

MSD[®] MULTI-SPOT Assay System

Inflammation Panel 1 (rat) Kit

1-Plate Kit
5-Plate Kit
25-Plate Kit

K15179C-1
K15179C-2
K15179C-4



MSD Toxicology Assays

Inflammation Panel 1 (rat) Kit

Lipocalin-2, TSP-1, TIMP-1, MCP-1

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

MESO SCALE DISCOVERY®

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Table of Contents

Introduction	4
Principle of the Assay	5
Reagents Supplied	6
Required Material and Equipment (not supplied)	6
Safety	7
Reagent Preparation.....	7
Assay Protocol.....	9
Analysis of Results	9
Assay Validation and Verification	10
Typical Data.....	11
Sensitivity.....	12
Precision	12
Spike Recovery.....	13
Dilution Linearity	15
Specificity	17
Samples	17
Assay Components	18
References.....	18
Summary Protocol.....	19
Plate Diagrams	21

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Introduction

The Inflammation Panel 1 (rat) Kit is designed to measure four important biomarkers of inflammation:

Lipocalin-2, also known as neutrophil gelatinase-associated lipocalin (NGAL), is a small extracellular protein that binds to a range of small hydrophobic molecules and soluble macromolecules.¹ Lipocalin-2 is induced in epithelial cells, renal tubular cells, and hepatocytes upon inflammation or injury.² In urine, Lipocalin-2 may be implicated in the progression of renal injury and protection from damage, especially to the proximal tubule.³ High concentrations of Lipocalin-2 in serum can indicate an inflammatory response.²

Thrombospondin 1 (TSP-1) is a key modulator of interactions between cells and the extracellular matrix.⁴ Platelet-bound TSP-1 influences macrophages, fibroblasts, and endothelial cells to participate in wound healing at sites of injury and bleeding.⁵

Tissue Inhibitor of Metalloproteinases I (TIMP-1) is an endogenous inhibitor of matrix metalloproteinases (MMPs).⁶ TIMPs have been implicated in direct regulation of cell growth and apoptosis,⁷ and TIMP-1 plays a role in pathologic processes associated with rheumatoid arthritis⁸ and cardiovascular diseases.⁹ TIMP-1 can bind to both activated and latent forms of MMP-9.¹⁰

Monocyte Chemoattractant Protein-1 (MCP-1) is the principal monocyte-selective chemotactic cytokine.¹¹ MCP-1 expression is linked to diseases such as atherosclerosis, multiple sclerosis, and rheumatoid arthritis, and it has been suggested that MCP-1 attracts specific leukocytes to sites of inflammation.¹²

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 Inflammation Panel 1 (rat) is a multiplex sandwich immunoassay (Figure 1). This panel has been qualified according to the principles outlined in “Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement” by Lee, J.W. et al.¹³ MSD provides a plate pre-coated with capture antibodies. The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) over the course of 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. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into a SECTOR® Imager where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures intensity of emitted light to provide a quantitative measure of analytes in the sample.

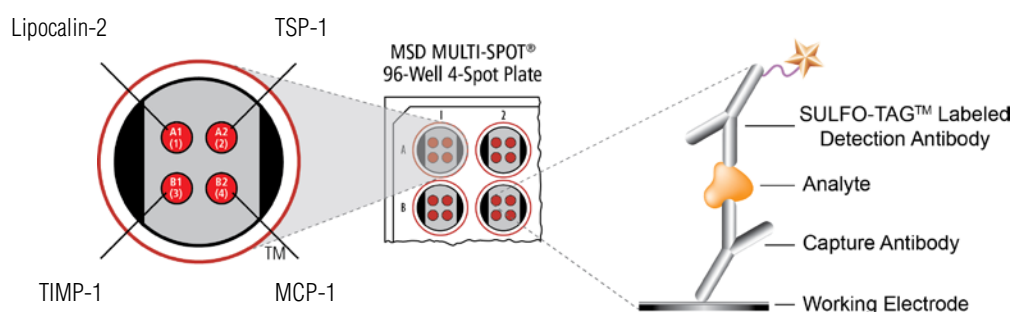


Figure 1. Spot diagram showing placement of analyte capture antibody. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing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

