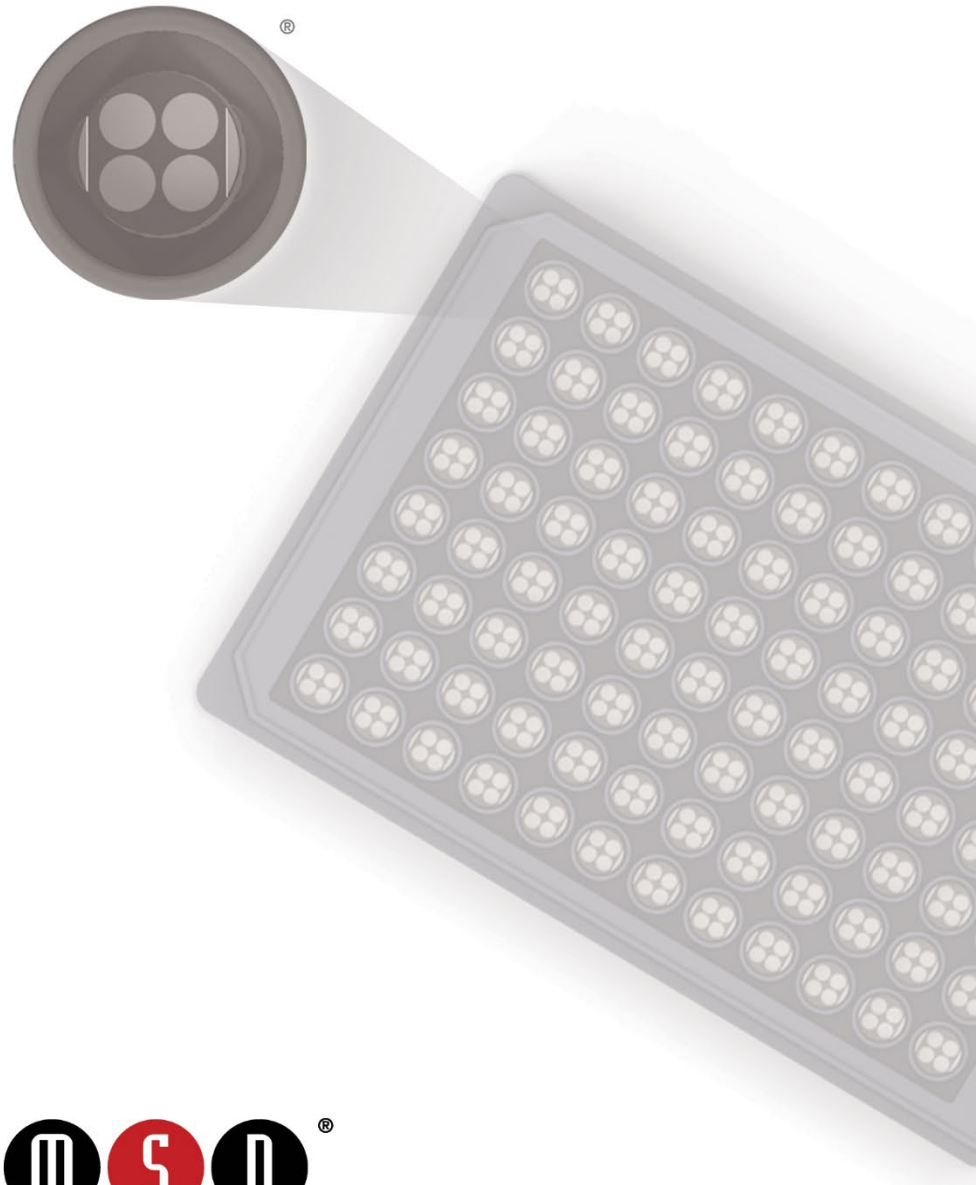
MSD® MULTI-SPOT Assay System

Kidney Injury Panel 1 (rat) Kit

1-Plate Kit	K15162C-1
5-Plate Kit	K15162C-2
25-Plate Kit	K15162C-4

T-PLEX®



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MSD Toxicology Assays

Kidney Injury Panel 1 (rat) Kit

NGAL/LCN2, Osteopontin, Albumin, HAVCR1/KIM-1

This product insert should be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Introduction

Traditional biomarkers in kidney injury research such as BUN and serum creatinine are not sensitive enough to detect subtle kidney damage and often do not correlate to damage measured by histopathology. MSD's kidney injury panels were designed to use traditional and novel biomarkers in kidney injury research that can overcome these shortcomings.

