# Inflammation Panel 1 (rat) Kit

| | |
|---|---|
| 1-Plate Kit | K15179C-1 |
| 5-Plate Kit | K15179C-2 |
| 25-Plate Kit | K15179C-4 |



MSD®

# 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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# Contact Information

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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 wtih rheumatoid arthritis[8] 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]

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# 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 an MSD instrument 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.

1. Lipocalin-2
2. TSP-1
3. TIMP-1
4. MCP-1
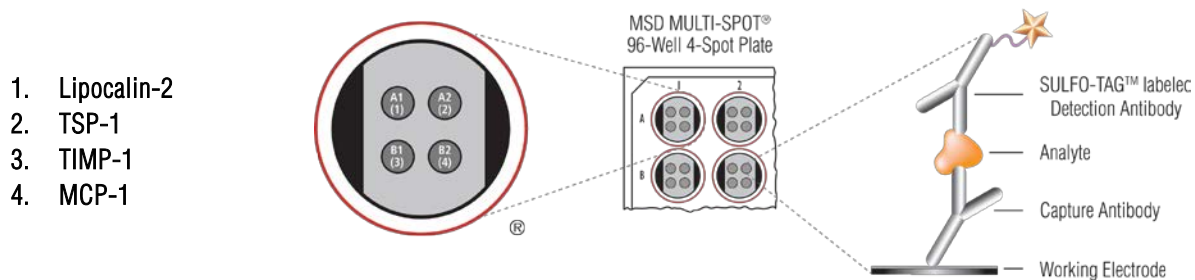


Figure 1. Spot diagram showing placement of analyte capture antibody. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

# 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<br>N45179A-1 | 2–8°C | 1 plate | 5 plates | 25 plates |
| SULFO-TAG Anti-rat Lipocalin-2 Antibody[1]<br>(50X) | 2–8°C | 1 vial<br>(75 µL) | 1 vial<br>(375 µL) | 5 vials<br>(375 µL ea) |
| SULFO-TAG Anti-rat TSP-1 Antibody[1]<br>(50X) | 2–8°C | 1 vial<br>(75 µL) | 1 vial<br>(375 µL) | 5 vials<br>(375 µL ea) |
| SULFO-TAG Anti-rat TIMP-1 Antibody[1]<br>(50X) | 2–8°C | 1 vial<br>(75 µL) | 1 vial<br>(375 µL) | 5 vials<br>(375 µL ea) |
| SULFO-TAG Anti-rat MCP-1 Antibody[1]<br>(50X) | 2–8°C | 1 vial<br>(75 µL) | 1 vial<br>(375 µL) | 5 vials<br>(375 µL ea) |
| Inflammation Panel 1 (rat) Calibrator Blend<br>(20X) | ≤-70°C | 1 vial<br>(20 µL) | 5 vials<br>(20 µL ea) | 25 vials<br>(20 µL ea) |
| Diluent 7<br>R54BB-4 (5 mL), R54BB-3 (50 mL) | ≤-10°C | 1 bottle<br>(5 mL) | 1 bottle<br>(50 mL) | 5 bottles<br>(50 mL ea) |
| Diluent 100<br>R50AA-4 (50 mL), R50AA-2 (200 mL) | 2–8°C | 1 bottle<br>(50 mL) | 2 bottles<br>(200 mL ea) | 10 bottles<br>(200 mL ea) |
| Read Buffer T (4X)<br>R92TC-3 (50 mL) | RT | 1 bottle<br>(50 mL) | 1 bottle<br>(50 mL) | 5 bottles<br>(50 mL ea) |

# Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene microcentrifuge tubes for preparing 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 suitable for dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate washing equipment: automated plate washer or multichannel pipette
- ❑ Microtiter plate shaker (rotary) capable of shaking at 500–1,000 rpm
- ❑ Adhesive plate seals
- ❑ Deionized water
- ❑ Vortex mixer

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

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# 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 as 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.
- Gently 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 prior to 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 inter-plate 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. The uncoated wells of a partially used plate may be sealed and stored up to 30 days at 2–8°C in the original foil pouch with desiccant. You may adjust volumes proportionally when preparing reagents.

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# 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 Calibrator Dilutions

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 (COA) supplied with the kit or available at www.mesoscale.com.

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) The recommended 8th standard is Diluent 100 (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.

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# 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 µL of 50X SULFO-TAG Anti-rat Lipocalin-2 Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-rat TSP-1 Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-rat TIMP-1 Antibody
- ❑ 60 µL 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 µL of Diluent 100 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

# 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.

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# Assay Protocol

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

### STEP 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 (500–1,000 rpm) at room temperature.

### STEP 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 (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 1X 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. No incubation in read buffer is required before reading the plate.

# 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.

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# 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
>
>> o 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.
>>
>> o 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.

>> o 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 COA.
>>
>> o 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 COA.

> ➤ 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 COA 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 COA enclosed with the kit.

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# 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.
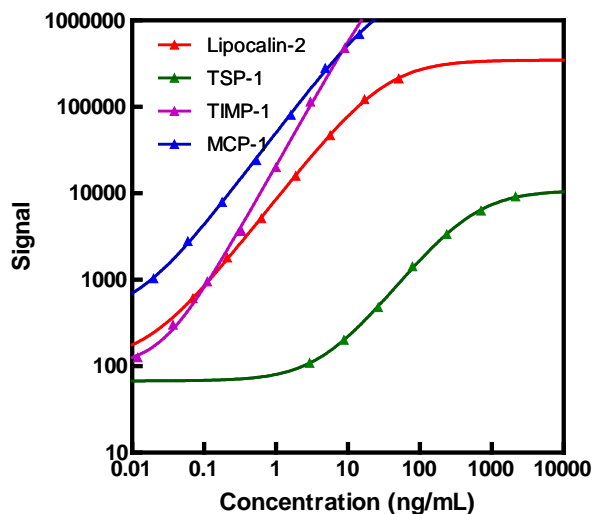


| Lipocalin-2 | | |
|---|---|---|
| Conc. (ng/mL) | Average Signal | %CV |
| 0 | 40 | 16.2 |
| 0.0700 | 608 | 8.3 |
| 0.210 | 1804 | 7.6 |
| 0.630 | 5129 | 6.2 |
| 1.89 | 15 934 | 5.5 |
| 5.67 | 46 911 | 4.3 |
| 17.0 | 121 978 | 3.3 |
| 51.0 | 211 776 | 4.5 |

| 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 |

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# 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 | *27.7* | *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 | *21.1* | *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 |

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| 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 |

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# 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).

% Recovery = % Recovery = (measured∗dilution factor)/expected∗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* |

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# 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 | 15 457 | 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 |

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# Assay Components

## Calibrators

The assay calibrator blend uses the following recombinant proteins:

Rat Lipocalin-2 expressed in mouse cells

Rat TIMP- 1 expressed in mouse cells

Rat MCP-1 expressed in mouse cells

Human TSP-1 expressed in mouse cells

## 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 |

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# 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:
  - o Dilute the stock calibrator blend 20-fold in Diluent 100.
  - o 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 µL/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 µL/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 µL/well of PBS-T.
- ❑ Add 25 µL/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 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.

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# Plate Diagrams