



## **KIM-1 ELISA**

For the quantitative determination of Kidney Injury Molecule in human serum, plasma, and urine.

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

**Catalog Number:** 41-KIMHU-E01

**Size:** 96 wells

**Version:** 4.2 - ALPCO 3.0

## INTENDED USE

The Kidney Injury Molecule (KIM-1) test kit is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for the determination of KIM-1 in human serum, plasma, and urine. For Research Use Only. Not for use in diagnostic or therapeutic procedures.

## INTRODUCTION

KIM-1 is a transmembrane structural glycoprotein in renal epithelial cells. These cells shed KIM-1 antigen into the urine during regeneration, which makes urinary KIM-1 a useful biomarker for researching kidney injury.

## 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 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 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.

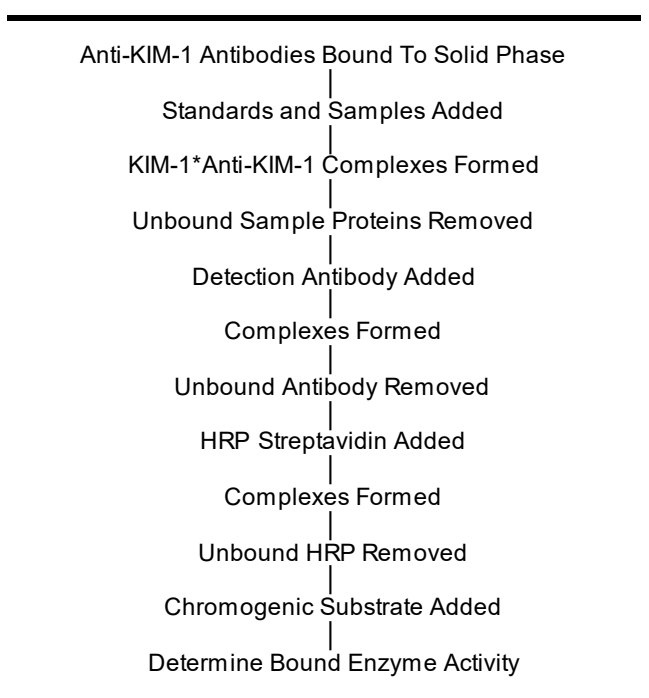


Figure 1.

## **LIMITATION OF THE PROCEDURE**

Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice. Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or deionized water, accuracy of reagent and sample pipetting, washing technique, and 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 this kit protocol.**

<b>Component</b>	<b>Description</b>	<b>Preparation</b>	<b>Storage</b>	<b>Stability</b>
<b>ELISA Microplate, antibody coated</b>	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.
<b>Detection Antibody 100X</b>	One vial of 150µL of 100X affinity purified antibody with biotin in a stabilizing buffer	Dilute 1:100 immediately prior to use.	2-8°C	Working solution must be diluted immediately prior to use and is stable up to 1 hour if stored in the dark, 100X conjugate is stable until the expiration date.
<b>HRP-Streptavidin, 100X</b>	One vial of 150µL of 100X horseradish peroxidase conjugated streptavidin in a stabilizing buffer	Dilute 1:100 immediately prior to use.	2-8°C	Working solution must be diluted immediately prior to use and is stable up to 1 hour if stored in the dark, 100X conjugate is stable until the expiration date.
<b>Calibrator</b>	One vial of calibrator	Refer to the Certificate of Analysis (CoA).	2-8°C for lyophilized calibrator. Aliquot and freeze if reconstituted. Avoid multiple freeze-thaw cycles.	The working standard solutions should be prepared immediately before use.
<b>Diluent Concentrate</b>	One 50 mL bottle of 5X diluent buffer	Dilute 1:5 to make 1X working solution.	2-8°C for both 1X working solution and 5X concentrate	The 1X working solution is stable for at least one week from the date of preparation. The 5X concentrate is stable until the expiration date.
<b>Wash Solution Concentrate</b>	One 50 mL bottle of 20x wash solution	Dilute 1:20 to make 1X working solution.	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 expiration date,

<b>Chromogen-Substrate Solution</b>	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.
<b>STOP Solution</b> <b><u>WARNING:</u></b> <b><u>Avoid Contact with Skin</u></b>	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 pipettes (2 µL to 100µL) for making and dispensing dilutions
- Test tubes
- Squirt bottle or Microtiter washer/aspirator
- Distilled or Deionized H<sub>2</sub>O
- Microtiter Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Centrifuge for sample collection
- Orbital plate shaker
- Anticoagulant for sample collection
- Timer

## 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. Serum must 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 cell debris. Assay immediately or aliquot and store samples at -80°C (preferably) or at -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 level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

- Plasma, Serum and Urine samples – Recommended starting dilution is 1:2. To prepare a 1:2 dilution of sample, transfer 150 µL of sample to 150 µL of 1X working diluent. Mix thoroughly.

## REAGENT PREPARATION

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

- **DILUENT CONCENTRATE**  
The Diluent Solution supplied is a 5X concentrate and must be diluted 1:5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH<sub>2</sub>O).
- **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 dH<sub>2</sub>O). 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.
- **DETECTION ANTIBODY 100X**  
Calculate the required amount of 1X working conjugate solution for each microtiter plate test strip by adding 10 µL detection antibody to 990 µL of 1X working 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 100X**  
Calculate the required amount of 1X working conjugate solution for each microtiter plate test strip by adding 10 µL HRP-streptavidin to 990 µL of 1X working 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. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.
- **HUMAN KIM-1 CALIBRATOR**  
Please refer to the Certificate of Analysis for preparation of the human KIM-1 calibrator.

## ASSAY PROCEDURE

1. **Bring all reagents to room temperature before use. All samples and standards should be assayed in duplicate.**
2. The standards and test samples should be loaded into the ELISA wells as quickly as possible to avoid a shift in OD readings. Using a multichannel pipette reduces this occurrence.

Pipette 100  $\mu$ L of

Standard 0	(0.0 pg/mL) in duplicate
Standard 1	(31.25 pg/mL) in duplicate
Standard 2	(62.5 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	(1,000 pg/mL) in duplicate

3. Pipette 100  $\mu$ L of sample (in duplicate) into pre-designated wells.
4. Incubate the microtiter plate while shaking on an orbital shaker at 400rpm at room temperature for one hundred and twenty ( $120 \pm 2$ ) 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 in a waste container. Follow this by sharply tapping the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.
7. Pipette 100  $\mu$ L of 1X working Detection Antibody to each well. Incubate the microtiter plate while shaking on an orbital shaker at 400rpm at room temperature for thirty ( $30 \pm 2$ ) 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  $\mu$ L of 1X working HRP-Streptavidin to each well. Incubate the microtiter plate while shaking on an orbital shaker at 400rpm at room temperature for thirty ( $30 \pm 2$ ) 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  $\mu$ L of TMB Substrate Solution into each well.

12. Incubate in the dark while shaking on an orbital shaker at 400rpm at room temperature for precisely ten (10) minutes. Keep plate covered in the dark and level during incubation.
13. After ten minutes, add 100  $\mu$ L of Stop Solution to each well.
14. Determine the absorbance (450 nm) of the contents of each well within 30 minutes. Calibrate the plate reader to the 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 curve. A second order polynomial (quadratic) or other curve fits may also be used, however, they will be a less precise fit of the data.
3. Interpolate test sample values from standard curve. Correct for sample dilution factor to arrive at the KIM-1 concentration in original samples.