KAMIYA BIOMEDICAL COMPANY

Dog KIM-1 ELISA

For the quantitative determination of Kidney Injury Molecule-1 (KIM-1) in dog biological samples

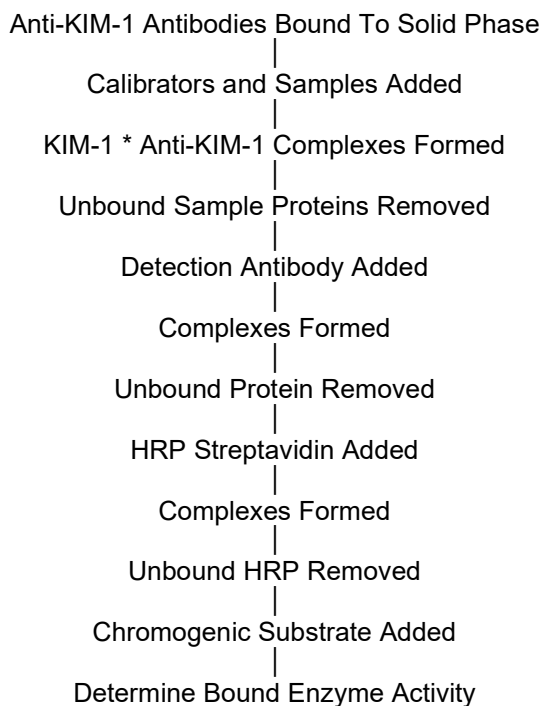## Cat. No. KT-687

For research use only.

PRODUCT INFORMATION

Dog KIM-1 ELISA
Cat. No. KT-687

INTENDED USE

The Dog KIM-1 ELISA is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for the quantitative determination of Kidney Injury Molecule-1 (KIM-1) in dog biological samples. For research use only.

PRINCIPLE

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the KIM-1 present in the sample 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 a wash 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 is proportional to 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 calibration curve constructed from the calibrators, and corrected for sample dilution.

Figure 1.

Anti-KIM-1 Antibodies Bound To Solid Phase

Calibrators and Samples Added

KIM-1 * Anti-KIM-1 Complexes Formed

Unbound Sample Proteins Removed

Detection Antibody Added

Complexes Formed

Unbound Protein Removed

HRP Streptavidin Added

Complexes Formed

Unbound HRP Removed

Chromogenic Substrate Added

Determine Bound Enzyme Activity

COMPONENTS

1. Diluent
   One bottle containing 60 mL of a 1X diluent running buffer.

2. Wash Solution Concentrate
   One bottle containing 50 mL of a 20X concentrated wash solution.

3. Detection Antibody 100X
   One vial containing 150 μL of affinity purified anti-Dog KIM-1 antibody conjugated with biotin in a stabilizing buffer.

Rev. 15696687

4. HRP-Streptavidin 100X
One vial containing 150 μL of HRP conjugated streptavidin in a stabilizing buffer.

5. TMB Substrate Solution
One bottle containing 12 mL of TMB and hydrogen peroxide in citric acid buffer at pH 3.3.

6. Stop Solution
One bottle containing 12 mL of 0.3 M sulfuric acid.
WARNING: Avoid contact with skin.

7. Microtiter Plate
Twelve removable eight-well micro strips in well holder frame.   Wells are coated with affinity-purified anti-dog KIM-1.