NGAL/LCN2 also known as neutrophil gelatinase-associated lipocalin (NGAL) and lipocalin-2 (LCN2) belongs to the calycin superfamily of proteins. It is a 25 kDa glycoprotein that acts as a transport protein carrying small hydrophobic molecules such as steroid hormones, vitamins, and metabolic products. NGAL/LCN2 is expressed in most tissues and is induced in epithelial cells upon inflammation. In the kidney, NGAL/LCN2 may be implicated in both the progress of and protection from renal injury.

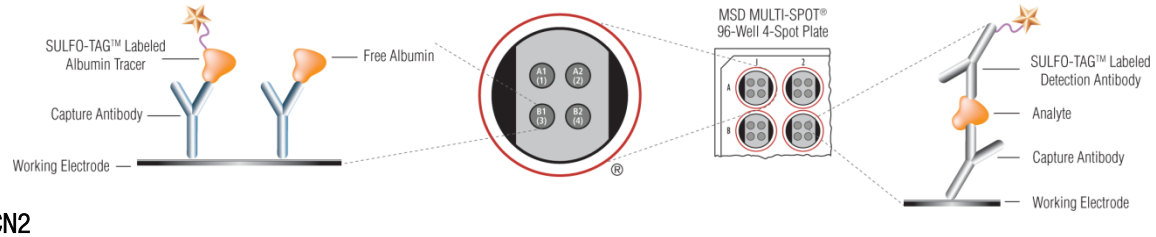
Osteopontin (OPN) is a secreted acidic and phosphorylated glycoprotein that is involved in bone metabolism, immune regulation, cell survival, and tumor progression. OPN is mostly expressed in bone, kidney, and epithelial tissues.

Albumin is the most abundant serum protein and acts as a transport protein for hemin and fatty acids. Albumin is produced in the liver and secreted into the bloodstream. Damage to the kidney can lead to albuminuria, which is the secretion of albumin into the urine.

HAVCR1/KIM-1 (hepatitis A virus cellular receptor 1; kidney injury molecule 1) also known as TIM-1 (T cell immunoglobulin- and mucin domain-containing molecule 1) is a type 1 transmembrane glycoprotein found on $CD4^+$ T cells and renal proximal tubule epithelial cells. The extracellular domain of HAVCR1/KIM-1 is made of an immunoglobulin-like domain topping a long mucin-like domain, suggesting a possible role in cell adhesion. HAVCR1/KIM-1 is released upon certain types of acute kidney injury and can be measured in urine, serum, or plasma.

Principle of the Assay

MESO SCALE DISCOVERY® toxicology assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. In the Kidney Injury Panel 1 (rat) Kit, albumin is a competition assay, and NGAL/LCN2, Osteopontin, and HAVCR1/KIM-1 are sandwich immunoassays (Figure 1). MSD provides a plate precoated with capture antibodies. The user adds the sample premixed with a solution containing albumin tracer (rat albumin conjugated with an electrochemiluminescent label, MSD SULFO-TAG™) and a solution containing NGAL/LCN2, Osteopontin, and HAVCR1/KIM-1 detection antibodies conjugated with SULFO-TAG labels throughout one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the detection antibodies by the bound analytes completes the sandwich. For the competition assay, free albumin (unconjugated) in the sample competes with the SULFO-TAG labeled albumin tracer for binding sites on the capture antibody; thus, the signal strength is inversely proportional to albumin levels in the sample. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample. This panel has been validated according to the principles outlined in "Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement" by J.W. Lee et al.[1]



