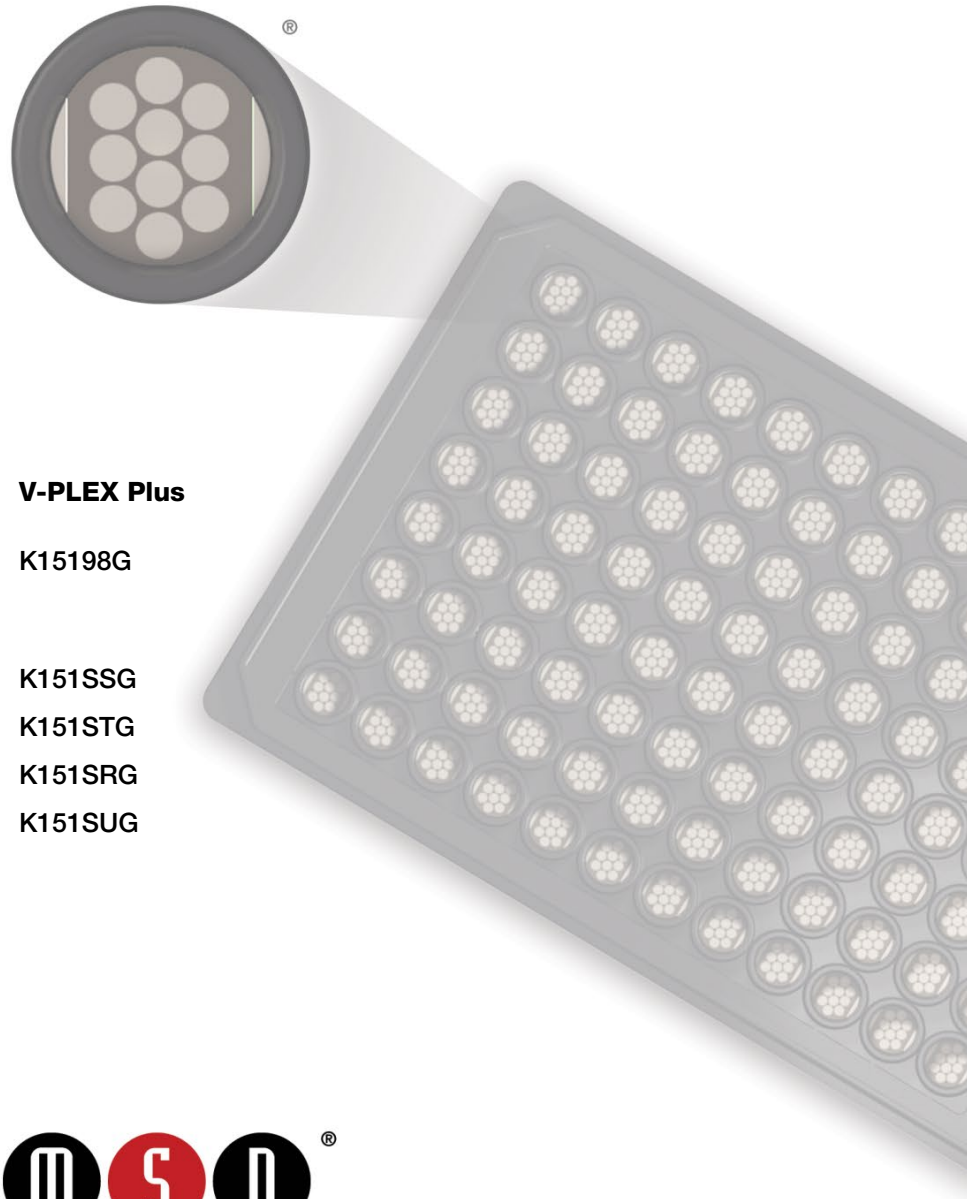
Vascular Injury Panel 2 (human) Kits

SAA, CRP, VCAM-1, ICAM-1

V-PLEX®



	V-PLEX®	V-PLEX Plus
Multiplex Kits	K15198D	K15198G
Individual Assay Kits		
Human SAA	K151SSD	K151SSG
Human CRP	K151STD	K151STG
Human VCAM-1	K151SRD	K151SRG
Human ICAM-1	K151SUD	K151SUG

MSD Biomarker Assays

Vascular Injury Panel 2 (human) Kits

SAA, CRP, VCAM-1, ICAM-1

For use with serum, plasma, cell culture supernatant, urine, and cerebral spinal fluid.