Reagents Supplied

Product Description	Storage	Quantity per Kit		
		K15179C-1	K15179C-2	K15179C-4
MULTI-SPOT 96-Well 4-Spot Inflammation Panel 1 (rat) Plate N45179A-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-rat Lipocalin-2 Antibody ¹ (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
SULFO-TAG Anti-rat TSP-1 Antibody ¹ (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
SULFO-TAG Anti-rat TIMP-1 Antibody ¹ (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
SULFO-TAG Anti-rat MCP-1 Antibody ¹ (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Inflammation Panel 1 (rat) Calibrator Blend (20X)	≤-70°C	1 vial (20 µL)	5 vials (20 µL ea)	25 vials (20 µL ea)
Diluent 7 R54BB-4 (5 mL), R54BB-3 (50 mL)	≤-10°C	1 bottle (5 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)
Diluent 100 R50AA-4 (50 mL), R50AA-2 (200 mL)	2–8°C	1 bottle (50 mL)	2 bottles (200 mL ea)	10 bottles (200 mL ea)
Read Buffer T (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

¹ SULFO-TAG conjugated detection antibodies should be stored in the dark.

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.

Reagent Preparation

Bring all reagents to room temperature. This is especially important for the Diluent 7, as some components are not soluble below room temperature. Thaw the stock calibrator on ice.

Important: Upon first thaw, separate Diluent 7 into aliquots appropriate for the size of your assay needs before refreezing. This diluent can go through up to three freeze-thaw cycles without significantly affecting the performance of the assay.

Prepare Standards

MSD supplies a blended calibrator for the Inflammation Panel 1 (rat) Kit at 20-fold higher concentration than the recommended highest standard. We recommend an 8-point standard curve with 3-fold serial dilution steps and a zero calibrator.

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 the calibrator, refer to the certificate of analysis (C of A) supplied with the kit. You may also find a copy of the lot-specific C of A at www.mesoscale.com by entering your kit's catalog number in the search box.

To prepare an 8-point standard curve for up to 4 replicates:

- 1) Prepare the highest standard by adding 10 μL of calibrator stock to 190 μL of Diluent 100. Mix well.
- 2) Prepare the next standard by transferring 80 μL of the highest standard to 160 μL of Diluent 100. Mix well. Repeat 3-fold serial dilutions 5 additional times to generate 7 standards.
- 3) Use Diluent 100 as the 8th standard (i.e. zero calibrator).

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

Dilute Samples

For rat serum and plasma samples, MSD recommends a 100-fold dilution in Diluent 100; however, you may adjust dilution factors for the sample set under investigation. Samples collected via cardiac puncture may require more than 100-fold dilution.

To dilute sample 100-fold, add 10 μL sample to 990 μL of Diluent 100.

Do not re-freeze unused serum samples.

Prepare Detection Antibody Solution

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

For one plate, combine:

- 60 μ L of 50X SULFO-TAG Anti-rat Lipocalin-2 Antibody
- 60 μ L of 50X SULFO-TAG Anti-rat TSP-1 Antibody
- 60 μ L of 50X SULFO-TAG Anti-rat TIMP-1 Antibody
- 60 μ L of 50X SULFO-TAG Anti-rat MCP-1 Antibody
- 2.76 mL of Diluent 100

Note: If you omit detection antibody for an analyte not being measured, add 60 μ L of Diluent 100 for each omitted antibody.

Prepare Read Buffer

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

For one plate, combine:

- 5 mL Read Buffer T (4X)
- 15 mL 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 pre-coated 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., pre-wetting) is required.

Assay Protocol

(Samples/calibrators should be diluted prior to step 1)

1. **Add Diluent 7:** Add 25 μL of Diluent 7 to each well. Seal the plate with an adhesive plate seal, and incubate for 30 minutes with vigorous shaking (300–1000 rpm) at room temperature.
2. **Add Sample or Calibrator:** Add 25 μL of calibrator or diluted sample per well. Seal the plate with an adhesive plate seal, and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
You may prepare detection antibody solution during incubation.
3. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with 300 μL /well of PBS-T. Add 25 μL of 1X detection antibody solution to each well. Seal the plate with an adhesive plate seal, and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
You may prepare diluted read buffer during incubation.
4. **Wash and Read:** Wash the plate 3 times with 300 μL /well of PBS-T. Add 150 μL of 1X Read Buffer T to each well. Analyze the plate on the SECTOR Imager. No incubation in read buffer is required before reading the plate.