1. NGAL/LCN2
2. Osteopontin
3. Albumin
4. HAVCR1/KIM-1

Figure 1. *Spot diagram showing the placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.*

Reagents Supplied

Product Description	Storage	Catalog #	Size	Quantity per Kit		
				K15162C-1	K15162C-2	K15162C-4
96-Well Kidney Injury Panel 1 (rat) Plate	2–8°C	N45730A	4-spot	1 plate	5 plates	25 plates
SULFO-TAG NGAL/LCN2 Antibody (50X)[1]	2–8°C	D23IJ-2	75 µL	1 vial	—	—
		D23IJ-3	375 µL	—	1 vial	5 vials
SULFO-TAG Osteopontin Antibody (50X)[1]	2–8°C	D23HJ-2	75 µL	1 vial	—	—
		D23HJ-3	375 µL	—	1 vial	5 vials
SULFO-TAG Albumin Tracer (200X)[1]	2–8°C	C23IK-2	25 µL	1 vial	—	—
		C23IK-3	125 µL	—	1 vial	5 vials
SULFO-TAG HAVCR-1/KIM-1 Antibody (50X)[1]	2–8°C	D23JH-2	75 µL	1 vial	—	—
		D23JH-3	375 µL	—	1 vial	5 vials
Kidney Injury Panel 1 (rat) Calibrator Blend (20X)	≤-70°C	C0162-2	15 µL	1 vial	5 vials	25 vials
Diluent 29	≤-10°C	R50HA-4	15 mL	1 bottles	—	—
		R50HA-3	40 mL	—	2 bottles	10 bottles
Blocker A Kit (Blocker A [dry] in 250 mL bottle and 50 mL bottle of 5X Phosphate Buffer)	RT	R93AA-2	250 mL	1 kit	1 kit	5 kits
Read Buffer T (4X)	RT	R92TC-3	50 mL	1 bottle	1 bottle	5 bottles

[1] SULFO-TAG conjugated detection antibodies should be stored in the dark.
Dash (—) = not applicable

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Microcentrifuge tubes for preparing serial dilutions
- ❑ Phosphate-buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing or MSD Wash Buffer (Catalog No. R61AA-1)
- ❑ Liquid-handling equipment for the desired throughput, capable of dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate-washing equipment: automated plate washer or multichannel pipette
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker (rotary) capable of shaking at 500–1,000 rpm
- ❑ Deionized water
- ❑ Vortex mixer

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

Additional product-specific safety information is available in the safety data sheet (SDS), which can be obtained from MSD Customer Service or at www.mesoscale.com.

Best Practices

- Bring frozen diluent to room temperature in a 22-25°C water bath. Thaw frozen calibrator (when applicable) on wet ice.
- Prepare Calibrator Standards and samples in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution and mix by vortexing after each dilution.
- Avoid prolonged exposure of the detection antibody (stock or diluted) to light. During the antibody incubation step, plates do not need to be shielded from light (except for direct sunlight).
- Avoid bubbles in wells during all pipetting steps because they may lead to variable results. Bubbles introduced when adding Read Buffer may interfere with signal detection.
- Use reverse pipetting when necessary to avoid the introduction of bubbles. For empty wells, pipette gently to the bottom corner.
- Plate shaking should be vigorous, with a rotary motion between 500 and 1,000 rpm. Binding reactions may reach equilibrium sooner if you use shaking at the middle of this range (~700 rpm) or above.
- When using an automated plate washer, rotate the plate 180 degrees between wash steps to improve assay precision.
- Tap the plate on a paper towel to remove residual fluid after washing.
- If an incubation step needs to be extended, leave the sample or detection antibody solution in the plate to keep the plate from drying out.
- Remove the plate seal before reading the plate.
- Make sure that the read buffer is at room temperature when added to a plate.
- Do not shake the plate after adding the read buffer.
- To improve interplate precision, keep time intervals consistent between adding the read buffer and reading the plate. Unless otherwise directed, read the plate as soon as possible after adding the read buffer.
- If the sample results are above the top of the calibration curve, dilute the samples and repeat the assay.
- When running a partial plate, seal the unused sectors to avoid contaminating unused wells. Remove all seals before reading. You may adjust volumes proportionally when preparing reagents.