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Introduction

MSD offers V-PLEX assays for customers who require unsurpassed performance and quality. V-PLEX products are developed under rigorous design control and are fully validated according to fit-for-purpose principles[1] following MSD's Quality Management System. They offer exceptional sensitivity, simple protocols, reproducible results, and lot-to-lot consistency. In addition to the analytical validation, robustness of the assay protocol is assessed during development along with the stability and robustness of the assay components and kits. V-PLEX assays are available in both single-assay and multiplex formats.

The V-PLEX assay menu is organized by panels. Grouping the assays into panels by species, analytical compatibility, clinical range, and expected use ensures optimal and consistent performance from each assay while still providing the benefits and efficiencies of multiplexing. V-PLEX panels are provided in MSD's MULTI-SPOT® 96-well plate format. The composition of each panel and the location of each assay (i.e., its spot within the well) are maintained from lot to lot. Multiplex assays and individual assays for human SAA, human VCAM-1, and human ICAM-1 are provided on the Vascular Injury Panel 2, 4-spot 96-well plate; the human CRP individual assay is provided on spot 1 of an MSD 4-spot, 96-well plate.

The Vascular Injury Panel 2 (human) measures four biomarkers that are important in acute inflammation and tissue damage as well as numerous other biological processes. These assays can detect secreted biomarkers in a variety of tissues and body fluids where over- or under-expression may indicate a shift in biological equilibrium. The Vascular Injury Panel 2 (human) measures biomarkers that are implicated in a number of disorders, including atherosclerosis, rheumatoid arthritis, Alzheimer's disease, cancer, cardiovascular disease, type 2 diabetes, and stroke.[2-9] As a result of their association with such a wide spectrum of diseases, these biomarkers are the subjects of drug discovery projects, diagnostics development, and basic research. The biomarkers constituting the Vascular Injury Panel 2 (human) kits are: **a)** serum amyloid A (SAA), **b)** c-reactive protein (CRP), **c)** vascular cell adhesion molecule-1 (VCAM-1/ CD106), and **d)** intercellular adhesion molecule-1 (ICAM-1/ CD54).

Principle of the Assay

MSD biomarker assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. The assays in the Vascular Injury Panel 2 (human) are sandwich immunoassays. MSD provides a plate pre-coated with capture antibodies on independent and well-defined spots as shown in the layout below. Multiplex assays and the individual SAA, VCAM-1, and ICAM-1 assays are provided on 4-spot plates (Figure 1); the individual CRP assay is provided on spot 1 of 4-spot plates (Figure 2). The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) 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. The user adds an MSD buffer that creates 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 (which is proportional to the amount of analyte present in the sample) and provides a quantitative measure of each analyte in the sample. V-PLEX assay kits have 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]

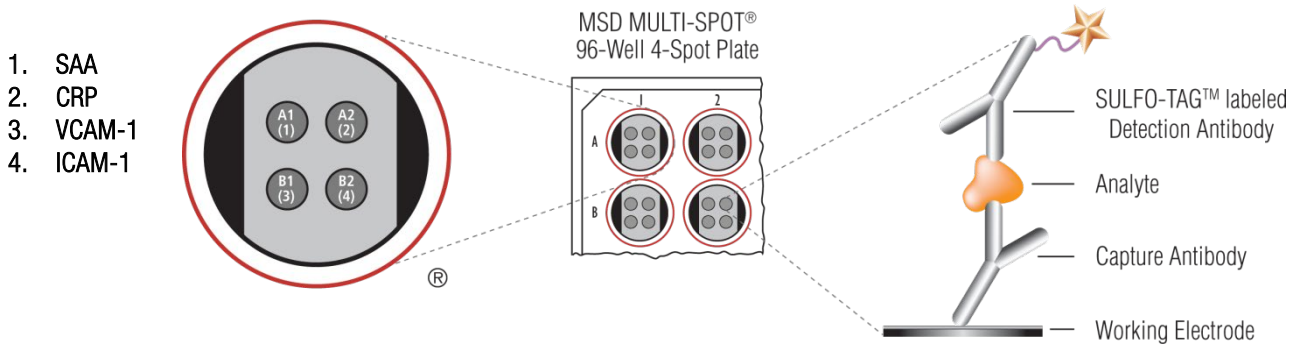

Figure 1. A 4-spot plate spot diagram showing placement of analyte capture antibodies for the Vascular Injury Panel 2 (human) multiplex assays and SAA, VCAM-1, and ICAM-1 individual assays. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

