PromedeusLab

Human Kidney Injury Molecule 1 ELISA

Cat. No.: PL1001

Enzyme Immunoassay for the quantitative determination Kidney Injury Molecule 1 (KIM-1) in human urine and serum.

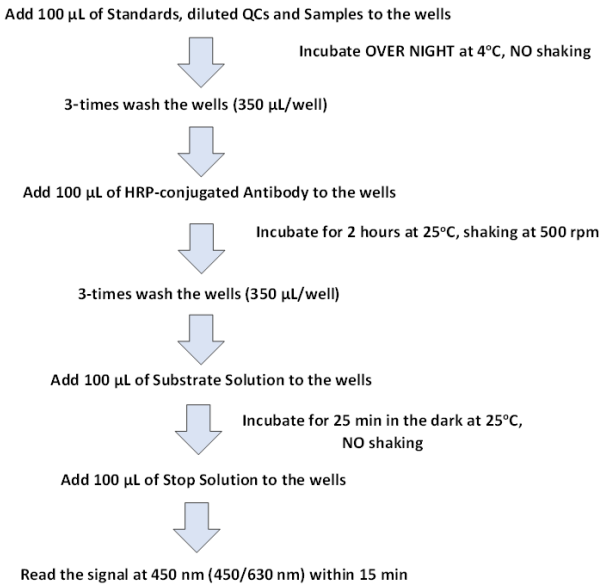
The urinary kidney injury molecule 1 (KIM-1) participates in renal tissue damage and repair and is proposed as a biomarker of early and subclinical AKI. KIM-1 is also elevated in the urine of a significant fraction of patients apparently recovered from an AKI. Besides its potential utility in the early and subclinical diagnosis of renal damage, this study suggests a new application of urinary KIM-1 in the non-invasive follow-up of renal repair after AKI.[1]

PRINCIPLE OF KIDNEY INJURY MOLECULE 1 ELISA

The microtiter plate is coated with the antibody specifically binding the Kidney Injury Molecule 1. The human serum or plasma is incubated in the plate with the capture antibody.

The specimen is washed out and the specifically bound protein is incubated with HRP-labelled detection antibody. Unbound reagent is then washed out. Horseradish peroxidase (HRP) bound in the complex reacts with the chromogenic substrate (TMB) creating the blue colour. The reaction is stopped by addition of STOP solution (H_2SO_4).

The absorbance values are measured at 450 nm (optionally 450/630 nm) and are proportional to the concentration of KIM-1 in the specimen. The concentration of KIM-1 in unknown samples is determined from the calibration curve which is created by plotting the absorbance values against the standard concentration values.



Kit Contents

Item	*Qty.*
Antibody Coated Microtiter Plate	96 wells
Antibody-HRP Conjugate	13 mL
Master Standard (lyophilized)	1 vial
Quality Control A (human serum, lyophilized)	1 vial
Quality Control B (human serum, lyophilized)	1 vial
Dilution Buffer	13 mL
Wash Buffer 15x conc.	50 mL
Substrate Solution	13 mL
STOP Solution	13 mL

Version: ENG.01

MATERIAL REQUIRED BUT NOT SUPPLIED

1. Glassware and test tubes
2. Microtiter plate washer
3. Precision pipettes (various volumes) with tips
4. Orbital shaker
5. Microtiter plate reader capable of measuring absorbance at 450 nm or 450/630 nm with software for data generation

WARNINGS AND PRECAUTIONS

1. For research use only
2. For professional laboratory use
3. The reagents with different lot numbers should not be mixed
4. To prevent cross sample contamination, use disposable labware and pipette tips
5. To protect laboratory stuff, wear protective gloves and protective clothing
6. The substrate solution should remain colourless, keep it protected from light
7. The test should be performed at standard laboratory conditions (temperature 25°C ±2°C).

STORAGE CONDITIONS

1. The kit must be stored at 2 – 8°C.
2. The opened components can be stored for one week at 2 – 8°C.

PREPARATION OF REAGENTS

- Use new pipette tip for pipetting different reagents and samples to prevent cross-contamination.
- All reagents and samples should be allowed to reach the temperature 25°C ±2°C.

Preparation of Standards

Reconstitute lyophilized Human KIM-1 Standard in Dilution Buffer, for the volume information see the Certificate of Analysis. Let it rehydrate for 15 min. The concentration of human KIM-1 in Master Standard is 1000 pg/mL,

Prepare set of Standard solution as follows:

Use the Master Standard to produce a dilution series (as below). Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as Blank.

	Volume of Standard	Dilution Buffer	Concentration
Std1	Standard 1000 pg/mL (lyophilised)	See CofA	1000 pg/mL
Std2	300 µL of Std1	300 µL	500 pg/mL
Std3	300 µL of Std2	300 µL	250 pg/mL
Std4	300 µL of Std3	300 µL	125 pg/mL
Std5	300 µL of Std4	300 µL	62.5 pg/mL
Std6	300 µL of Std5	300 µL	31.25 pg/mL
Std7	300 µL of Std6	300 µL	15.625 pg/mL
Blank	-	250 µL	0 ng/mL

Preparation of Quality Control A and B

Reconstitute the lyophilized human serum Quality Controls in deionized/distilled water, for the volume information see the Certificate of Analysis. Let the QCs rehydrate for 15 min and dilute them 1:2 in Dilution Buffer, prior to use, see Preparation of samples.

Preparation of Wash Buffer 1x

Prepare a working solution of Wash Buffer by adding 50 mL of Wash Buffer 15x conc. to 700 mL of deionized/ distilled water (dH_2O). Mix well. Store at 4°C for two weeks or at -20°C for long term storage.

