Product Manual

KIM-1 (human) ELISA Kit

Catalog #ADI-900-226

96-Well Enzyme Immunoassay Kit

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Please read entire booklet before proceeding with the assay.

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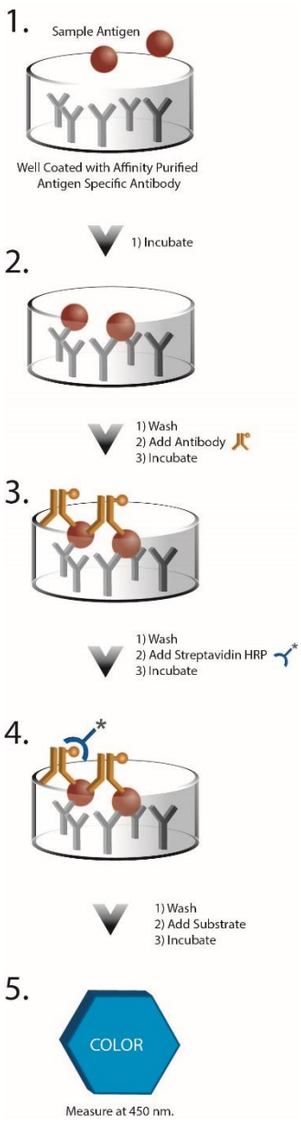
BACKGROUND

The human KIM-1 (kidney injury molecule-1) ELISA kit is a complete kit for the quantitative determination of KIM-1 in urine. Please read the complete kit insert before performing this assay.

Chronic kidney disease (CKD) is a major public health problem, as one in nine American adults have CKD. There have been numerous studies focused on this disease over the years but there remains a need for improved therapeutics and identifying and/or predicting patient outcomes[3]. One protein that has been identified as playing an important role in kidney disease is Kidney Injury Molecule-1 (KIM-1). KIM-1 is a 30KDa, type 1 membrane protein with an ectodomain that contains immunoglobulin (Ig) and highly *O-glycosylated* mucin subdomains as well as multiple *N-glycosylation* sites[4]. It is the most highly upregulated protein in the proximal tubule of the injured kidney[5]. It exists in very low levels in normal kidneys but when upregulated during injury, it is detectable in urine in a wide variety of human diseases. KIM-1 may be a biomarker for renal injury, which would suggest it has great importance in various kidney diseases and disorders, such as chronic kidney disease (as mentioned above), as well as acute tubular necrosis and acute kidney failure.

This product is licensed from the General Hospital Corporation and is protected by US patents 6,664,385 B1, 7,041,290 B2 and 7,696,321.

PRINCIPLE



1. The kit uses a monoclonal antibody to KIM-1 immobilized on a microtiter plate to bind the KIM-1 in the standards or sample.

2. After a short incubation the excess sample or standard is washed out and a biotinylated monoclonal antibody to KIM-1 is added. This antibody binds to the KIM-1 captured on the plate.

3. After a short incubation the excess antibody is washed out and Streptavidin conjugated to Horseradish peroxidase is added, which binds to the biotin on the monoclonal antibody. The plate is then incubated.

4. Once the incubation is complete, excess conjugate is washed out and TMB substrate solution is added. An HRP-catalyzed reaction generates a blue color in the solution.

5. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450 nm. The amount of signal is directly proportional to the level of KIM-1 in the sample.

MATERIALS SUPPLIED

Do not mix components from different kit lots or use beyond the expiration date of the kit.

1. **KIM-1 Microtiter plate**

   One plate of 96 wells, Product No. 80-2557

   A clear plate of break-apart strips coated with a monoclonal antibody specific to KIM-1

2. **KIM-1 Standard, 25 ng/mL**

   0.5ml, Product No. 80-2558

   One vial containing 25 ng/mL of recombinant KIM-1

3. **KIM-1 Detector Antibody**

   10 mL, Product No. 80-2559

   A yellow solution of biotinylated monoclonal antibody to KIM-1

4. **KIM-1 Conjugate**

   10 mL, Product No. 80-2560

   A blue solution of Streptavidin conjugated to Horseradish peroxidase

5. **Assay Buffer 13**

   60 mL, Product No. 80-1500

   Tris buffered saline containing BSA and detergents

Protect substrate from prolonged exposure to light.