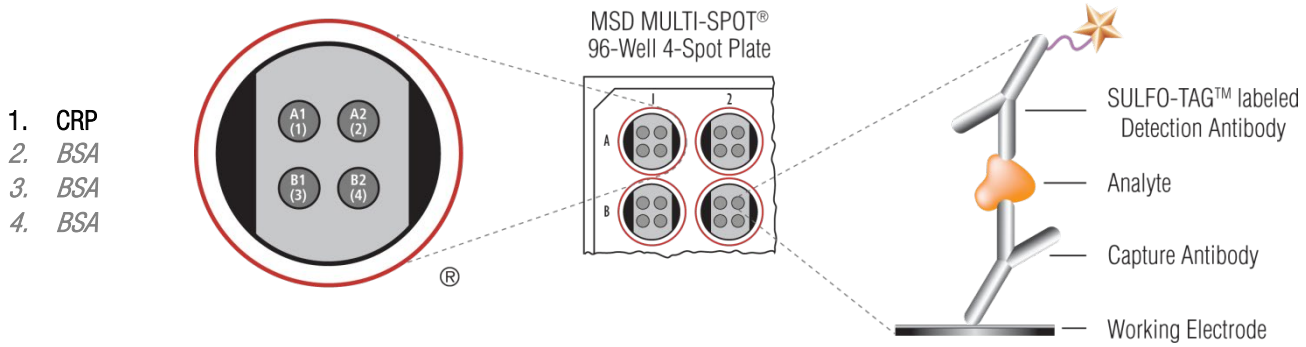

Figure 2. A 4-spot plate diagram showing placement of analyte capture antibody for the CRP individual assay.

Kit Components

Vascular Injury Panel 2 (human) assays are available as a 4-spot multiplex kit, as single assay kits, or as custom V-PLEX kits with subsets of assays selected from the full panel. All kits share common reagents except for the detection antibodies and the plate type. V-PLEX Plus kits include additional items (controls, wash buffer, and plate seals). See Tables 1 through 4 for details.

See **Catalog Numbers** section for complete kits.

Reagents Supplied With All Kits

Table 1. Reagents that are supplied with V-PLEX and V-PLEX Plus Kits

Reagents	Storage	Catalog No.	Size	Quantity Supplied			Description
				1-Plate Kit	5-Plate Kit	25-Plate Kit	
Vascular Injury Panel 2 (human) Calibrator Blend (20X)*	≤–70 °C	C0687-2	20 µL	1 vial	5 vials	25 vials	Four recombinant human proteins in diluent. Individual analyte concentration is provided in the lot-specific certificate of analysis (COA).
Diluent 100‡	2–8 °C	R50AA-4	50 mL	2 bottles	-	-	Diluent for initial-fold sample dilution.
		R50AA-2	200 mL	-	2 bottles	7 bottles	
Diluent 101	≤–10 °C	R51AD-2	30 mL	1 bottle	-	-	Diluent for samples, calibrator, and detection antibody; contains protein, blockers, and preservatives.
		R51AD-5	150 mL	-	1 bottle	5 bottles	
Read Buffer T (4X)	RT	R92TC-3	50 mL	1 bottle	1 bottle	5 bottles	Buffer to catalyze the electro-chemiluminescence reaction.

*The calibrator blend containing native CRP protein (catalog number: C0198-2) was discontinued and replaced by a calibrator blend containing recombinant CRP protein (catalog number: C0687-2).
‡Diluent 100 is now included with the kit. Diluent 100, along with Diluent 101 is recommended for sample dilution (see "Dilute Samples" section).