8. Dog KIM-1 Calibrator
One vial containing a lyophilized Dog KIM-1 Calibrator.

MATERIALS REQUIRED BUT NOT PROVIDED
- Precision pipettes (2 μL to 200 μL) for making and dispensing dilutions
- Test tubes
- Squirt bottle or Microplate washer/aspirator
- Distilled or de-ionized H_2O
- Microplate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Microplate shaker
- Centrifuge for sample collection
- Anticoagulant for plasma collection

PRECAUTIONS
1. Read the instructions carefully before beginning the assay.
2. This kit is for research use only.
3. Great care has been taken to ensure the quality and reliability of this product. However, it is possible that in certain cases, unusual results may be obtained due to high levels of interfering factors.
4. Azide and thimerosal at concentrations higher than 0.1% inhibit the enzyme reaction.
5. Other precautions:
    - Do not interchange kit components from different lots.
    - Do not use kit components beyond the expiration date.
    - Protect reagents from direct sunlight.
    - Do not pipette by mouth.
    - Do not eat, drink, smoke or apply cosmetics where reagents are used.
    - Avoid all contact with the reagents by using gloves.
    - Stop solution contains diluted sulfuric acid. Irritation to eyes and skin is possible. Flush with water after contact.

REAGENT PREPARATION
1. Diluent Solution
Ready to use as supplied.

2. Wash Solution Concentrate
The Wash Solution supplied is a 20X concentrate and must be diluted 1:20 with distilled or de-ionized water. Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

3. Detection Antibody 100X
Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10 μL detection antibody to 990 μL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming. Dilute immediately before use and protect from light.

4. HRP-Streptavidin 100X
Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10 μL HRP-Streptavidin to 990 μL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming. Dilute immediately before use and protect from light.

Rev. 15696687

5. TMB Substrate Solution
    Ready to use as supplied.

6. Stop Solution
    Ready to use as supplied.

7. Microtiter Plate
    Ready to use as supplied. Unseal Microtiter Pouch and remove plate from pouch. Remove all strips and wells that <u>will not</u> be used in the assay and place back in pouch and re-seal along with desiccant.

8. Dog KIM-1 Calibrator
    Add 1.0 mL of distilled or de-ionized water to the lyophilized Dog KIM-1 Calibrator and mix gently until dissolved. The calibrator is now at a concentration of 30.25 ng/mL **(the reconstituted calibrator should be aliquoted and frozen if future use is intended).** Dog KIM-1 Calibrators need to be prepared immediately before use (see chart below). Mix well between each step. Avoid foaming.

Calibrator	Concentration (pg/mL)	Calibrator Volume added to 1X Diluent ⟶	Volume of 1X Diluent
6	1,000	20 μL Dog KIM-1 Calibrator	585 μL
5	500	0.3 mL Calibrator 6	0.3 mL
4	250	0.3 mL Calibrator 5	0.3 mL
3	125	0.3 mL Calibrator 4	0.3 mL
2	62.50	0.3 mL Calibrator 3	0.3 mL
1	31.25	0.3 mL Calibrator 2	0.3 mL
0	0		0.6 mL

STORAGE AND STABILITY

1. Complete Kit
    The expiration date for the kit is stated on the outer label. The recommended storage temperature is 4°C. **Note: See long term storage recommendations below for the Dog KIM-1 Calibrator.**

2. Diluent
    The 1X Diluent Solution is stable until the expiration date and should be stored at 4°C.

3. Wash Solution
    The 20X Wash Solution Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4°C.

4. Detection Antibody 100X
    Undiluted Biotin conjugated anti-KIM-1 should be stored at 4°C and diluted immediately prior to use. The working conjugate solution is stable for up to 1 hour when stored in the dark. The undiluted conjugate is stable until the expiration date.

5. HRP-Streptavidin 100X
    Undiluted horseradish peroxidase conjugated streptavidin should be stored at 4°C and diluted immediately prior to use. The working conjugate solution is stable for up to 1 hour when stored in the dark. The undiluted conjugate is stable until the expiration date.

6. TMB Substrate Solution
    The TMB Substrate Solution should be stored at 4°C in the dark and is stable until the expiration date. Protect from light.

7. Stop Solution
    The Stop Solution should be stored at 4°C and is stable until the expiration date.

8. Microtiter Plate
    Anti-dog KIM-1 coated wells are stable until the expiration date and should be stored at 4°C in the sealed foil pouch with a desiccant pack.