6. **TMB Substrate**

   10 mL, Product No. 80-0350

   A solution of 3,3’5,5’ tetramethylbenzidine (TMB) and hydrogen peroxide

7. **Stop Solution 2**

   10 mL, Product No. 80-0377

   A 1N solution of hydrochloric acid in water

Stop solution is caustic. Keep tightly capped.

8. **Wash Buffer Concentrate**

   100 mL, Product No. 80-1287

   20x Tris buffered saline containing detergent

9. **KIM-1 Assay Layout Sheet**

   1 each, Product No. 30-0315

10. **Plate Sealer**

    3 each, Product No. 30-0012

STORAGE

All kit components are stable at 4°C until the kit's expiration date. Shipping conditions may not reflect storage conditions.

OTHER MATERIALS NEEDED

1. Deionized or distilled water
2. Precision pipets for volumes between 5 µL and 1,000 µL
3. Repeater pipet for dispensing 100 µL
4. Disposable beakers for diluting buffer concentrates
5. Graduated cylinders
6. A microplate shaker
7. Adsorbent paper for blotting
8. Microplate reader capable of reading a 450 nm
9. Software (such as AssayBlaster™ catalog number ADI-28-0002) for extrapolating sample values from optical density readings utilizing a four parameter logistic curve fit.

If buffers other than those provided are used, the end-user must determine the appropriate dilution and assay variation.

SAMPLE HANDLING

The KIM-1 ELISA is compatible with KIM-1 samples in urine. Samples diluted sufficiently into Assay Buffer 13 can be read directly from a standard curve. Urine samples must be diluted at least 1:4 with Assay Buffer 13 in order to remove matrix interference effects. The minimal recommended dilution may not be optimal for all urine samples for the levels of endogenous KIM-1 could vary between sample groups. Therefore it is up to each end user to optimize the dilution for their unique set of samples.