V-PLEX Plus Kits: Additional Components

Table 2. Additional components that are supplied with V-PLEX Plus Kits

Reagents	Storage	Catalog No.	Size	Quantity Supplied			Description
				1-Plate Kit	5-Plate Kit	25-Plate Kit	
Vascular Injury Control 1*	≤-70 °C	C4687-1	1 vial	1 vial	5 vials	25 vials	Multi-analyte controls in diluent.** The concentration of the controls is provided in the lot-specific COA.
Vascular Injury Control 2*	≤-70 °C		1 vial	1 vial	5 vials	25 vials	
Vascular Injury Control 3*	≤-70 °C		1 vial	1 vial	5 vials	25 vials	
Wash Buffer (20X)	RT	R61AA-1	100 mL	1 bottle	1 bottle	5 bottles	20-fold concentrated phosphate buffered solution with surfactant.
Plate Seals	-	-	-	3	15	75	Adhesive seals for sealing plates during incubations.

*Provided as components in the Vascular Injury Control Pack 1.
**The control pack containing native CRP protein (catalog number: C4198-1) was discontinued and replaced by a control pack containing recombinant CRP protein (catalog number: C4687-1).

Kit-Specific Components

Table 3. Components that are supplied with specific kits

Plates	Storage	Catalog No.	Size	Quantity Supplied			Description
				1-Plate Kit	5-Plate Kit	25-Plate Kit	
Vascular Injury Panel 2 (human) Plate	2–8 °C	N45198B-1	4-spot	1	5	25	96-well plate, foil sealed, with desiccant.
Human SAA Plate	2–8 °C	N45198B-1	4-spot	1	5	25	
Human CRP Plate	2–8 °C	N451STB-1	4-spot	1	5	25	
Human VCAM-1 Plate	2–8 °C	N45198B-1	4-spot	1	5	25	
Human ICAM-1 Plate	2–8 °C	N45198B-1	4-spot	1	5	25	

Table 4. Kits are supplied with individual detection antibodies for each assay ordered

SULFO-TAG Detection Antibody	Storage	Catalog No.	Size	Quantity Supplied			Description
				1-Plate Kit	5-Plate Kit	25-Plate Kit	
Anti-hu SAA Antibody (50X)	2–8 °C	D21SS-2	75 µL	1	-	-	SULFO-TAG conjugated antibody
		D21SS-3	375 µL	-	1	5	
Anti-hu CRP Antibody (50X)	2–8 °C	D21ST-2	75 µL	1	-	-	SULFO-TAG conjugated antibody
		D21ST-3	375 µL	-	1	5	
Anti-hu VCAM-1 Antibody (50X)	2–8 °C	D21SR-2	75 µL	1	-	-	SULFO-TAG conjugated antibody
		D21SR-3	375 µL	-	1	5	
Anti-hu ICAM-1 Antibody (50X)	2–8 °C	D21SU-2	75 µL	1	-	-	SULFO-TAG conjugated antibody
		D21SU-3	375 µL	-	1	5	

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene microcentrifuge tubes for preparing dilutions
- ❑ Liquid-handling equipment for 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
- ❑ Microtiter plate shaker (rotary) capable of shaking at 500–1,000 rpm.
- ❑ Phosphate-buffered saline (PBS) plus 0.05% Tween-20 for plate washing or MSD Wash Buffer, catalog # R61AA-1 (included in V-PLEX Plus kit)
- ❑ Adhesive plate seals (3 per plate included in V-PLEX Plus kits)
- ❑ Deionized water
- ❑ Vortex mixer

Optional Materials and Equipment

- ❑ Vascular Injury Control Pack 1, available for separate purchase from MSD, catalog # C4687-1 (included in V-PLEX Plus kit)
- ❑ Centrifuge (for sample preparation)