Rev. 15696687

9. Dog KIM-1 Calibrator
    The lyophilized Dog KIM-1 calibrator should be stored at 4°C or frozen until reconstituted. The reconstituted calibrator should be aliquoted out and stored frozen (Avoid multiple freeze-thaw cycles). The working calibrator solutions should be prepared immediately prior to use.

INDICATIONS OF INSTABILITY
If the test is performing correctly, the results observed with the calibrator solutions should be within 20% of the expected values.

SPECIMEN COLLECTION AND HANDLING
Blood should be collected by venipuncture and the serum separated from the cells, after clot formation, by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results. For urine samples, collect mid-stream using sterile or clean urine collector. Centrifuge to remove cell debris. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freezing/thawing.

For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

ASSAY PROTOCOL

Dilution of Samples
The assay for quantification of KIM-1 in samples requires that each test sample be diluted before use. For a single step determination a dilution of 1:5 is appropriate for most serum/plasma samples. A dilution at 1:40 is appropriate for most urine samples. For absolute quantification of samples that yield results outside the range of the calibration curve, a lesser or greater dilution might be required. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

To prepare a 1:5 dilution of serum/plasma sample, transfer 60 μL of sample to 240 μL of 1X Diluent. This gives you a 1:5 dilution. To prepare a 1:40 dilution of urine sample, transfer 10 μL of sample to 390 μL of 1X Diluent. This gives you a 1:40 dilution.   Mix thoroughly.

Procedure
1. Bring all reagents to RT before use.

The Calibrators and the test 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.

2. Pipette 100 μL of
        Calibrator 0 (0.0 pg/mL) in duplicate
        Calibrator 1 (31.25 pg/mL) in duplicate
        Calibrator 2 (62.50 pg/mL) in duplicate
        Calibrator 3 (125 pg/mL) in duplicate
        Calibrator 4 (250 pg/mL) in duplicate
        Calibrator 5 (500 pg/mL) in duplicate
        Calibrator 6 (1,000 pg/mL) in duplicate

3. Pipette 100 μL of diluted sample (in duplicate) into pre-designated wells.

4. Incubate the Microtiter Plate while shaking at 400 rpm on an orbital shaker at 22°C (RT) for two hours (120 ± 2) minutes. Keep plate covered and level during incubation.

5. Following incubation, aspirate the contents of the wells.

6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually; completely fill wells with wash buffer, invert the plate and pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of 4 washes.

7. Pipette 100 μL of appropriately diluted detection antibody to each well. Incubate while shaking at 400 rpm on an orbital shaker at 22°C (RT) for twenty (20 ± 2) minutes. Keep plate covered in the dark and level during incubation.

8. Wash and blot the wells as described in Steps 5 and 6.

9. Pipette 100 µL of appropriately diluted HRP-streptavidin to each well. Incubate while shaking at 400 rpm on an orbital shaker at 22°C (RT) for twenty (20 ± 2) minutes. Keep plate covered in the dark 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 while shaking at 400 rpm on an orbital shaker at RT for precisely ten (10) minutes. Keep plate covered in the dark and level during incubation.

13. After ten (10) minutes, add 100 µL of Stop Solution to each well.

14. Determine the absorbance at 450 nm of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturer's specifications.

The absorbance of the final reaction mixture can be measured up to 2 hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

RESULTS

1. Subtract the average background value from the test values for each sample.

2. Using the results observed for the calibrators construct a calibration curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.

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

QUALITY CONTROL

In accord with good laboratory practice, the assays for specific KIM-1 require meticulous quality control. Each laboratory should use routine quality control procedures to establish inter- and intra-assay precision and performance characteristics.

LIMITATION OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.

2. Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or de-ionized water, and accuracy of reagent and sample pipettings, washing technique, incubation time or temperature.

FOR RESEARCH USE ONLY

KAMIYA BIOMEDICAL COMPANY

12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068   Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com

Rev. 15696687