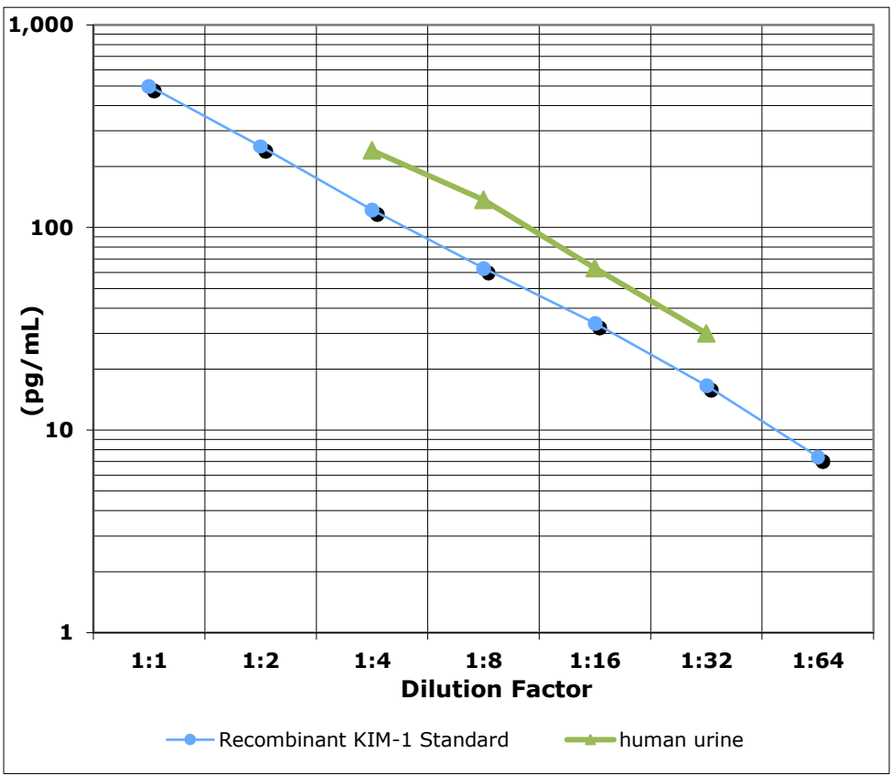
Linearity

The minimum required dilution for urine is 1:4. This was determined by serially diluting kidney disease-state urine samples into the provided assay buffer and identifying the dilution at which linearity was observed.

Dilutional Linearity	
Dilution	**Urine**
Neat	---
1:4	100%
1:8	108%
1:16	95%
1:32	88%

Parallelism

To assess parallelism, human urine was serially diluted into assay buffer and run in the assay. The KIM-1 concentration in each sample was assigned using the standard curve. Assigned concentrations were plotted as a function of sample dilution. Parallelism of the curves demonstrates that the antigen binding characteristics are similar enough to allow the accurate determination of native analyte levels in diluted samples of human origin.



Spike and Recovery

After diluting each individual sample to read within the dynamic range of the assay, recombinant KIM-1 was spiked at a high concentration into neat urine, diluted 1:4 and then serially (1:2) into assay buffer. Endogenous KIM-1 was subtracted from the spiked values and the recovery in each of the spiked specimens was compared to the recovery of identical spikes in the assay buffer. The percent recovery of each concentration is indicated below for human urine.

Sample Matrix	Dilution	Spike Concentration (pg/mL)	% Recovery of Spike
Human urine	1:4	400	89
		200	109
		100	118
		50	127

REAGENT PREPARATION

Sample handling procedures should be completed prior to reagent preparation.

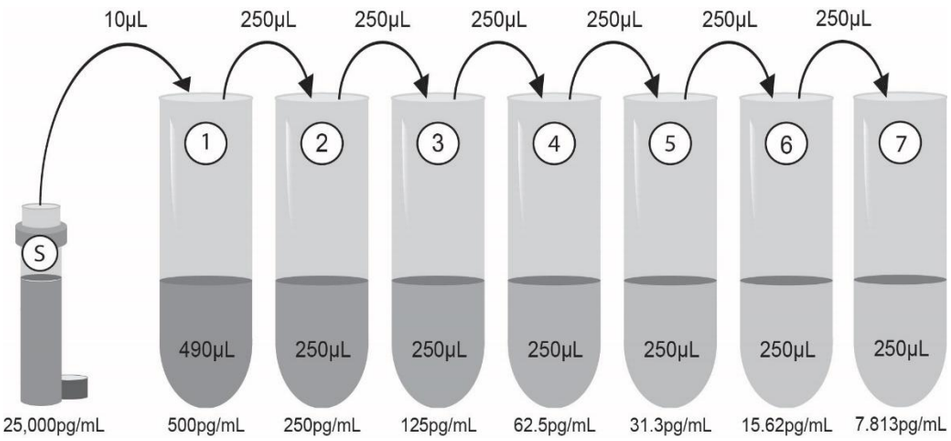
1. **Wash Buffer**

   Prepare Wash buffer by diluting 50 mL of the supplied Wash Buffer concentrate with 950 mL of deionized water. Store the diluted wash buffer at room temperature. Diluted wash buffer should be used within 3 months.

Polypropylene tubes may be used for standard preparation. Avoid polystyrene.

2. **KIM-1 Standard**

   Allow the KIM-1 standard to warm to room temperature. Label seven 12x75 mm polypropylene tubes #1 through #7. Pipet 490 µL of Assay Buffer 13 into tube #1. Pipet 250 µL of Assay Buffer 13 into tube #2 through tube #7. Add 10 µL of 25,000 pg/mL KIM-1 standard stock to tube #1. Add 250 µL of tube #1 into tube #2 and vortex. Add 250 µL of tube #2 to tube #3 and vortex thoroughly. Continue this for tubes #4 through #7.



