Meso Scale Discovery®

MULTI-SPOT® Assay System

Kidney Injury Panel 1 (rat) Assay Kit

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

This product insert should be used with kit lot # K0040049

MSD Toxicology Assays

Kidney Injury Panel 1 (rat) Assay Kit

Lipocalin-2, Osteopontin, Albumin, TIM-1

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.

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Ordering Information

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Introduction

Traditional clinical markers for kidney injury 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. This product insert describes multiplex panels of traditional and novel biomarkers for kidney injury that can overcome these shortcomings.

Lipocalin-2 belongs to calycin superfamily of proteins and also known as Neutrophil Gelatinase-associated Lipocalin (NGAL). It is a 25 kDa glycoprotein that acts as transport protein carrying small hydrophobic molecules such as steroid hormones, vitamins and metabolic products. Lipocalin-2 is expressed in most tissues and is induced in epithelial cells upon inflammation. In the kidney, Lipocalin-2 may be implicated in both progress 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 that acts as a transport protein for hemin and fatty acids. Albumin is produced in the liver and secreted in the bloodstream. Damage to the kidney can lead to albuminuria, secretion of albumin into the urine.

TIM-1 (T cell immunoglobulin and mucin domain containing molecule 1) also known as *KIM-1* (Kidney Injury Molecule-1) or *HAVCR* (Hepatitis A Virus Cellular Receptor 1) is a type 1 transmembrane glycoprotein found on CD4+ T cells and renal proximal tubule epithelial cells. The extracellular domain of TIM-1 is made of an immunoglobulin-like domain topping a long mucin-like domain, suggesting a possible role in cell adhesion.TIM-1 is released upon certain types of acute kidney injury and can be measured in urine, serum, or plasma.

Principle of the Assay

MSD® toxicology assays provide a rapid and convenient method for measuring the levels of protein targets within a single small-volume sample. The assays are available in both singleplex and multiplex formats. In a singleplex assay, an antibody for a specific protein target is coated on one electrode (or "spot") per well. In a multiplex assay, an array of capture antibodies against different targets is patterned on distinct spots in the same well. Our Kidney Injury Panel 1 (rat) Assay detects lipocalin-2, osteopontin, and TIM-1 in a sandwich immunoassay and uses a competitive assay format to detect Albumin (Figure 1). MSD provides a plate that has been pre-coated with four capture antibodies on spatially distinct spots—antibodies for lipocalin-2, osteopontin, albumin, and TIM-1. The user adds the sample, premixed with Albumin Tracer, and a solution containing the labeled detection antibodies—anti-lipocalin-2, anti-osteopontin, and anti-TIM-1 labeled with an electrochemiluminescent compound, MSD SULFO-TAG™ label—over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface. In the sandwich assay, recruitment of the labeled detection antibodies by bound analytes completes the sandwich. In the competition assay, the Albumin Tracer will compete with the sample albumin for binding to the capture antibody. This format yields a decreasing signal with increasing sample albumin concentration. The user adds an MSD read buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD SECTOR® instrument for analysis. Inside the SECTOR instrument, a voltage applied to the plate electrodes causes the labels bound to the electrode surface to emit light. The instrument measures intensity of emitted light to afford a quantitative measure of lipocalin-2, osteopontin, albumin, and TIM-1 present in the sample.
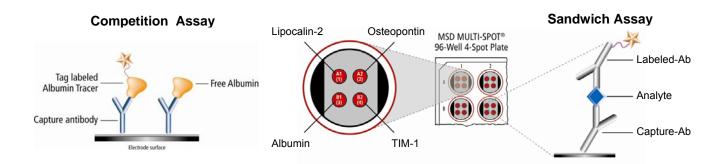

Figure 1. *Spot diagram showing placement of analyte capture antibody. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.*