Reagent Preparation

Bring all reagents to room temperature. Thaw the stock calibrator blend on ice.

Important: Upon the first thaw, separate Diluent 29 into aliquots appropriate for the size of your needs before refreezing. The diluent can go through 3 freeze-thaw cycles without significantly affecting the performance of the assay.

Prepare Blocker A Solution

Follow the Blocker A instructions included in the kit.

Prepare Albumin Tracer Solution

MSD provides albumin tracer as a 200X stock solution. The working tracer solution is 1X.

For one plate, combine:

- ❑ 20 µL of 200X SULFO-TAG Albumin Tracer
- ❑ 3,980 µL of Diluent 29

Prepare Calibrator Solutions

MSD supplies blended calibrator for the Kidney Injury Panel 1 (rat) Kit at 20-fold higher concentration than the recommended highest standard. We recommend a 7-point standard curve with 3-fold serial dilution steps and a zero calibrator blank. Signals from the blank should be excluded when generating the curve. Thaw the stock calibrator and keep on ice, then add to diluent at room temperature to make the standard curve solutions. For the actual concentration of each calibrator in the blend, refer to the certificate of analysis (COA) supplied with the kit or available at www.mesoscale.com.

To prepare 7 standard solutions plus a zero calibrator for up to 3 replicates:

Prepare the highest standard by adding 10 µL of stock calibrator to 190 µL of Diluent 29. Mix well.

1) Prepare the next standard by transferring 80 µL of the highest standard to 160 µL of Diluent 29. Mix well. Repeat 3-fold serial dilutions 5 additional times to generate 7 standards.
2) Use Diluent 29 as the blank.
3) Calibrators should be prepared at room temperature no more than 20 minutes before combining with 1X albumin tracer.

Prepare Samples

For rat urine samples, MSD recommends 10-fold dilution in Diluent 29; however, you may need to adjust the dilution factor for the sample set under investigation.

Prepare Premix of Standards and Samples with 1X Albumin Tracer

Combine equal volumes of diluted sample and 1X albumin tracer solution.

For two replicates, combine:

- ❑ 75 µL of diluted sample/standards
- ❑ 75 µL of 1X albumin tracer solution

For three replicates, combine:

- ❑ 100 µL of diluted sample/standards
- ❑ 100 µL of 1X albumin tracer solution

Incubate the prepared standards and samples at room temperature without shaking for at least 30 minutes before adding to the assay plate.

Prepare Detection Antibody Solution

MSD provides each detection antibody as a 50X stock solution. The working detection antibody solution is 1X.

For one plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-rat NGAL/LCN2 Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-rat Osteopontin Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-rat HAVCR1/KIM-1 Antibody
- ❑ 2,820 µL of Diluent 29

You may omit detection antibody for any analyte not being measured; add 60 µL of Diluent 29 for each omitted antibody.

Prepare Wash Buffer

MSD provides Wash Buffer as a 20X stock solution. The working solution is 1X PBS + 0.05% Tween-20 can be used instead.

For one plate, combine:

- ❑ 15 mL of MSD Wash Buffer (20X)
- ❑ 285 mL of deionized water

1X MSD Wash Buffer can be stored at room temperature for up to two weeks.

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 1X.

For one plate, combine:

- ❑ 5 mL of Read Buffer T (4X)
- ❑ 15 mL of deionized water

You may prepare diluted read buffer in advance and store it at room temperature in a tightly sealed container.

Prepare MSD Plate

MSD plates are precoated with capture antibodies (Figure 1) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Plates can be used as delivered; no additional preparation (e.g., prewetting) is required.

Protocol

Note: Follow **Reagent Preparation** before beginning this assay protocol.