Diluted standards should be used within 60 minutes of preparation. Discard any unused standard dilutions.

All other kit components should be brought to room temperature prior to use in the assay.

ASSAY PROCEDURE

Bring all reagents to room temperature for at least 30 minutes prior to opening.

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove the wells not needed for the assay and return them, with the desiccant, to the mylar bag and seal. Store unused wells at 4°C.

1. Pipet 100 µL of Assay Buffer 13 into the S0 (0 pg/mL standard) and NSB wells. Leave the Blank wells empty.
2. Pipet 100 µL of standards #1 through #7 to the bottom of the appropriate wells.
3. Pipet 100 µL of the samples into the appropriate wells.
4. Seal the plate. Incubate for 30 minutes with mixing* on a plate shaker at room temperature.
5. Empty the contents of the wells and wash by adding ~300 µL of 1X Wash Buffer to each well. Empty or aspirate the wells and repeat the wash 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

All standards and samples should be run in duplicate.

Pipet the reagents to the sides of the wells to avoid possible contamination.

6. Pipet 100 µL of yellow Antibody into each well, except the NSB and blank wells. Add 100 µL Assay Buffer 13 into NSB wells and leave Blank wells empty.
7. Seal the plate and incubate for 30 minutes with mixing on a plate shaker at room temperature.
8. Empty the contents of the wells and wash by adding ~300 µL of 1X Wash Buffer to each well. Empty or aspirate the wells and repeat the wash 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
9. Add 100 µL of blue Conjugate to each well, except the Blank.
10. Seal the plate and incubate for 30 minutes with mixing on a plate shaker at room temperature
11. Empty the contents of the wells and wash by adding ~300 µL of 1X Wash Buffer to each well. Empty or aspirate the wells and repeat the wash 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
12. Pipet 100 µL of TMB solution into each well.
13. Seal the plate. Incubate for 20 minutes with shaking on a plate shaker at room temperature.

14. Pipet 100 µL of Stop Solution into each well.
15. After blanking the plate reader against the substrate, read optical density at 450 nm. If the plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

*** Note:** The optimal speed for each shaker will vary and may range from 120-700 rpm. The speed must be set to ensure adequate mixing of the wells, but not so vigorously that the contents of the wells splash out and contaminate other wells.

CALCULATION OF RESULTS

Be sure to multiply sample concentrations by the dilution factor used during sample preparation.

Several options are available for the calculation of the concentration of KIM-1 in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic (4PL) curve fitting program. Assay Blaster! Data analysis software (Prod. no. ADI-28-0002) is an easy-to-use and cost effective program that provides the options of point-to-point, 4PL and 5PL curve fitting options. The concentration of KIM-1 can be calculated as follows:

1. Calculate the average net OD for each standard and sample by subtracting the average NSB OD from the average OD for each standard and sample.