Notes

Shaking the plate typically accelerates capture at the working electrode.

You may keep excess diluted read buffer in a tightly sealed container at room temperature for later use.

Bubbles introduced when adding read buffer will interfere with imaging of the plate and produce unreliable data. Use reverse pipetting technique to avoid creating bubbles.

Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

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 analyte 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 DISCOVERY WORKBENCH[®] 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.

Assay Validation and Verification

The performance of this kit meets levels of consistency and robustness outlined in “Fit -for-Purpose Method Development and Validation for Successful Biomarker Measurement” by Lee, J.W. et al.¹³

Bioanalytical and functional characterizations of calibrators, antibodies and assay components are completed to allow for bridging of reagents between lots. This includes plate coating uniformity and reagent and component specificity testing for individual kit lots. Control samples for specific matrices are designed and tested to meet the accuracy, precision and sensitivity criteria for a kit that has completed the validation process. Spike recovery and dilution linearity of endogenous samples, pooled and individual matrices, are tested across the assay range.

➤ Sensitivity, Range, and Curve Fitting

- Sample range and assay sensitivity are established from 4-PL fitted calibration curves with $1/Y^2$ weighting. Percent recovery of calibrators and controls between the upper limit of quantification (ULOQ) and lower limit of quantification (LLOQ) must have calculated concentration %CV of less than 20% and accuracy within 20% of the expected concentration.
- The limits of quantification defined in the product insert are verified for each lot as part of the lot verification and quality control release.

➤ Accuracy and Precision

High, mid, and low controls made in matrix (defined on a kit-by-kit basis) are run to measure accuracy and precision.

- Validation – The assay is tested over multiple days (>6 days) and multiple runs per day for a total of 15-20 runs of complete kits. Precision is measured for the standard curve for intra- and inter-day coefficients of variance (CVs) of less than 20%. The typical specification includes a calculated concentration CV of less than 20%, accuracy within 20% of expected concentration, and a total error of less than 30%. The kit specifications for this lot are provided in the enclosed Certificate of Analysis (C of A).
- Verification – A multi-day (2-3 days) analysis with multiple runs per day of 6-12 total plates is performed as part of the release testing for each lot. The specifications for release are provided in the C of A.

➤ Robustness and Stability

Freeze-thaw testing and accelerated stability studies performed during assay development (calibrators, antibodies, controls) are augmented with real-time stability studies on complete kits out to 18 months from the date of manufacture.

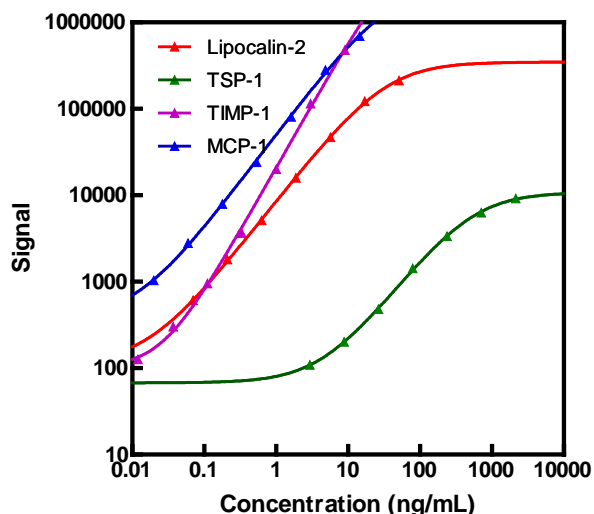
All acceptance criteria and verification conformance are defined in the C of A for all kit lots. Presented below are representative data from the assay validation for this assay that meets the criteria described above. The kit lot-specific standard curve and measured limits of quantification can be found in the C of A enclosed with the kit.

Typical Data

The following standard curves illustrate the dynamic range of the assay. The actual signals may vary. Run a standard curve on each plate for the best quantification of unknown samples.

Some variation in the concentration of the highest calibrator is permissible between kit lots. The table below details the acceptable range of the highest calibrator concentration. For each kit lot, refer to the C of A for the actual concentration of the calibrator.