Reagents Supplied

Product Description	Storage	Quantity per Kit		
		K15162C-1	K15162C-2	K15162C-4
MULTI-SPOT® 96-well Kidney Injury Panel 1 (rat) Plate N45162A	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG™ Anti-rat Lipocalin-2 Antibody (50X)[1]	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
SULFO-TAG Anti-rat Osteopontin Antibody (50X)[1]	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
SULFO-TAG Anti-rat TIM-1 Antibody (50X)[1]	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Kidney Injury Panel 1 (rat) Calibrator Blend (20X) Lot A0040020 Lipocalin-2: 3620 ng/mL Osteopontin: 964 ng/mL Albumin: 936000 ng/mL TIM-1: 49.2 ng/mL	≤ -70°C	1 vial (15 µL)	5 vials (15 µL ea)	25 vials (15 µL ea)
SULFO-TAG Albumin Tracer (200X) Stock concentration: 50 µg/mL	2-8°C	1 vial (25 µL)	1 vial (125 µL)	5 vials (125 µL ea)
Blocker A Kit R93AA-2 (250 mL)	RT	1 bottle (250 mL)	1 bottle (250 mL)	5 bottles (250 mL ea)
Diluent 5 R52BA-5 (25 mL)	≤ -10°C	1 bottle (25 mL)	3 bottles (25 mL ea)	15 bottles (25 mL ea)
Read Buffer T (with surfactant), 4X R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)

Required Materials and Equipment - not supplied

- Deionized water for diluting concentrated buffers
- 50 mL tubes for reagent preparation
- 15 mL tubes for reagent preparation
- Microcentrifuge tubes for preparing serial dilutions
- Phosphate buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing
- Appropriate liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL into a 96-well microtiter plate
- Plate washing equipment: automated plate washer or multichannel pipette
- Adhesive plate seals
- Microtiter plate shaker

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[1] Some SULFO-TAG labeled detection antibodies may be light-sensitive, so they should be stored in the dark.

V Safety

Safe laboratory practices and personal protective equipment such as gloves, safety glasses, and lab coats should be used at all times during the handling of all kit components. All hazardous samples should be handled and disposed of properly, in accordance with local, state, and federal guidelines.

VI Reagent Preparation

Bring all reagents to room temperature. The stock calibrator blend should be thawed on ice.

> **Important:** Upon first thaw, separate Diluent 5 into aliquots appropriate to the size of your assay needs. The diluent can go through up to three freeze-thaw cycles without significantly affecting the performance of the assay.

Prepare Blocker A Solution

Follow instructions included with the Blocker A Kit.

Prepare Albumin Tracer Solution

Prepare a solution of 1X Albumin Tracer in Diluent 5. For one plate dilute the stock tracer 200-fold in Diluent 5.   Approximately 40 μL per well of this solution will be required. For one plate, add 20 μL of stock albumin tracer to 3.98 mL of Diluent 5.

Prepare Calibrator and Control Solutions

Calibrators for the Kidney Injury Panel 1(rat) are supplied at 20-fold higher concentration than the recommended highest calibrator. For each assay, an 8-point standard curve is recommended with 3-fold serial dilution steps and a zero Calibrator. The Calibrators are supplied as a blend. The concentrations provided are for Calibrator Lot A0040020. The table below shows the concentrations of the 8-point standard curve:

Standard	Lipocalin (ng/mL)	Osteopontin (ng/mL)	Albumin (ng/mL)	TIM-1 (ng/mL)	Dilution Factor
Stock Cal. Vial	3620	964	936000	49.2	
STD-01	181	48.2	46800	2.46	20
STD-02	60.3	16.1	15600	0.82	3
STD-03	20.1	5.36	5200	0.27	3
STD-04	6.70	1.79	1733	0.091	3
STD-05	2.23	0.60	578	0.030	3
STD-06	0.74	0.20	193	0.010	3
STD-07	0.25	0.066	64.2	0.0034	3
STD-08	0	0	0	0	n/a

To prepare this 8-point standard curve:

1) Prepare the highest Calibrator by adding 10 µL of the Calibrator stock vial to 190 µL of Diluent 5 (20-fold dilution).
2) Prepare the next Calibrator by transferring 75 µL of the diluted Calibrator to 150 µL of Diluent 5. Repeat 3-fold serial dilutions 5 additional times to generate 7 Calibrators.
3) Reserve 150 µL of Diluent 5 to be used as zero calibrator.

Calibrators should be prepared at room temperature no more than 20 minutes before use.

