used; however, they will be a less precise fit of the data.

   Interpolate test sample values from standard curve. Correct for the dilution factor to arrive at the KIM-1 concentration in original samples.