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ab235081 – Human KIM-1 ELISA Kit (TIM-1)

For the detection and quantification of KIM-1 in human urine.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Abcam's human KIM-1 (kidney injury molecule-1) ELISA kit is a complete kit for the colorimetric detection and quantification of KIM-1 in urine.

This kit uses a monoclonal antibody to KIM-1 immobilized on a microtiter plate to bind KIM-1 in the sample/standard. Using the colorimetric assay the level of KIM-1 can be quantified using the absorbance values and the KIM-1 standard curve.

Prepare all reagents, samples, and standards as instructed.

Incubate the standards/samples in the KIN-1 immobilized microtiter plate.

Remove and gently wash out excess standard/sample and add the biotinylated antibody to KIM-1.

Gently wash out excess antibody and add streptavidin (HRP) and incubate.

Gently wash out excess conjugate and add TMB substrate. This will generate a blue color in the solution.

Add stop solution, resulting in a yellow color and read plate at 450 nm.

2. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

3. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

4. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

5. Materials Supplied

Item	Amount	Storage Condition
KIM-1 Microtiter Plate	1 unit	4°C
KIM-1 Standard (25 ng/mL)	0.5 mL	4°C
KIM-1 Detector Antibody	10 mL	4°C
KIM-1 Conjugate	25 mL	4°C
Assay Buffer	60 mL	4°C
TMB Substrate	10 mL	4°C
Stop Solution	10 mL	4°C
Wash Buffer Concentrate	100 mL	4°C
Plate Sealer	1 x 3 units	4°C

6. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Precision pipettes for volumes between 5 µl and 1,000 µl.
- Repeater pipette for dispensing 100 µl.
- Graduated cylinders.
- A microplate shaker.
- Absorbent paper for blotting.
- Microplate reader capable of reading at 450 nm.

7. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.

8. Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) for 4 hours prior to use.

Prepare only as much reagent as is needed on the day of the experiment.

8.1 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.

8.2 KIM-1 standard

See section 10.

8.3 All other Reagents in this kit are ready to use as supplied.

9. Sample Handling

- The KIM-1 ELISA is compatible with KIM-1 samples in urine. Samples diluted sufficiently into Assay Buffer 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.

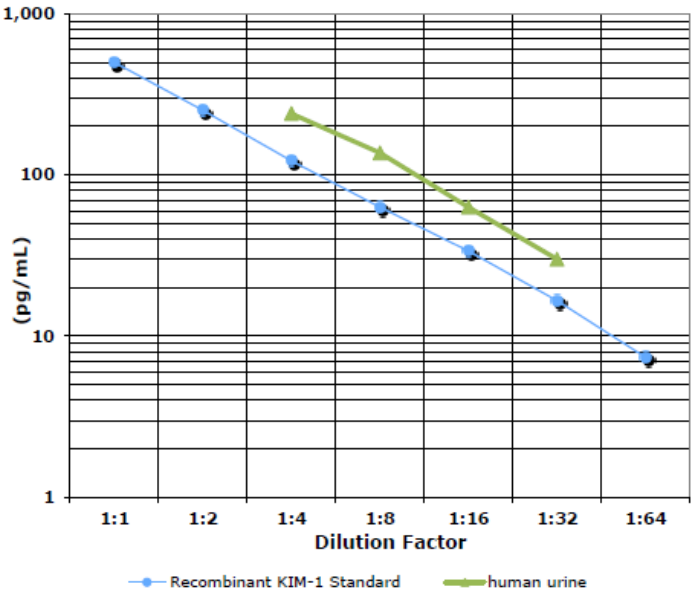


Figure 1: KIN-1 standard curve.

Spike and recovery

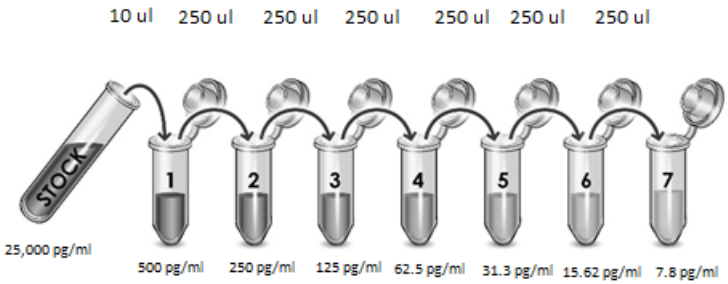
After diluting each individual sample to read within the dynamic range of the assay, recombinant KIM-1 was spiked at a high concentration into neat urine, diluted 1:4 and then serially (1:2) into assay buffer. Endogenous KIM-1 was subtracted from the spiked values and the recovery in each of the spiked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

10. Standard preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1.1 Allow the the KIM-1 standard to warm to room temperature. Label 7 tubes, standards 1-7.
10.1.2 Pipet 490 µl of Assay Buffer into tube 1.
10.1.3 Pipet 250 µl of Assay buffer into tubes 2-7.
10.1.4 Add 10 µl of 25 ng/mL KIM-1 standard stock into tube number 1.
10.1.5 Add 250 µl of tube 1 into tube 2 and mix well.
10.1.6 Add 250 µl of tube 2 into tube 3 and mix well.
10.1.7 Continue this for tubes 4-7.



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

11. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all controls and samples in duplicate.
- 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.

ΔNote: 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.

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.

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.

12. Calculations

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.

The concentration of KIM-1 can be calculated as follows:

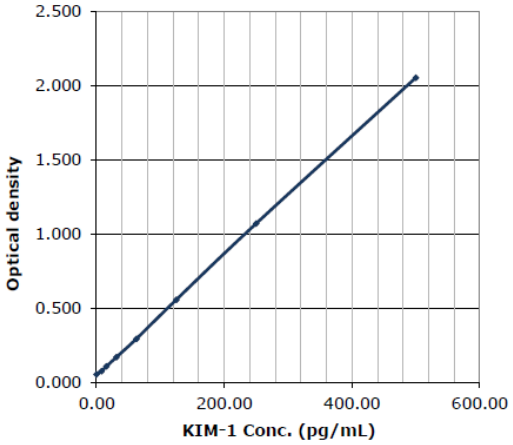
- Calculate the average net OD for each standard and sample by subtracting the average NSB OD from the average OD for each standard and sample.

  Average Net OD = Average OD - Average NSB OD

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

13. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.



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

Figure 2: Example of human KIM-1 standard curve and raw data values shown in the table. The KIM-1 standard curve was prepared as described in section 10.

14. Typical sample values

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.

Intra-assay precision-

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-

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

15. Assay Specificity

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%

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	≤ 1 mM
Aprotinin	≤ 100 ug/ml

16. Troubleshooting

Problem	Reason	Solution
Difficulty pipetting lysate; viscous lysate.	Genomic DNA solubilized	Prepare 1X Cell Extraction Buffer PTR (without enhancer). Add enhancer to lysate after extraction.
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB Development Solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

17. Notes

Technical Support

For all technical and commercial enquires please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)