	Highest Calibrator Concentration	
	Target	Acceptable range
Lipocalin-2 (ng/mL)	54.0	45.9–62.1
TSP-1 (ng/mL)	2463	2093–2832
TIMP-1 (ng/mL)	9.36	7.96–10.8
MCP-1 (ng/mL)	15.0	12.8–17.3



Lipocalin-2		
Conc. (ng/mL)	Average Signal	%CV
0	40	16.2
0.0700	608	8.3
0.210	1804	7.6
0.630	5129	6.2
1.89	15 934	5.5
5.67	46 911	4.3
17.0	121 978	3.3
51.0	211 776	4.5

TSP-1		
Conc. (ng/mL)	Average Signal	%CV
0	67	11.1
2.94	109	6.4
8.81	201	8.2
26.4	480	7.1
79.3	1423	5.4
238	3352	4.1
713	6283	4.9
2140	9150	6.4

TIMP-1		
Conc. (ng/mL)	Average Signal	%CV
0	41	10.5
0.0123	126	8.9
0.0370	302	11.7
0.111	951	11.6
0.333	3659	6.9
1.00	20 061	7.1
3.00	114 424	6.2
9.00	477 118	5.3

MCP-1		
Conc. (ng/mL)	Average Signal	%CV
0	216	5.3
0.0199	1035	7.0
0.0597	2799	9.1
0.179	7928	7.0
0.537	24 125	7.5
1.61	80 489	6.8
4.83	280 734	4.6
14.5	693 324	3.7

Sensitivity

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

A multi-plate, multi-day study was performed to measure the reproducibility of the assay. The lower limit of quantification (LLOQ) and upper limit of quantification (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 25% and the percent recovery of the standard is between 75% and 125%.

	Lipocalin-2 (ng/mL)	TSP-1 (ng/mL)	TIMP-1 (ng/mL)	MCP-1 (ng/mL)
LLOD	0.00234	1.42	0.00300	0.000840
LLOQ	0.0330	29.7	0.0567	0.0447
ULOQ	27.6	766	6.71	11.0

Precision

Rat serum-based controls (high and mid controls) and diluent-based control (low control) were measured in quadruplicate on 16 runs over 5 days.

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

Inter-plate %CV is the variability of controls across 16 runs over 5 days.

Inter-lot %CV is the variability of controls across 2 kit lots.

	Control	Runs	Average Conc. (ng/mL)	Average Intra-plate %CV	Inter-plate %CV	Inter-lot %CV
Lipocalin-2	High	16	8.81	4.6	6.4	4.6
	Mid	16	1.10	3.5	6.1	4.5
	Low	16	0.253	3.5	8.3	5.9
TSP-1	High	16	1118	10.9	14.5	7.1
	Mid	16	707	6.6	9.8	8.7
	Low	16	70.0	5.7	8.6	3.7
TIMP-1	High	16	3.84	3.9	5.2	5.2
	Mid	16	0.518	3.5	8.2	4.6
	Low	16	0.242	3.8	8.3	4.6
MCP-1	High	16	8.64	4.6	5.1	6.1
	Mid	16	0.806	5.9	7.5	9.4
	Low	16	0.0956	6.5	9.4	7.8

Spike Recovery

Normal rat serum, EDTA plasma, and heparin plasma were diluted 100-fold then spiked with calibrators at multiple levels throughout the range of the assay. Values in italics were below the assay LLOQ.