STEP 1: Add Blocker A Solution

- ❑ Add 150 µL of Blocker A solution to each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (500–1,000 rpm) at room temperature.

STEP 2: Wash and Add Sample

- ❑ Wash the plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 50 µL of the sample (standards, controls, or unknowns) – premixed with 1X albumin tracer – per well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (500–1,000 rpm) at room temperature.

  You may prepare detection antibody solution during incubation.

STEP 3: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (500–1,000 rpm) at room temperature.

  You may prepare diluted read buffer during incubation.

STEP 4: Wash and Read

- ❑ Wash the plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 150 µL of 1X Read Buffer T to each well. Analyze the plate on an MSD instrument. Incubation in read buffer is not required before reading the plate.

Validation and Verification

MSD's validation testing is conducted according to fit-for-purpose principles[1] through a design-control process.

Validation. Bioanalytical and functional characterizations of calibrators, antibodies, and other assay components are completed to ensure the quality and consistency of reagents between lots. This includes plate coating uniformity and reagent and component specificity testing for individual kit lots. Multiple control sample replicates in the specified matrices are tested to ensure the assay meets MSD's accuracy, precision, and sensitivity criteria.

Verification. Multiday analysis with multiple runs per day using 6–12 plates is performed as part of the release testing for each lot.

- **Curve Fitting**

  Calibration curve fitting methods, including weighting functions and 4- or 5-parameter logistic models, are evaluated on multiple runs to select the best curve-fitting algorithm.

- **Sensitivity and Dynamic Range**

  - **Sensitivity.** The lower limit of detection (LLOD) is established based on runs throughout assay development. It is a calculated concentration based on a signal 2.5 standard deviations above the average reading from the blank calibrators. This results in a signal that is significantly higher than the background.
  - **Dynamic Range.** The dynamic (quantitative) range is established based on multiple runs from multiple lots. The limits of the range—lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ)—are the lowest and highest concentrations that can be measured with acceptable levels of precision and accuracy.

  The limits of quantification defined in this product insert are verified for each lot as part of the lot verification and quality control release.

- **Precision and Accuracy**

  Control samples made in the specified matrix are tested over multiple days to measure intra-run, and inter-run, accuracy and precision. Coefficient of variance (CV) information is presented in the product insert. During the validation process, the assay is tested over multiple days with multiple runs per day.

  - **Precision.** The typical specification for precision is a CV of less than 20% for controls on both intra-and inter-day runs.
  - **Accuracy.** The typical specification for accuracy includes a calculated concentration CV of less than 20%, accuracy within 20% of expected concentration.

- **Robustness and Stability**

  Freeze-thaw testing and accelerated stability studies for calibrators, antibodies, and controls are performed during assay development and are augmented with real-time stability studies on complete kits out to 24 months from the date of manufacture.

- **Specificity, Spike Recovery, and Dilution Linearity**

  Assays are tested in the targeted matrix for nonspecific bindings. Spike recovery and dilution linearity are tested across the assay range to evaluate sample matrix effects.

- **Tested Samples**

  Normal samples for the specified species are tested to determine the normal range of biomarker concentration detected with the assay.

