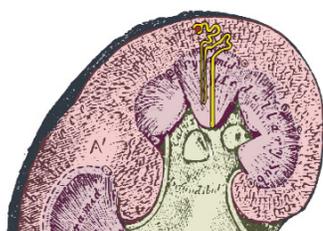


# Kidney Injury Molecule-1 (KIM-1) - early transmembrane biomarker of acute kidney injury



It is well known that kidney injuries can tend to accompany many severe illnesses such as cardiac diseases, trauma and sepsis, and is an increasingly common and devastating complication in hospitalized patients.

Despite improvements in health outcomes in many areas during recent years, mortality and morbidity rates associated with acute kidney injury (AKI) remain very high. Currently, the diagnosis of AKI is predominantly based on creatinine measurement in serum/plasma. However, creatinine levels start to increase late after the onset of disease, which leads to unavoidable delays in AKI diagnosis and makes treatment largely ineffective (1). Furthermore, creatinine concentration is considerably influenced by muscle mass and other factors that render its clinical use unreliable. Several new early biomarkers of AKI have been suggested recently and kidney injury molecule-1 (KIM-1) is among the most promising ones.

KIM-1 is expressed on the surface of tubular epithelial cells in the kidney. KIM-1 levels are undetectable in normal kidneys, whereas elevated KIM-1 expression was detected in the ischemic kidney in the animal model of disease (2), as well as in humans (3-5).

KIM-1 concentration in the urine of healthy humans is less than 1 ng/ml. Meanwhile, following the ischemic kidney injury it could be elevated up to

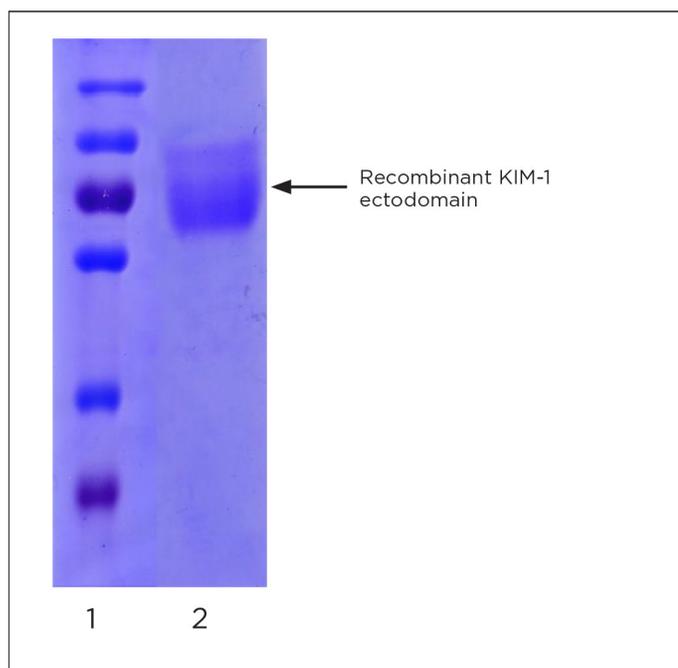
3-7 ng/ml. KIM-1 levels begin to increase as early as 6 hours after an ischemic insult and remain elevated for a period of 48 hours post-injury (6). KIM-1 is not only a sensitive diagnostic marker but also has predictive value for AKI in patients undergoing cardiac surgery (7). Kidney tissue may suffer from ischemia as a result of drug-related response. Accordingly, KIM-1 could be utilized as a nephrotoxicity biomarker in preclinical studies of drug candidates (8) and the Food and Drug Administration has recently recognized KIM-1 as an appropriate biomarker for renal injury in preclinical studies of pharmacologic agents (9). According to some publications, KIM-1 could also be used for detecting certain types of cancer (10, 11).

Kidney injury molecule-1 is a transmembrane glycoprotein (339 amino acid residues in length) with an N-terminal ectodomain (270 a.a.r.) that contains immunoglobulin-like and mucin domains. Ectodomain of KIM-1 could be shed into urine upon ischemic insult and could be detected with the help of specific antibodies. Due to its simplicity and high specificity, immunodetection of KIM-1 is the method of choice for clinical setting.

Advanced ImmunoChemical offers two monoclonal antibodies specific to ectodomain of human KIM-1 (Cat.# 2-KIM1) and recombinant human KIM-1 antigen (Cat.# 8-KIM-rh). Antibodies constitute a pair that is suitable for the measurement of KIM-1 levels in urine by the sandwich ELISA.

## Recombinant KIM-1

Advanced ImmunoChemical offers recombinant ectodomain of human KIM-1 that are expressed in the baculovirus expression system. Recombinant human KIM-1 ectodomain contains 7 additional amino acid residues on the N-terminus and these additional residues serve as an affinity tag for purification. Recombinant human KIM-1 is purified from cell culture fluid using several chromatography steps and migrates as relatively broad protein band (this is a characteristic of the majority of glycoproteins) on Coomassie-stained gel after SDS-electrophoresis in reducing conditions (Fig. 1). The apparent molecular weight of recombinant human KIM-1 ectodomain lies in the region 60-90 kDa.