Dilution of Samples

Some rat samples may need to be diluted prior to the assay in order to get the analyte levels into the detection range. If this is the case, Diluent 5 should be used to dilute samples. 10X dilution of urine samples is recommended for this multiplex panel. Depending on the sample set under investigation, higher or lower dilution factors may be necessary.

Prepare Premix of Samples and Calibrators with the 1X Albumin Tracer

Combine equal amounts of sample or standard with 1X Albumin Tracer Solution. For duplicate measurements, mix 75 µL of diluted sample or standard with 75 µL of 1X Albumin Tracer Solution. For triplicate measurements, mix 100 µL of diluted sample or standard with 100 µL of 1X Albumin Tracer Solution. These solutions should be prepared at RT for at least 30 minutes before addition to the assay plate.

Prepare Detection Antibody Solution

The Detection Antibodies are provided as a 50X stock solution. The working Detection Antibody Solution should contain 1X as final concentration of each antibody.
In a 15 mL tube combine (per plate):

- ❑ 60 µL of 50X SULFO-TAG Anti-rat Lipocalin-2 Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-rat Osteopontin Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-rat TIM-1 Antibody
- ❑ 2820 µL of Diluent 5

Prepare Read Buffer

The Read Buffer should be diluted in deionized water to make a final concentration of 1X Read Buffer T. Add 5 mL of stock Read Buffer T (4X) to 15 mL of deionized water for each plate.

Prepare MSD Plate

This plate has been pre-coated with antibody for the analytes shown in Figure 1. The plate can be used as delivered; no additional preparation (e.g., pre-wetting) is required. The plate has also been exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies.

VII Assay Protocol

1. **Addition of Blocker A Solution:** Dispense 150 µL of Blocker A Solution into each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (300-1000 rpm) at room temperature.

2. **Wash and Addition of Premixed Sample or Calibrator:** Wash the plate 3X with PBS-T. Dispense 50 µL of sample or Calibrator Blend—premixed with Albumin Tracer—into the appropriate wells of the MSD plate. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300-1000 rpm) at room temperature.

   Shaking a 96-well MSD MULTI-SPOT plate typically accelerates capture at the working electrode.

3. **Wash and Addition of the Detection Antibody Solution:** Wash the plate 3X with PBS-T. Dispense 25 µL of the 1X Detection Antibody Solution into each well of the MSD plate. Seal the plate and incubate for 2 hours with vigorous shaking (300-1000 rpm) at room temperature.

4. **Wash and Read:** Wash the plate 3X with PBS-T. Add 150 µL of 1X Read Buffer T to each well of the MSD plate. Analyze the plate on the SECTOR Imager. Plates may be read immediately after the addition of Read Buffer.

   Bubbles in the fluid will interfere with reliable reading of MULTI-SPOT plate. Use reverse pipetting techniques to insure bubbles are not created when dispensing the Read Buffer.

VIII Analysis of Results

The calibrators should be run in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have a wide dynamic range (3-4 logs) which allows accurate quantitation in many samples without the need for dilution. The MSD Discovery Workbench® analysis software utilizes a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting functionality is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.
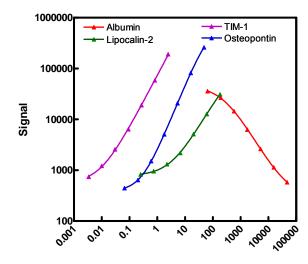
Typical Standard Curve

typical standard curve

The following standard curve is an example of the dynamic range of the assay. The actual signals may vary and a standard curve should be run for each set of samples and on each plate for the best quantitation of unknown samples.