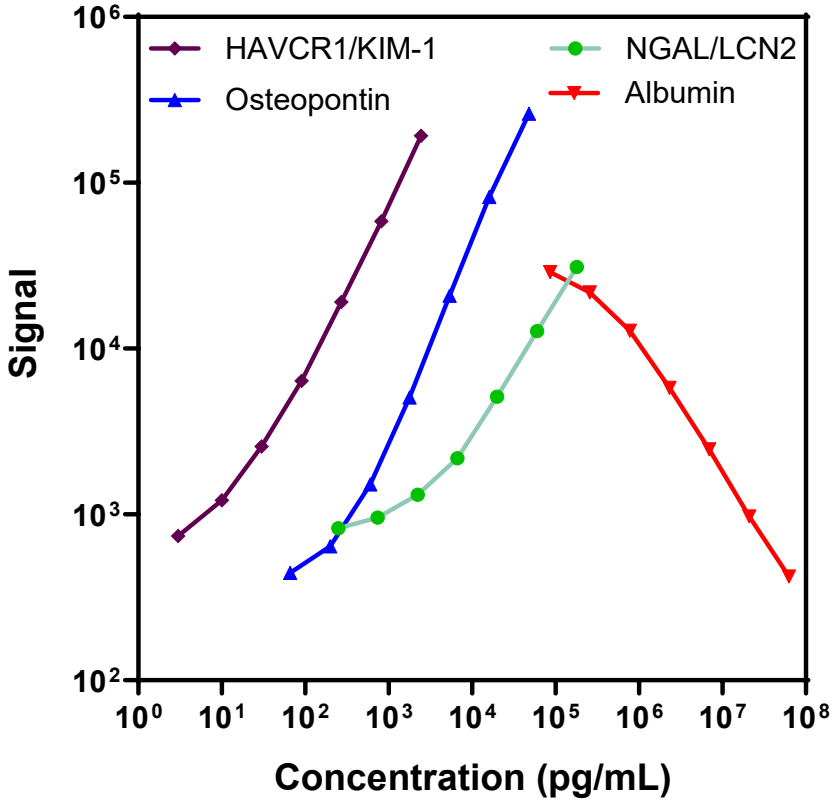
Representative data from this kit's validation process are presented below. The lot-specific standard curve and measured limits of quantification can be found in the COA enclosed with each kit. You can also find a copy of the lot-specific COA at www.mesoscale.com.

Analysis of Results

Run at least one set of calibrators in duplicate to generate the standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from the calibrators can be used to calculate the concentration of analytes in the samples. The assays have a wide dynamic range (3–4 logs) which allows accurate quantification without the need for dilution in many cases. The MSD analysis software uses a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting function is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

Typical Data

The following standard curve graph illustrates the dynamic range of the assay. Actual signals will vary. The best quantification of unknown samples will be achieved by generating a standard curve for each plate using a minimum of 2 replicates of standards.



Sensitivity

A multiplate, multiday study was performed to measure the reproducibility of the assay. The lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) were established based on the results of multiple plate runs from multiple kit lots.

NGAL/LCN2, Osteopontin, and HAVCR1/KIM-1

The lower limit of detection (LLOD) is a calculated concentration based on a signal 2.5 standard deviations above the background (zero calibrator blank).

The LLOQ is the lowest concentration where the %CV of the calculated concentration is less than 20% and the percent recovery is between 80% and 120% of the known value. For NGAL/LCN2, the percent recovery is between 70% and 130%.

The ULOQ is the highest concentration where the %CV of the calculated concentration is less than 20% and the percent recovery of the standard is between 80% and 120% of the known value.

	NGAL/LCN2	Osteopontin	HAVCR1/KIM-1
Average LLOD (ng/mL)	0.317	0.064	0.002
LLOQ (ng/mL)	3.67	0.390	0.020
ULOQ (ng/mL)	150	37.5	1.88

Albumin

The LLOD was set at 80% of the maximum signal at 0 ng/mL. The LLOQ is the lowest concentration where the %CV of the calculated concentration is less than 20% and the percent recovery is between 80% and 120% of the known value. The upper limit was observed at 20% of the signal over the highest calibrator.

	Albumin
Average LLOD (ng/mL)	64.0
LLOQ (ng/mL)	459
Upper Limit (ng/mL)	33,200

Precision

Controls were made by spiking calibrator into rat urine at levels throughout the range of the assay. Analyte levels were measured using a minimum of 3 replicates on 13 runs over 4 days.

Average intra-run %CV is the average %CV of the control replicates within an individual run.

Inter-run %CV is the variability of controls across 13 runs.