% Recovery=measured/expected*100

Sample	Lipocalin-2				TSP-1			
	Spike Conc. (ng/mL)	Measured Conc. (ng/mL)	Measured Conc. %CV	% Recovery	Spike Conc. (ng/mL)	Measured Conc. (ng/mL)	Measured Conc. %CV	% Recovery
Serum 1	0	1.22	4.4		0	129	8.9	
	1.18	2.33	6.9	97	54.4	173	7.4	94
	3.53	4.65	8.4	98	163	264	1.5	90
	10.6	10.9	3.3	92	490	496	3.0	80
Serum 2	0	0.670	1.3		0	139	3.5	
	1.18	1.79	4.8	97	54.4	183	2.5	95
	3.53	3.95	0.6	94	163	283	4.6	94
	10.6	10.2	5.6	91	490	496	8.2	79
Serum 3	0	0.638	6.1		0	147	3.5	
	1.18	1.65	3.8	91	54.4	192	3.5	95
	3.53	3.84	4.3	92	163	281	3.7	91
	10.6	10.8	2.6	96	490	510	6.0	80
EDTA Plasma 1	0	1.44	2.8		0	<i>27.7</i>	2.7	
	1.18	2.34	2.3	89	54.4	72.2	1.9	88
	3.53	4.42	5.7	89	163	168	7.6	88
	10.6	10.2	6.3	85	490	350	10.8	68
EDTA Plasma 2	0	1.03	1.1		0	<i>21.1</i>	7.2	
	1.18	2.09	8.0	95	54.4	79.0	14.3	105
	3.53	4.07	3.4	89	163	185	9.6	100
	10.6	10.2	10.7	88	490	459	6.2	90
EDTA Plasma 3	0	0.518	4.7		0	33.0	14.4	
	1.18	1.62	7.1	95	54.4	90.4	7.7	103
	3.53	3.61	2.3	89	163	186	5.9	94
	10.6	9.58	6.1	86	490	397	8.7	76
Heparin Plasma 1	0	1.54	2.4		0	31.9	3.5	
	1.18	2.61	4.3	96	54.4	79.4	3.7	92
	3.53	4.78	4.1	94	163	179	2.5	91
	10.6	10.9	6.1	90	490	407	1.9	78
Heparin Plasma 2	0	0.833	5.2		0	46.5	2.8	
	1.18	1.94	0.3	97	54.4	103	5.6	102
	3.53	4.24	3.3	97	163	202	4.8	96
	10.6	10.8	1.8	95	490	481	7.5	90
Heparin Plasma 3	0	0.841	5.0		0	38.9	4.1	
	1.18	1.98	6.4	98	54.4	87.5	9.5	94
	3.53	4.02	5.2	92	163	182	6.1	90
	10.6	10.6	8.3	93	490	407	5.7	77

Sample	TIMP-1				MCP-1			
	Spike Conc. (ng/mL)	Measured Conc. (ng/mL)	Measured Conc. %CV	% Recovery	Spike Conc. (ng/mL)	Measured Conc. (ng/mL)	Measured Conc. %CV	% Recovery
Serum 1	0	0.0910	8.3		0	0.0550	0.6	
	0.186	0.270	3.2	98	0.307	0.319	4.5	88
	0.559	0.620	4.4	95	0.920	0.931	12.0	95
	1.68	1.77	3.0	100	2.76	2.76	4.6	98
Serum 2	0	0.0790	12.8		0	0.0710	2.6	
	0.186	0.264	5.0	99	0.307	0.386	7.8	102
	0.559	0.624	2.1	98	0.920	0.902	1.7	91
	1.68	1.83	4.2	104	2.76	2.79	6.0	99
Serum 3	0	0.0940	3.1		0	0.0950	3.4	
	0.186	0.269	3.9	96	0.307	0.376	0.8	94
	0.559	0.599	3.8	92	0.920	0.967	9.1	95
	1.68	1.82	6.4	103	2.76	2.98	5.7	105
EDTA Plasma 1	0	0.0790	2.5		0	0.0550	15.7	
	0.186	0.214	8.7	81	0.307	0.321	11.1	89
	0.559	0.570	12.9	89	0.920	0.921	4.4	94
	1.68	1.58	9.9	90	2.76	2.63	2.6	93
EDTA Plasma 2	0	0.101	7.0		0	0.0460	4.2	
	0.186	0.270	9.8	94	0.307	0.309	11.5	88
	0.559	0.614	7.2	93	0.920	0.819	4.5	85
	1.68	1.83	12.3	103	2.76	2.63	2.3	94
EDTA Plasma 3	0	0.0700	7.3		0	0.054	1.8	
	0.186	0.225	6.0	88	0.307	0.325	12.5	90
	0.559	0.566	8.1	90	0.920	0.876	6.7	90
	1.68	1.71	8.2	98	2.76	2.82	4.9	100
Heparin Plasma 1	0	0.0540	5.5		0	0.0450	8.3	
	0.186	0.216	6.8	90	0.307	0.307	2.1	87
	0.559	0.519	6.9	85	0.920	0.894	13.7	93
	1.68	1.75	7.7	101	2.76	2.55	4.3	91
Heparin Plasma 2	0	0.0510	9.6		0	0.0570	4.7	
	0.186	0.220	6.5	92	0.307	0.343	3.0	94
	0.559	0.539	8.6	88	0.920	0.934	5.5	96
	1.68	1.70	10.5	98	2.76	2.94	1.0	104
Heparin Plasma 3	0	0.0610	3.1		0	0.0410	5.5	
	0.186	0.223	9.6	90	0.307	0.327	10.3	94
	0.559	0.544	11.0	88	0.920	0.772	5.1	80
	1.68	1.71	7.8	99	2.76	2.46	1.8	88