Preparation of samples

Human serum or plasma may be used with this assay. For long-term storage the samples should be frozen at minimum -70°C. Lipemic or haemolytic samples may cause false results.

Recommended dilution of samples is 1:2, i.e., 70 µL of sample + 70 µL of Dilution Buffer for singlets and 150 µL of sample + 150 µL of Dilution Buffer for duplicates.

Do not store the diluted samples.

ASSAY PROCEDURE

1. Prepare the reagents as described in the previous chapter.
2. Pipette 100 µL of set of Standards, Quality Controls, diluted Samples and Dilution Buffer = Blank into each well. Incubate **OVER NIGHT** at 4°C ±2°C, NO shaking.
3. Wash the wells 3-times with 1x Wash Buffer (350 µL/well). When finished, tap the plate against the paper towel to remove the liquid completely.
4. Pipette 100 µL of HRP-labelled Antibody Conjugate into each well. Incubate for **2 hours** at 25°C ±2°C, shaking at **500 rpm**.
5. Wash the wells as described in point 3.
6. Pipette 100 µL Substrate solution, incubate for **25 min,** at 25°C ±2°C**.** Avoid exposure to the light during this step.
7. Pipette 100 µL of STOP solution.
8. Read the signal at 450 or 450/630 nm within 15 min.

Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1		Bckg	Sa 4	Sa 8	Sa 12	Sa 16	Sa 20	Sa 24	Sa 28	Sa 32	Sa 36
B	Std 2											
C	Std 3		Sa 1	Sa 5	Sa 9	Sa 13	Sa 17	Sa 21	Sa 25	Sa 29	Sa 33	Sa 37
D	Std 4											
E	Std 5		Sa 2	Sa 6		Sa 10	Sa 14	Sa 18	Sa 22	Sa 26	Sa 30	Sa 34	Sa 38
F	Std 6											
G	QCA		Sa3	Sa 7		Sa 11	Sa 15	Sa 19	Sa 23	Sa 27	Sa 31	Sa 35	Sa 39
H	QCB											

PERFORMANCE CHARACTERISTICS

Samples used in the tests were diluted 1:2 as recommended and assayed. The results are multiplied by the dilution factor.

1. Sensitivity

The limit of detection, defined as a concentration of human KIM-1 giving absorbance higher than absorbance of blank + 3 standard deviations, is better than 45 pg/mL of sample.

2. Precision

Intra-assay

Sample	Mean (pg/mL)	SD	CV (%)
1	519	8.8	1.7
2	287	15.6	5.4

Inter-assay (Run – to – run)

Sample	Mean (pg/mL)	SD	CV (%)
1	284	16	5.7
2	114	15	13.3

3. Accuracy

Dilution linearity

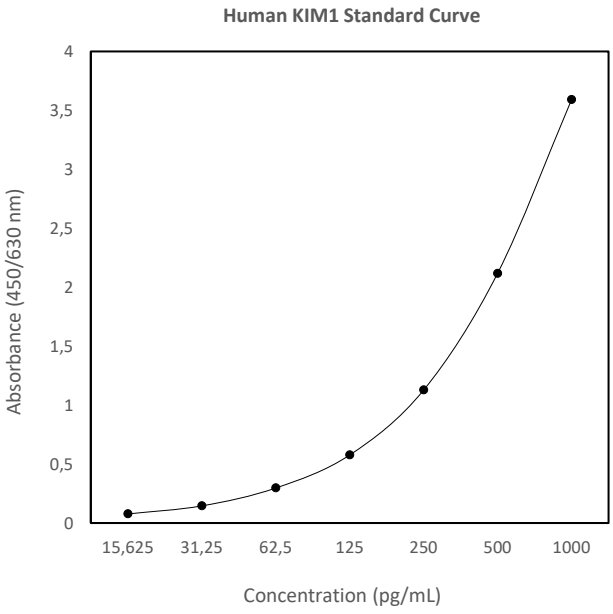
Sample	Dilution	Measured concentration (pg/mL)	Expected concentration (pg/mL)	Yield (%)
1		509	-	-
	2x	230	254	90
	4x	106	127	83
	8x	52	64	82
2		448	-	-
	2x	196	224	87
	4x	99	112	89
	8x	54	56	96

Spiking Recovery

Sample	Spike (ng/mL)	Measured concentration (pg/mL)	Expected concentration (pg/mL)	Yield (%)
1	-	236	-	-
	360	663	596	111
	180	446	416	107
	90	341	326	105

Typical standard curve

The standard curve needs to be measured in every test. Most of the microplate reader can automatically calculate the analyte concentration using 4-parameter algorithm or alternative functions to fit the standard points properly. The concentrations need to be multiplied by the dilution factor, either automatically by reader or manually.



RESOURCES

[1] Cuesta C, Fuentes-Calvo I, Sancho-Martinez SM, Valentijn FA, Düwel A, Hidalgo-Thomas OA, Agüeros-Blanco C, Benito-Hernández A, Ramos-Barron MA, Gómez-Alamillo C, Arias M, Nguyen TQ, Goldschmeding R, Martínez-Salgado C, López-Hernández FJ. Urinary KIM-1 Correlates with the Subclinical Sequelae of Tubular Damage Persisting after the Apparent Functional Recovery from Intrinsic Acute Kidney Injury. Biomedicines. 2022 May 10;10(5):1106. doi: 10.3390/biomedicines10051106. PMID: 35625842; PMCID: PMC9139078.