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

- Do not mix or substitute reagents from different sources or different kit lots. Lot information is provided in the lot-specific COA.
- Assay incubation steps should be performed between 20 °C to 26 °C to achieve the most consistent signals between runs.
- Bring frozen diluent to room temperature in a 24 °C water bath. Thaw other reagents on wet ice and use them as directed immediately.
- Prepare calibrators, samples, and controls in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution, and vortex after each dilution before proceeding.
- Avoid prolonged exposure of 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 at all pipetting steps. Bubbles may lead to variable results; bubbles introduced when adding Read Buffer T 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.
- Washing plates with a high volume of wash buffer, 3 times with 300 µL/well, may provide improvement in assay precision for certain assays without impacting the analytical parameters, including limit of quantifications (LOQs), control recovery, and sample quantification.
- Gently tap the plate on a paper towel to remove residual fluid after washing.
- Read buffer should be at room temperature when added to the plate.
- Keep time intervals consistent between adding read buffer and reading the plate to improve inter-plate precision. Unless otherwise directed, read the plate as soon as possible after adding read buffer.
- Do not shake the plate after adding read buffer.
- If an incubation step needs to be extended, avoid letting the plate dry out by keeping the sample or detection antibody solution in the plate.
- Remove the plate seals before reading the plate.
- If assay results are above the top of the calibration curve, dilute the samples and repeat the assay.
- We do not recommend attempting to use a partial plate when running this panel.

Reagent Preparation

Bring diluents and buffers to room temperature.

Important: Upon the first thaw, separate Diluent 101 into aliquots appropriate for the size of your needs before refreezing.

Prepare Calibrator Dilutions

MSD supplies blended calibrator for the Vascular Injury Panel 2 (human) Kit at a 20-fold higher concentration than the recommended highest calibrator. Thaw the stock calibrator and keep on ice, then add to the assay diluent to make the calibrator solutions as described below. The material is intended for a one-time use; however, the calibrator blend can tolerate 3 freeze-thaw cycles.

To prepare 7 calibrator solutions plus a zero calibrator for up to 6 replicates (Figure 3):

1) Prepare the highest calibrator by adding 10 µL of stock calibrator to 190 µL of Diluent 101. Mix well by vortexing.
2) Prepare the next calibrator by transferring 40 µL of the highest calibrator to 160 µL of Diluent 101. Mix well by vortexing. Repeat 5-fold serial dilutions 5 additional times to generate 7 calibrators.
3) Use Diluent 101 as the zero calibrator.

For the lot-specific concentration of each calibrator in the blend, refer to the COA supplied with the kit. You can also find a copy of the COA at www.mesoscale.com.

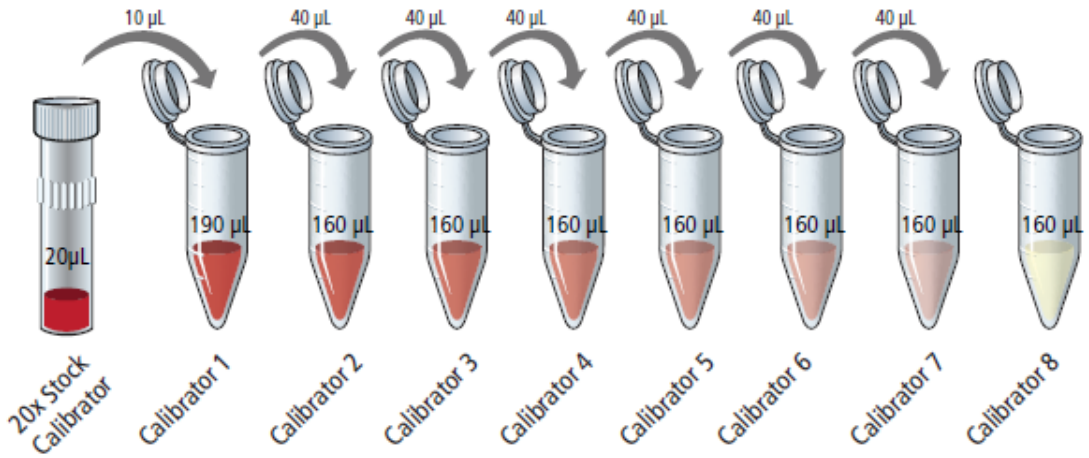

Figure 3. Dilution schema for preparation of Calibrator Standards.

Sample Collection and Handling

Below are general guidelines for human sample collection, storage, and handling. If possible, use published guidelines.[10-13] Evaluate sample stability under the selected method as needed.