Dilution Linearity

To assess linearity, normal rat serum, EDTA plasma, and heparin plasma samples were diluted 50-fold, 100-fold, 200-fold, and 400-fold. 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 measured from the previous dilution (expected).

$$\% \text{ Recovery} = \frac{\text{measured} \times \text{dilution factor}}{\text{expected}} \times 100$$

Sample	Fold Dilution	Lipocalin-2			TSP-1		
		Conc. (ng/mL)	Conc. %CV	% Recovery	Conc. (ng/mL)	Conc. %CV	% Recovery
Serum 4	50	229	4.3		17 636	2.8	
	100	264	4.0	115	20 665	2.6	117
	200	247	2.8	93	21 758	8.7	105
	400	258	2.2	105	23 791	8.5	109
Serum 5	50	611	3.2		14 201	7.3	
	100	652	3.6	107	17 991	8.6	127
	200	615	5.3	94	17 685	9.3	98
	400	609	1.6	99	20 327	6.6	115
Serum 6	50	79.2	1.4		20 285	3.4	
	100	83.7	4.4	106	25 683	7.5	127
	200	81.1	0.9	97	28 047	2.4	109
	400	87.5	5.7	108	29 018	2.0	103
EDTA Plasma 4	50	183	2.9		5568	4.0	
	100	189	5.3	103	6428	1.9	115
	200	188	3.0	99	7270	4.4	113
	400	189	0.8	101	7023	8.7	97
EDTA Plasma 5	50	158	9.0		3031	2.5	
	100	173	5.0	110	3640	8.1	120
	200	172	6.4	99	3394	4.4	93
	400	171	6.1	99	3362	5.2	99
EDTA Plasma 6	50	107	3.9		5135	2.5	
	100	112	4.3	105	6032	6.1	117
	200	110	3.3	98	6309	7.9	105
	400	110	1.5	100	5895	8.0	93
Heparin Plasma 4	50	218	1.4		2344	10.0	
	100	210	4.1	96	2548	5.6	109
	200	199	3.2	95	2272	1.5	89
	400	186	2.4	93	2091	11.8	92
Heparin Plasma 5	50	165	0.9		5704	2.9	
	100	177	1.9	107	6890	4.0	121
	200	178	2.9	100	6761	2.2	98
	400	171	1.1	96	6452	10.8	95
Heparin Plasma 6	50	8.68	1.4		1594	3.4	
	100	8.86	3.5	102	1808	3.9	113
	200	9.24	1.9	104	1443	6.9	80
	400	9.13	9.6	99	1414	25.3	98

Sample	Fold Dilution	TIMP-1			MCP-1		
		Conc. (ng/mL)	Conc. %CV	% Recovery	Conc. (ng/mL)	Conc. %CV	% Recovery
Serum 4	50	11.7	0.4		13.6	9.0	
	100	13.1	2.8	112	15.2	8.5	112
	200	13.5	5.4	103	14.1	2.4	93
	400	15.3	4.4	113	16.9	6.5	120
Serum 5	50	17.0	4.0		13.0	1.3	
	100	18.2	5.0	107	15.3	3.6	118
	200	18.2	2.7	100	14.1	4.0	92
	400	20.0	3.2	110	15.1	0.9	107
Serum 6	50	10.6	3.0		12.5	9.8	
	100	11.8	6.9	112	13.2	5.0	105
	200	13.5	2.1	115	13.7	7.5	104
	400	15.0	4.6	111	16.0	3.6	117
EDTA Plasma 4	50	9.76	6.1		14.1	2.2	
	100	10.2	8.1	104	12.8	8.3	91
	200	12.0	4.2	118	13.8	6.7	108
	400	12.8	6.8	107	14.1	2.2	103
EDTA Plasma 5	50	11.1	6.3		5.75	5.7	
	100	12.5	5.8	113	6.13	6.0	107
	200	13.4	9.4	107	6.42	5.4	105
	400	15.0	8.6	111	6.71	3.2	105
EDTA Plasma 6	50	9.52	2.4		13.7	1.4	
	100	10.5	3.8	111	14.6	1.4	107
	200	11.1	4.1	106	16.0	5.0	109
	400	12.8	2.5	115	16.8	3.8	105
Heparin Plasma 4	50	9.39	2.3		9.80	9.0	
	100	9.64	10.1	103	9.15	5.0	93
	200	10.6	7.7	109	9.48	10.7	104
	400	10.2	9.8	97	9.01	3.1	95
Heparin Plasma 5	50	6.68	4.7		4.98	4.2	
	100	7.40	5.1	111	5.46	1.3	110
	200	8.18	1.8	111	5.68	7.1	104
	400	8.78	6.4	107	5.91	3.3	104
Heparin Plasma 6	50	8.59	9.5		5.96	12.3	
	100	9.43	1.0	110	5.89	8.4	99
	200	10.3	2.8	110	6.08	4.5	103
	400	11.6	1.6	113	6.68	8.0	110