Albumin		
Conc. (ng/mL)	Average Counts	%CV
0	42800	3.1
64.2	35810	3.1
193	26630	3.3
578	14481	3.8
1733	6303	2.7
5200	2623	4.2
15600	1140	2.3
46800	581	6.5

Lipocalin-2		
Conc. (ng/mL)	Average Counts	%CV
0	742	5.1
0.25	825	3.6
0.74	955	4.6
2.23	1313	4.5
6.70	2178	2.7
20.1	5139	3.2
60.3	12783	3.7
181	31025	5.0

TIM-1		
Conc. (ng/mL)	Average Counts	%CV
0	526	7.7
0.0034	740	4.1
0.010	1216	3.5
0.030	2564	3.6
0.091	6405	3.6
0.27	19095	2.7
0.82	58497	2.0
2.46	191919	2.9

Osteopontin		
Conc. (ng/mL)	Average Counts	%CV
0	365	7.4
0.066	444	6.5
0.20	643	6.0
0.60	1514	5.4
1.79	5068	5.0
5.36	20862	6.1
16.1	82237	3.3
48.2	261007	4.1

Ⅹ Sensitivity

s e n s i t i v i t y

The lower limit of detection (LLOD) is measured as the concentration at 2.5 standard deviations over the background for TIM-1, NGAL, and OPN or at 80% of the maximum signal for albumin.

A multi-plate, multi-day study was performed to measure the reproducibility of the assay. The lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ) were established from the multiple plate run.

The LLOQ is determined as the lowest concentration where the %CV of the calculated concentration is less than 20% and the percent recovery of the standard is between 80% and 120%.

The ULOQ is determined as 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%.

For the albumin assay, which is a competitive assay, the LLOD was set at 80% of the 0 pg/mL signal. The upper limit of albumin was based on 20% of the signal over the highest calibrator. The estimated LLOQ for albumin is the point on the standard curve where the %CV of the standard is less than 20% and the percent recovery of the standard was between 80% and 120%.

	TIM-1	Lipocalin-2	Osteopontin
LLOD (ng/mL)	0.0016	0.32	0.064
LLOQ (ng/mL)	0.02	3.12	0.39
ULOQ (ng/mL)	1.88	150	37.5

	Albumin
LLOD (ng/mL))	76.3
LLOQ (ng/mL)	781
ULOQ (ng/mL)	33648

XI Precision

A multi-day, multi-plate study over 9 plates was performed to show reproducibility. In addition to the standard curves, control samples of a high, mid, and low levels of each analyte were measured on each plate. Each sample was run in triplicate. The average intra-plate %CV and inter-plate %CV of the concentrations are shown below.

	Control	Plates	Concentration (ng/mL)	Average Intra-day % CV	Inter-day % CV
Albumin	High	9	9629	13.7	15.9
	Mid	9	4845	14.1	15.7
	Low	9	1593	17.5	20.5
TIM-1	High	9	0.80	7.4	8.3
	Mid	9	0.15	10.5	13.4
	Low	9	0.04	10.3	11.8
Lipocalin-2	High	9	58.4	10.3	10.3
	Mid	9	10.8	14.2	16.0
	Low	9	2.71	12.7	15.7
Osteopontin	High	9	3.99	11.0	12.1
	Mid	9	2.16	9.8	13.6
	Low	9	2.00	8.5	11.5

XII Spike Recovery

Rat urine samples were spiked with the calibrators at multiple values throughout the range of the assay. The spiked samples were tested at a 10-fold dilution into the assay diluent. The recombinant osteopontin may be bound to proteins in the urine, making it under-recover. Diluting the sample did not show linear dilution of the osteopontin. At a single dilution, the %CV of the samples are very consistent; therefore, with the large dynamic range of the assay, we can measure both control samples and treated samples without diluting the samples differently.   % Recovery = measured / expected x 100

Albumin Spike Level (ng/mL)	Conc. (ng/mL)	Conc. %CV	% Recovery
7812	10412	11.0	94
1953	4846	1.1	94
0	3225		

Lipocalin-2 Spike Level (ng/mL)	Conc. (ng/mL)	Conc. %CV	% Recovery
62.5	60.6	8.7	81
15.6	25.8	2.8	91
0	12.7	5.3	

TIM-1 Spike Level (ng/mL)	Conc. (ng/mL)	Conc. %CV	% Recovery
6.25	5.913	0.7	94
1.56	1.584	2.0	99
0.39	0.430	2.4	102
0.098	0.131	7.8	102
0.024	0.058	0.4	105
0	0.031	5.0	

Osteopontin Spike Level (ng/mL)	Conc. (ng/mL)	Conc. %CV	% Recovery
4.88	4.6	3.4	68
1.2	2.3	2.8	72
0	1.9	0.8	