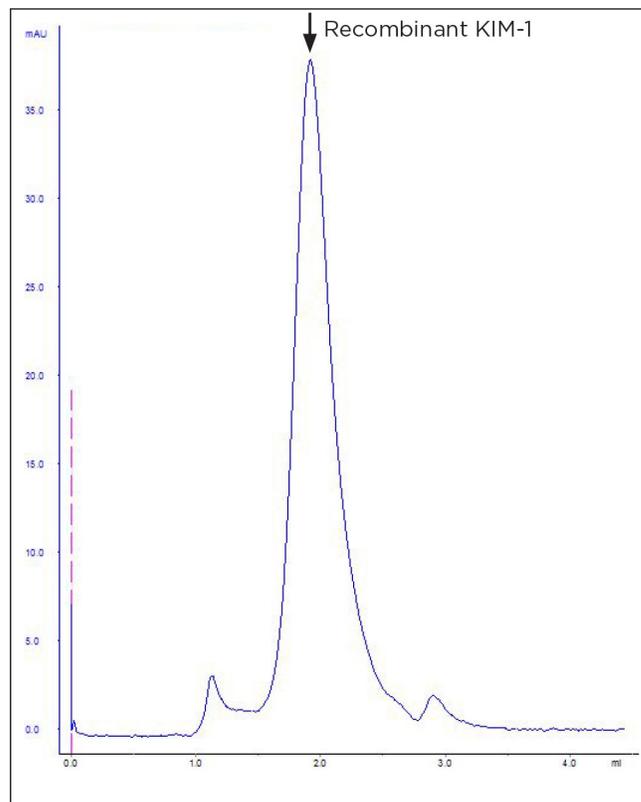
**Figure 1. SDS-gel electrophoresis of human recombinant KIM-1 ectodomain expressed in baculovirus system, reducing conditions.**

Lane 1: Molecular weight standards, Fermentas (130, 100, 70, 55, 35, 25 kDa)

Lane 2: Human recombinant KIM-1 ectodomain expressed in baculovirus system, 2 µg

Gel staining: Coomassie Brilliant Blue R-250

The homogeneity of KIM-1 was further tested by size-exclusion chromatography in tris-buffered saline (Fig. 2). KIM-1 elutes as a single symmetrical peak at conditions outlined in the figure legend, which indicates that recombinant KIM-1 is homogenous.



**Figure 2. Size-exclusion chromatography of purified recombinant human KIM-1 ectodomain.**

Chromatography was conducted using the AKTA purifier system on Superdex 200 5/150 column. Eluent: 50 mM tris, pH 8, 150 mM NaCl. Protein load 17 µg. Blue line – optical density at 280 nm wavelength.

Recombinant human KIM-1 (cat. # 8-KIM-rh) ectodomain can be utilized as a calibrator in immunoassays for detecting human KIM-1 in vitro as well as in urine samples (Fig. 4).

## Ordering Information:

### ANTIGEN

Product Name	Cat #	Purity	Source
Kidney injury molecule-1 (KIM-1), ectodomain, recombinant	8-KIM-rh	>92%	Recombinant

## Anti-human KIM-1 monoclonal antibodies

Host animal:	Mice Balb/c
Cell line used for fusion:	Sp2/0
Antigen:	Recombinant human KIM-1 ectodomain
Purification method:	Protein A affinity chromatography
Presentation:	MAB solution in PBS with 0.1% sodium azide
Applications:	Human KIM-1 immunoassay, sandwich human KIM-1 immunoassay

Hybridoma clones were derived from the hybridization of Sp2/0 myeloma cells with spleen cells of Balb/c mice that were immunized with the human recombinant KIM-1 ectodomain. Two anti-human KIM-1 MABs,

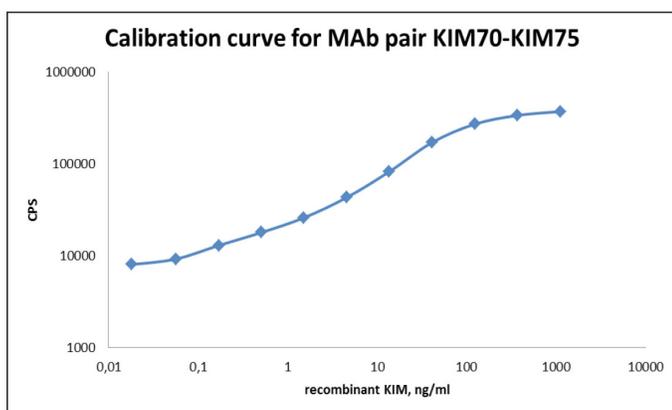
KIM70 and KIM75 (cat. # 2-KIM-1), were selected with regard to the specificity and sensitivity of their interaction with KIM-1 in ELISA with antigen coated on to the plate surface.