Specificity

To assess specificity of the detection antibodies, the Inflammation Panel 1 (rat) was run using blended calibrators (17.0 ng/mL Lipocalin-2; 713 ng/mL TSP-1; 3.00 ng/mL TIMP-1; 4.83 ng/mL MCP-1) with individual detection antibodies. The table below shows the % cross-reactivity for the individual detection antibodies.

Spot	Blended Calibrator and Single Detection Antibody % Cross-Reactivity			
	Lipocalin-2	TSP-1	TIMP-1	MCP-1
Lipocalin-2	100	0.4	< 0.1	< 0.1
TSP-1	0.2	100	0.2	< 0.1
TIMP-1	< 0.1	0.2	100	< 0.1
MCP-1	< 0.1	0.9	< 0.1	100

Samples

Serum, EDTA plasma, and heparin plasma samples were collected from normal Sprague-Dawley rats, diluted 100-fold, and tested with the Inflammation Panel 1 (rat). Median and range of concentrations for each sample set are displayed below. Concentrations are corrected for sample dilution.

Sample	Statistic	Lipocalin-2	TSP-1	TIMP-1	MCP-1
Serum	Median (ng/mL)	140	15457	9.73	12.7
	Range (ng/mL)	66.3–2681	11 540–22 288	7.19–17.5	6.39–30.5
	N	12	12	12	12
EDTA Plasma	Median (ng/mL)	129	3330	9.06	6.22
	Range (ng/mL)	5.40–245	1712–6225	7.11–13.4	3.14–13.9
	N	13	13	13	13
Heparin Plasma	Median (ng/mL)	132	5011	6.53	5.31
	Range (ng/mL)	4.30–235	1842–8273	4.70–10.6	3.65–10.6
	N	14	14	14	14

Assay Components

Calibrator

The assay calibrator blend uses the following recombinant proteins:

- Rat Lipocalin-2 expressed in mouse cells
- Rat TIMP-1/MCP-1 expressed in mouse cells
- Rat MCP-1 expressed in mouse cells
- Rat TSP-1 expressed in insect cells using a baculovirus expression system

Antibodies

Analyte	Source Species	
	MSD Capture Antibody	MSD Detection Antibody
Lipocalin-2	Mouse Monoclonal	Goat Polyclonal
TSP-1	Mouse Monoclonal	Mouse Monoclonal
TIMP-1	Mouse Monoclonal	Goat Polyclonal
MCP-1	Goat Polyclonal	Goat Polyclonal