XIII Linearity
linearity

Urine samples were tested at 5, 10, and 20-fold dilution to measure linearity.
The concentrations shown below have been corrected for dilution (concentration = measured x dilution factor). Percent recovery is calculated as the measured concentration divided by the concentration for the previous dilution (expected).
% Recovery = (measured x dilution factor) / expected x 100

Sample Dilution Factor	Albumin (pooled control urine)			TIM-1 (urine from treated rat)		
	Conc. (ng/mL)	Conc. %CV	% Recovery	Conc. (ng/mL)	Conc. %CV	% Recovery
5	25957	23.3		0.74	2.5	
10	30928	4.1	119	0.74	17.9	100
20	30482	32.4	99	0.86	17.6	116

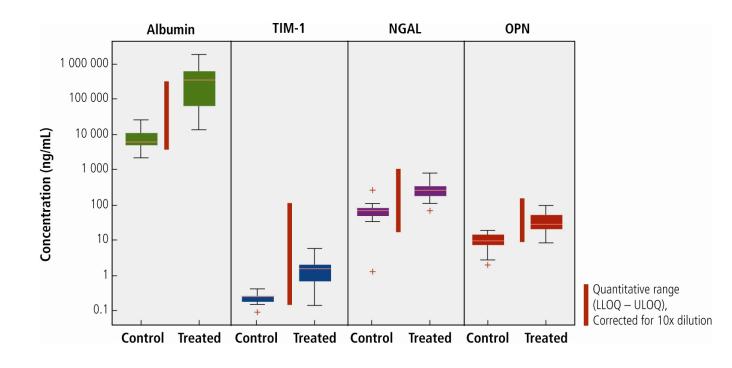| Sample Dilution Factor	Lipocalin-2 (urine from treated rat)			Osteopontin (pooled control urine)		
	Conc. (ng/mL)	Conc. %CV	% Recovery	Conc. (ng/mL)	Conc. %CV	% Recovery
5	73.7	12.7		20.2	8.5	
10	94.9	0.9	129	41.5	3.0	206
20	126	2.2	133	67.9	2.6	163

XIV Samples
samples

Rat urine samples were assayed at 10-fold dilution on the Kidney Injury Panel 1. The "treated" samples tested were from rats exposed to known nephrotoxicants prior to sample collection. *High Tox* designated samples that assayed above the ULOQ were assayed again at 40-fold dilution. For all of the analytes on the panel, significant correspondence between histopathology score and abundance is observed. Concentrations in gray were below the LLOQ for the analyte designated.

Animal #	Designation	Histopathology Score	Albumin (ng/mL)	TIM-1 (ng/mL)	Lipocalin-2 (ng/mL)	Osteopontin (ng/mL)
1	Control	0	6380	0.26	79.4	2.0
2	Control	0	4676	0.22	81.8	10.6
3	Control	0	5639	0.15	54.0	6.8
4	Control	0	2172	0.09	50.9	5.7
5	Control	0	7981	0.21	80.2	9.5
6	Control	0	13063	0.27	32.8	10.4
7	Control	0	4099	0.15	68.2	9.3
8	Control	0	25787	0.24	43.6	8.7
9	Control	0	6010	0.25	68.6	17.7
10	Control	0	10219	0.16	48.1	14.7
11	Mild Tox	1	24359	0.14	68.9	8.5
12	Mild Tox	1	582857	1.66	216	20.1
13	Mild Tox	1	347477	2.43	185	18.7
14	Mild Tox	2	63056	1.99	185	25.8
15	Mild Tox	2	190213	0.84	110	18.1
16	Mild Tox	3	593019	1.34	184	28.1
17	Mild Tox	2	409532	1.56	264	47.6
18	Mild Tox	2	1375229	1.92	242	59.7
19	Mild Tox	2	438888	2.00	325	53.4