	Control	Runs	Average Conc. (ng/mL)	Average Intra-run %CV	Inter-run %CV
NGAL/LCN2	Mid	13	91.5	6.4	9.0
	Low	13	42.3	5.6	8.2
Osteopontin	High	13	15.6	5.0	6.4
	Mid	13	6.06	4.5	6.4
Albumin	High	13	9,100	2.8	3.2
	Mid	13	4,810	4.0	4.7
	Low	13	2,775	2.2	2.3
HAVCR1/KIM-1	High	13	0.960	3.5	4.4
	Mid	13	0.329	3.8	5.7
	Low	13	0.133	4.2	9.9

Dilution Linearity/Parallelism

To assess linearity, urine samples were diluted 5-fold, 10-fold, 20-fold, and 40-fold before testing. Percent recovery at each dilution was normalized to the dilution-adjusted 10-fold concentration.

% Recovery = (measured concentration/expected concentration) ∗100

	NGAL/LCN2					Osteopontin				
Fold Dilution	N	Conc. Ave (ng/mL)	Conc. CV Average, %	% Recovery Average	% Recovery Range	N	Conc. Ave (ng/mL)	Conc. CV Average, %	% Recovery Average	% Recovery Range
5	3	175	12.0	69	64-75	3	3.69	4.80	53	45-61
20	3	315	6.8	112	106-120	3	19	6.5	187	NA
40	3	347	8.6	116	106-135	3	35	5.1	337	NA

	Albumin					HAVCR1/KIM-1				
Fold Dilution	N	Conc. Ave (ng/mL)	Conc. CV Average, %	% Recovery Average	% Recovery Range	N	Conc. Ave (ng/mL)	Conc. CV Average, %	% Recovery Average	% Recovery Range
5	8	29,740	1.8	104	99-112	3	7.71	3.9	90	84-97
20	8	28,300	2.4	99	97-103	3	9.0	4.5	101	98-104
40	8	29,000	8.6	101	94-106	3	13	3.9	99	99-99

Spike Recovery

Normal Sprague-Dawley rat urine samples were diluted 10-fold and spiked with calibrators at multiple levels throughout the range of the assay. We observed that recombinant rat Osteopontin is under-recovered when spiked into urine samples, as shown below. Osteopontin has been found to bind to calcium oxalate crystals in the urine, causing inaccurate measurements by ELISA.[4]

% Recovery = (measured concentration/expected concentration) ∗100

NGAL/LCN2						Osteopontin					
Spike Conc. (ng/mL)	N	Conc. Average (ng/mL)	Average Conc. %CV	% Recovery Average	% Recovery Range	Spike Conc. (ng/mL)	N	Conc. Average (ng/mL)	Average Conc. %CV	% Recovery Average	% Recovery Range
0	3	29.6	7.5	NA	NA	0	3	1.10	5.5	NA	NA
4.44	3	36.4	3.5	103	96-109	1.11	3	1.46	2.1	63	57-71
13.3	3	49.0	4.9	106	96-121	3.33	3	2.34	3.3	53	50-57
40.0	3	72.7	4.7	99	91-115	10.0	3	4.14	2.6	38	31-42

Albumin						HAVCR1/KIM-1					
Spike Conc. (ng/mL)	N	Conc. Average (ng/mL)	Average Conc. %CV	% Recovery Average	% Recovery Range	Spike Conc. (ng/mL)	N	Conc. Average (ng/mL)	Average Conc. %CV	% Recovery Average	% Recovery Range
0	8	29,430	5.1	NA	NA	0	3	1.79	2.0	NA	NA
1,470	8	28,506	2.8	93	83-108	0.056	3	1.67	4.2	95	90-99
4,410	8	30,097	2.1	90	83-101	0.167	3	1.82	2.1	101	93-111
13,200	8	39,845	1.1	94	83-106	0.50	3	2.22	1.4	104	97-113

Specificity

To assess the specificity of the individual assays, the Kidney Injury Panel 1 (rat) was run using blended calibrators and single detection antibodies. Dilute the stock calibrator blend 60-fold in diluent 29 before use. No significant cross-reactivity (<0.2%) was observed, except with the binding of the NGAL/LCN2 detection antibody to the OPN calibrator (<5%). The extent of binding of the NGAL/LCN2 detection antibody to the OPN calibrator varies due to inconsistent behavior of diluent raw materials.