References

1. Lee J-H, Kye KC, Seo E-Y, Lee K, Lee S-K, Lim J-S, Seo Y-J, Kim CD, Park J-K. Expression of Neutrophil Gelatinase-Associated Lipocalin in Calcium-Induced Keratinocyte Differentiation. *J Korean Med Sci.* 2008 Apr; 23(2):302-6
2. De-xiu Bu, Anne-Louise Hemdahl, Anders Gabrielsen, Jonas Fuxe, Chaoyong Zhu, Per Eriksson, Zhong-qun Yan. Induction of Neutrophil Gelatinase-Associated Lipocalin in Vascular Injury via Activation of Nuclear Factor- κ B. *Am J Pathol.* 2006 Dec;169(6):2245-53
3. Mishra J, Ma Q, Prada A, Mitsnefes M, Zahedi K, Yang J, Barasch J, Devarajan P. Identification of Neutrophil Gelatinase-Associated Lipocalin as a Novel Early Urinary Biomarker for Ischemic Renal Injury. *J Am Soc Nephrol.* 2003 Oct;14(10):2534-43.
4. Bornstein, P. Diversity of Function Is Inherent in Matricellular Proteins: An Appraisal of Thrombospondin 1. *J Cell Biol.* 1995 Aug; 130(3):503-6.
5. Bornstein, P. Thrombospondins as matricellular modulators of cell function. *J Clin Invest.* 2001 Apr;107(8):929-34.
6. Chirco R, Liu X-W, Jung K-K, Kim H-RC. Novel functions of TIMPs in cell signaling. *Cancer Met Rev.* 2006 Mar; 25(1):99-113
7. Stetler-Stevenson WG. Tissue inhibitors of metalloproteinases in cell signaling: metalloproteinase-independent biological activities. *Sci Signal.* 2008 Jul 8;1(27):rev6.Review.
8. Giannelli G, Erriquez R, Iannone F, Marinosci F, Lapadula G, Antonaci S. MMP-2, MMP-9, TIMP-1 and TIMP-2 levels in patients with rheumatoid arthritis and psoriatic arthritis. *Clin Exp Rheumatol.* 2004 May-Jun; 22(3):335-8
9. Sundström J, Evans JC, Benjamin EJ, Levy D, Larson MG, Sawyer DB, Siwik DA, Colucci WS, Wilson PWF, Vasan RS. Relations of plasma total TIMP-1 levels to cardiovascular risk factors and echocardiographic measures: the Framingham heart study. *Eur Heart J.* 2004 May; 25(17):1509-16.
10. Roderfeld M, Graf J, Giese B, Salguero-Palacios R, Tschuschner A, Müller-Newen G, Roeb E. Latent MMP-9 is bound to TIMP-1 before secretion. *Biol Chem.* 2007 Nov; 388(11):1227-34.
11. Gerdprasert O, O'Bryan MK, Nikolic-Paterson DJ, Sebire K, Kretser DM, Hedger MP. Expression of monocyte chemoattractant protein-1 and macrophage colony-stimulating factor in normal and inflamed rat testis. *Mol Hum Reprod.* 2002 Jun;8(6):518-524
12. Gu L, Rutledge B, Fiorillo J, Ernst C, Grewal I, Flavell R, Gladue R, Rollins B. In vivo properties of monocyte chemoattractant protein-1. *J Leuk Bio.* 1997 Nov;62(5):577-580
13. Lee JW, Devanarayan V, Barrett YC, Weiner R, Allinson J, Fountain S, Keller S, Weinryb I, Green M, Duan L, Rogers JA, Millham R, O'Brien PJ, Sailstad J, Khan M, Ray C, Wagner JA. Fit-for-purpose method development and validation for successful biomarker measurement. *Pharm Res.* 2006 Feb;23(2):312-28.

Summary Protocol

MSD 96-well MULTI-SPOT Inflammation Panel 1 (rat) Kit

*MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing
the Inflammation Panel 1 (rat) assays.*

Sample and Reagent Preparation

Bring all reagents to room temperature, and thaw the calibrator on ice.

Prepare an 8-point standard curve using the supplied calibrator:

- Dilute the stock calibrator blend 20-fold in Diluent 100.
- Perform a series of 3-fold dilution steps and prepare a zero calibrator blank.

Dilute samples 100-fold in Diluent 100 before adding to the plate.

Prepare combined detection antibody solution by diluting each stock detection antibody 50-fold in Diluent 100.

Prepare 1X Read Buffer T by diluting stock 4X Read Buffer T 4-fold with deionized water.

Step 1 : Add Diluent 7

Add 25 μ L/well of Diluent 7.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 30 minutes.

Step 2 : Add Sample or Calibrator

Add 25 μ L/well of calibrator or diluted sample.

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

Step 3 : Wash and Add Detection Antibody Solution

Wash plate 3 times with 300 μ L/well of PBS-T.

Add 25 μ L/well of 1X detection antibody solution.

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

Step 4 : Wash and Read Plate

Wash plate 3 times with 300 μ L/well of PBS-T.

Add 150 μ L/well of 1X Read Buffer T.

Analyze plate on SECTOR Imager.

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