Animal #	Designation	Histopathology Score	Albumin (ng/mL)	TIM-1 (ng/mL)	Lipocalin-2 (ng/mL)	Osteopontin (ng/mL)
20	High Tox	3	1398916	1.91	334	32.5
21	High Tox	3	814377	0.64	345	41.4
22	High Tox	3	1822741	1.29	308	39.5
23	High Tox	3	141078	0.37	134	17.0
24	High Tox	3	122467	0.39	144	24.0
25	High Tox	4	352434	0.45	297	24.7
26	High Tox	3	20037	3.38	689	40.8
27	High Tox	3	13009	1.18	309	75.7
28	High Tox	3	25402	5.11	493	27.8
29	High Tox	3	71925	5.76	800	67.7
30	High Tox	3	42825	4.00	665	96.0
31	Control	0	10563	0.26	261	2.7
32	Control	0	4724	0.25	51.8	12.8
33	Control	0	26265	0.18	109	7.1
34	Control	0	5412	0.27	73.2	17.4
35	Control	0	3501	0.19	96.5	8.3
36	Control	0	5178	0.25	1.3	7.9
37	Control	0	5138	0.34	56.7	12.5
38	Control	0	10322	0.41	70.1	19.0
39	Control	0	12564	0.26	41.9	15.9

XV Calibrators

calibrators

Recombinant rat lipocalin-2 (residues 21–198) with an N-terminal signal peptide and a C-terminal 6xHis tag was expressed in murine myeloma cells.

Recombinant rat osteopontin (full-length) with an N-terminal 6xHis tag was expressed in *E.coli*.

Recombinant rat TIM-1 (residues 18–238) was expressed in murine myeloma cells.

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

These analytes were calibrated against internal controls, diluted, and pooled to make the Kidney Injury Panel 1 (rat) Calibrator Blend.

XVI References

references

1. Mishra J., Ma, Q., Prada, A., Mitsnefes, M., Zahedi, K., Yang, J., Barasch, J., Devarajan, P. (2003) *Identification of Neutrophil Gelatinase-Associated Lipocalin as a Novel Early Urinary Biomarker for Ischemic Renal Injury*. J Am Soc Nephrol. 14:2534–2543

2. Dieterle F, Marrer E, Suzuki E, Grenet O, Cordier A, Vonderscher J. (2008) *Monitoring kidney safety in drug development: emerging technologies and their implications.* Curr. Opin. Drug Discov. Devel. 11(1): 60-71

Summary Protocol

MSD 96-well MULTI-SPOT Kidney Injury Panel 1 (rat) Assay Kit

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

Step 1 : Sample and Reagent Preparation

Bring appropriate diluents and plates to room temperature

Urine samples should be diluted in Diluent 5. (10X is recommended)

Prepare Blocker A Solution according to the kit instructions.

Prepare Assay Diluent by diluting 200X Albumin Detection/Tracer in Diluent 5 to a final concentration of 1X.

Prepare an 8-point standard curve using supplied calibrators:

■ The Calibrator Blend should be diluted in Diluent 5.

■ Dilute the stock Calibrator Blend 1:20 in Diluent 5, then perform a series of 3-fold dilution steps and a no calibrator blank.

Prepare a mix of each Calibrator or diluted sample solution with 1X Albumin Tracer by adding equal amounts of sample and tracer solutions.

Prepare a Detection Antibodiy Solution by diluting the supplied Detection Antibodies stock to 1X concentration of each antibody in Diluent 5 (per plate). Each Detection Antibody is supplied as 50X stock solution.

Prepare 20 mL of 1X Read Buffer T by diluting 4X MSD Read Buffer T (with surfactant) with deionized water.

Step 2 : Add Blocker A Solution

Dispense 150 µL/well Blocker A Solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 3 : Wash and Add Sample or Calibrator Premix Solutions

Wash plate 3X with PBS-T.

Dispense 50 µL/well Calibrator or Sample premix solutions.

Incubate at room temperature with vigorous shaking (300-1000 rpm) for 2 hours.

Step 4 : Wash and Add Detection Antibody Solution

Wash plate 3X with PBS-T.

Dispense 25 µL/well 1X Detection Antibody Solution.

Incubate at room temperature with vigorous shaking (300-1000 rpm) for 2 hours.

Step 5 : Wash and Read Plate

Wash plate 3X with PBS-T.

Dispense 150 µL /well 1X Read Buffer T.

Analyze plate on SECTOR instrument.