## Applications

### Quantitative sandwich immunoassay

Selected MABs were tested in sandwich fluoroimmunoassay as capture and detection antibodies with recombinant human ectodomain KIM-1 as an antigen. The recommended MAB pair is KIM70-KIM75 (Fig. 3).

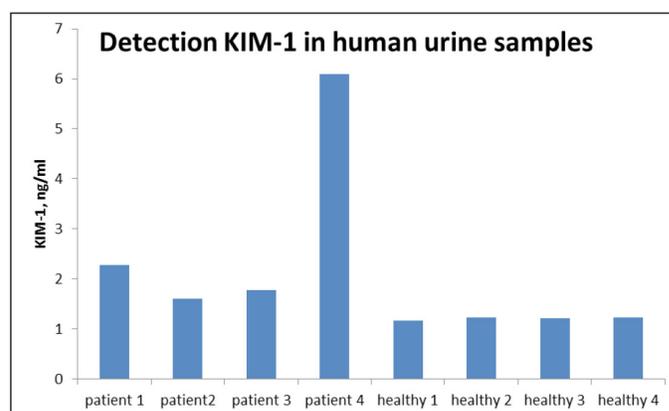
The KIM70-KIM75 assay was tested for its ability to recognize native KIM-1 in urine specimens of patients with cardiorenal syndrome, trauma and pyelonephritis, as well as in the urine of healthy volunteers (Fig. 4).



**Figure 3. Calibration curve for KIM70-KIM75 fluoroimmunoassay.**

The MAb KIM70 was used for capture (1 µg/well), detection MAb KIM75 was labeled with stable Eu<sup>3+</sup> chelate (0.2 µg/well). Recombinant human KIM-1 ectodomain antigen in the buffer containing 0.1% sodium deoxycholate and 10 mM glucose was utilized as an antigen.

The limit of detection of the KIM70-KIM75 fluoroimmunoassay is approximately 0.2 ng/ml.



**Figure 4. Detection of KIM-1 in human urine samples by the KIM70-KIM75 sandwich fluoroimmunoassay.**

Urine samples were taken from patients with cardiorenal syndrome (patients 3 and 4), trauma (patient 1) and pyelonephritis (patient 2), as well as from apparently healthy volunteers (healthy 1, 2, 3, 4). These samples were diluted 1:1 with a buffer containing 0.1% sodium deoxycholate and 10 mM glucose. Recombinant human KIM-1 ectodomain was used as a calibrator.

## Immunodetection of recombinant human KIM-1 ectodomain in Western blotting

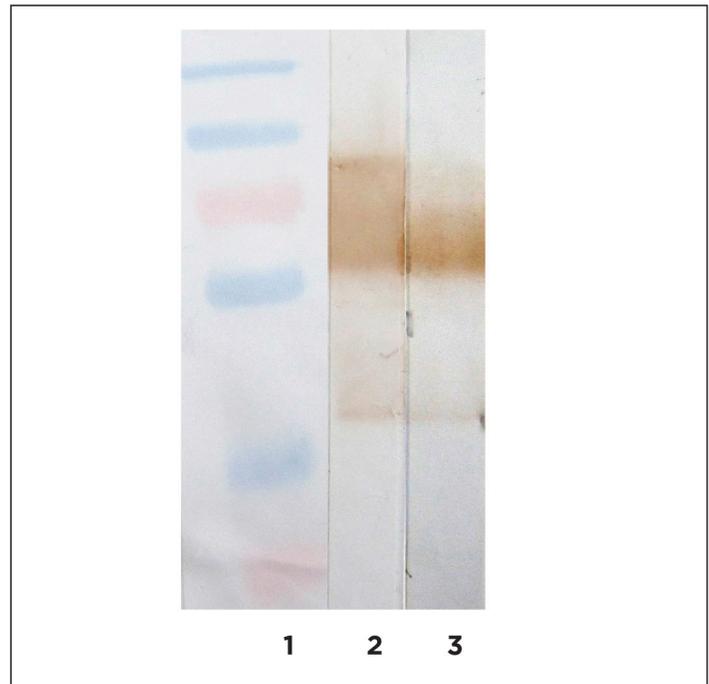
The MAbs KIM70 and KIM75 are capable of recognizing recombinant human KIM-1 ectodomain in Western blotting (Fig. 5).

**Figure 5. Immunodetection of recombinant human KIM-1 ectodomain in Western blotting following SDS-electrophoresis in reducing conditions.**

Lane 1: Molecular weight standards, Fermentas (130, 100, 70, 55, 35, 25 kDa)

Lane 2: Human recombinant KIM-1 ectodomain expressed in baculovirus system, 0.5 µg, stained with MAb KIM70

Lane 3: Human recombinant KIM-1 ectodomain expressed in baculovirus system, 0.5 µg, stained with MAb KIM75



## Ordering Information:

### MONOCLONAL ANTIBODIES

Product Name	Cat #	MAb	Subclass	Remarks
Kidney injury molecule-1 (KIM-1)	2-KIM-1	KIM70	IgG1	EIA, WB
		KIM75	IgG2b	EIA, WB

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