   Average Net OD = Average OD - Average NSB OD

2. Using data analysis software, plot the Average Net OD for each standard versus KIM-1 concentration in each standard.

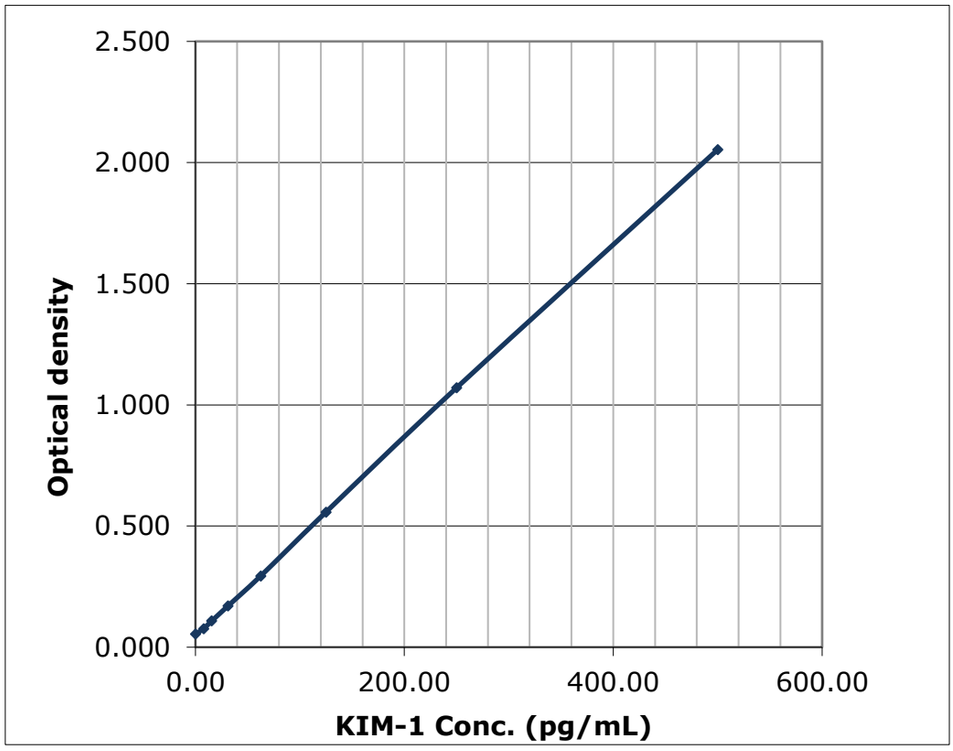
TYPICAL RESULTS

The results shown below are for illustration only and should not be used to calculate results.

Sample	Mean OD	Net OD	KIM-1 (pg/mL)
Blank	(0.001)		
NSB	0.004		
S0	0.058	0.054	**0**
S1	2.058	2.054	**500**
S2	1.075	1.071	**250**
S3	0.562	0.558	**125**
S4	0.298	0.294	**62.5**
S5	0.175	0.171	**31.3**
S6	0.113	0.109	**15.625**
S7	0.081	0.077	**7.813**

TYPICAL STANDARD CURVE

Typical standard curves are shown below. These curves must not be used to calculate KIM-1 concentrations; each user must run a standard curve for each assay.



PERFORMANCE CHARACTERISTICS

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols[6].

Specificity

The cross reactivities of related compounds were determined by diluting the cross reactant in the kit assay buffer at a concentration of ten times the high standard and then measuring in the assay.

Analyte	Cross Reactivity
TIM-3	≤0.02%
TIM-4	≤0.02%

Sensitivity

The sensitivity or limit of detection of the assay is 1.279 pg/mL, determined by interpolation at 2 standard deviations away from the mean signal of 10 replicates of 0 pg/mL. Data was used from 4 standard curves.

Interference

Protease inhibitors commonly used in clinical specimens were analyzed for interference in the assay and the tolerance was determined.

Protease inhibitor	Assay Tolerance
PIC	≤2.5%
PMSF	≤1mM
Aprotinin	≤100 ug/mL

Intra-assay precision was determined by assaying 20 replicates of three buffer controls containing KIM-1 in a single assay.

Intra-assay precision	
pg/mL	**%CV**
385.5	1.8
93.1	2.3
39.3	2.6

Inter-assay precision was determined by measuring buffer controls of varying KIM-1 concentrations in multiple assays over several days.

Inter-assay precision	
pg/mL	**%CV**
397.5	6.2
99.8	6.4
39.7	1.9

REFERENCES

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4. Ichimura T., et al., “Kidney injury molecule-1: a tissue and urinary biomarker for nephrotoxicant-induced renal injury”, Am. Journal of Renal Physiology. 2004. 286: F522-F563.
5. Ichimura T., Brooks C., Bonventre J., “Kim-1/Tim-1 and immune cells: shifting sands”, Kidney International. 2012 May; 81(9): 809-811.
6. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

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