Tested Samples

Urine samples from untreated rats were diluted 10-fold prior to testing.

	NGAL/LCN2	Osteopontin	Albumin	HAVCR1/KIM-1
Number of Samples	30	30	30	30
Average (ng/mL)	2,960	1.6	71,100	1.5
Range (ng/mL)	529–11,800	0.66–3.6	24,400–256,000	0.44–4.3
% Recovered	63	20	100	100

Assay Components

Calibrators

The assay calibrator blend uses the following recombinant rat proteins:

NGAL/LCN2 (residues 21–198), expressed in murine myeloma cells

Osteopontin (full-length), expressed in Chinese Hamster Ovary-derived cells

HAVCR1/KIM-1 (residues 18–238), expressed in murine myeloma cells

Albumin, isolated from the serum of Sprague-Dawley rats and treated to be essentially globulin-free

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
NGAL/LCN2	Goat Polyclonal	Goat Polyclonal
Osteopontin	Goat Polyclonal	Rabbit Polyclonal
Albumin	Rabbit Polyclonal	n/a
HAVCR1/KIM-1	Goat Polyclonal	Goat Polyclonal

References

1. Lee JW, et al. Fit-for-purpose method development and validation for successful biomarker measurement. Pharm Res. 2006 Feb;23(2):312-28.
2. Mishra J, et al. Identification of Neutrophil Gelatinase-Associated Lipocalin as a Novel Early Urinary Biomarker for Ischemic Renal Injury. J Am Soc Nephrol. 2003;14:2534–2543.
3. Dieterle F, et al. Monitoring kidney safety in drug development: emerging technologies and their implications. Curr. Opin. Drug Discov. Devel. 2008;11(1):60-71.
4. Thurgood LA, Grover PK, Ryall RL. High calcium concentration and calcium oxalate crystals cause significant inaccuracies in the measurement of urinary osteopontin by enzyme linked immunosorbent assay. Urol Res. 2008;36:103-110.

Summary Protocol

Kidney Injury Panel 1 (rat) Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol before performing the Kidney Injury Panel 1 (rat) assays.

Sample and Reagent Preparation

- ❑ Bring all reagents to room temperature and thaw the calibrator on ice.
- ❑ Prepare Blocker A solution.
- ❑ Prepare 7 standard solutions using the supplied calibrator:
  - Dilute the stock calibrator blend 20-fold in Diluent 29. Perform a series of 3-fold dilution steps and prepare a zero calibrator blank.
- ❑ Dilute samples 10-fold (or as needed) in Diluent 29.
- ❑ Mix each standard, control, or diluted sample with an equal volume of 1X albumin tracer. Incubate for 30 minutes at room temperature.
- ❑ Prepare combined detection antibody solution by diluting each stock detection antibody 50-fold in Diluent 29.
- ❑ Prepare 1X Read Buffer T by diluting stock 4X Read Buffer T 4-fold with deionized water.

STEP 1: Add Blocker A Solution

- ❑ Add 150 µL/well of Blocker A solution.
- ❑ Incubate at room temperature with vigorous shaking (500–1,000 rpm) for 1 hour.

STEP 2: Wash and Add Sample

- ❑ Wash plate 3 times with 300 µL/well of PBS-T.
- ❑ Add 50 µL/well of sample (standards, controls, or unknowns) premixed with 1X albumin tracer.
- ❑ Incubate at room temperature with vigorous shaking (500–1,000 rpm) for 2 hours.

STEP 3: Wash and Add Detection Antibody Solution

- ❑ Wash plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 25 µL/well of 1X detection antibody solution.
- ❑ Incubate at room temperature with vigorous shaking (500–1,000 rpm) for 2 hours.

STEP 4: Wash and Read Plate

- ❑ Wash plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 150 µL/well of 1X Read Buffer T.
- ❑ Analyze plate on an MSD instrument.

Plate Diagrams