- Serum and plasma. When preparing serum, allow samples to clot for 2 hours at room temperature, then centrifuge for 20 minutes at 2,000 × *g* before using or freezing. Centrifuge plasma for 20 minutes at 2000 × *g* before using or freezing. If no particulates are visible, you may not need to centrifuge.
- Other samples. Use immediately or freeze.

Freeze all samples in suitably sized aliquots; they may be stored at ≤–10 °C until needed. Repeated freezing and thawing of samples is not recommended. After thawing, centrifuge samples at 2,000 × *g* for 3 minutes to remove particulates before sample preparation.

Dilute Samples

For human serum and plasma samples, MSD recommends a 1000-fold dilution. Dilute serum and plasma samples with Diluent 100 and Diluent 101 as shown below. For cerebrospinal fluid (CSF) and urine samples, MSD recommends a 5-fold dilution. Depending on the sample set under investigation, higher or lower dilution factors may be necessary. Tissue culture supernatants may require additional dilution based on stimulation and analyte concentrations in the sample. MSD recommends dilution of samples that require higher dilutions in two or more dilution steps. Perform the initial dilution using Diluent 100 and subsequent dilutions using Diluent 101. Dilute CSF samples with Diluent 101. The kit includes sufficient diluent for running samples in duplicate. Additional diluent can be purchased at www.mesoscale.com.

Human serum and plasma samples should be prepared in two dilution steps as follows:

1) Add 10 µL of sample to 490 µL of Diluent 100 (50-fold dilution).
2) Add 10 µL of the 50-fold diluted sample into 190 µL of Diluent 101 (20-fold dilution).

Prepare Controls

Three levels of multi-analyte controls are available for separate purchase from MSD in the Vascular Injury Control Pack 1, catalog # C4687-1. (Controls are included in V-PLEX Plus Kits.) Vascular Injury Controls 1, 2, and 3 are prepared by spiking known levels of human SAA, CRP, VCAM-1, and ICAM-1 into diluent. The controls are supplied frozen.

Thaw controls on wet ice and use as provided; no further dilution is required. The material is intended for one-time use; however, the controls can tolerate three freeze-thaw cycles. Discard unused material after the third freeze-thaw cycle. Refer to the Vascular Injury Control Pack 1 COA for analyte levels. A copy of the COA is available at www.mesoscale.com.

Prepare Detection Antibody Solution

MSD provides each detection antibody separately as a 50X stock solution. The working solution is 1X. Prepare the detection antibody solution immediately before use.

Vascular Injury Panel 2 (human) kit

For one plate, combine the following detection antibodies and add to 2,760 µL of Diluent 101:

- ❑ 60 µL of 50X SULFO-TAG Anti-hu SAA Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-hu CRP Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-hu VCAM-1 Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-hu ICAM-1 Antibody

Custom multiplex kits

For one plate, combine 60 µL of each supplied detection antibody, then add Diluent 101 to bring the final volume to 3,000 µL.

Individual assay kits

For one plate, add 60 µL of the supplied 50X detection antibody to 2,940 µL of Diluent 101.

Prepare Wash Buffer

MSD provides 100 mL of Wash Buffer as a 20X stock solution in V-PLEX Plus Kits. Dilute the stock solution to 1X before use. 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 T

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 keep excess, diluted read buffer in a tightly sealed container at room temperature for up to one month.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (Figures 1 and 2) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Pre-wash plates before use as recommended in the assay protocol.

Protocol

1. **Wash and Addition of Sample:** Wash the plate 3 times with at least 150 µL/well of wash buffer. Add 25 µL of diluted sample, calibrator, or control per well. Seal the plate with an adhesive plate seal and incubate with shaking for 2 hours at room temperature.

2. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with at least 150 µL/well of wash buffer. Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 1 hour.

3. **Wash and Read:** Wash the plate 3 times with at least 150 µL/well of wash buffer. Add 150 µL of 1X Read Buffer T to each well. Read the plate on the MSD instrument. No incubation in read buffer is required before reading the plate.

Alternate Protocol

The suggestion below may be useful as an alternate protocol; however, it was not tested using multiple kit lots.

Reduced Wash: The wash step before sample addition can be omitted to reduce the total number of wash steps. See Appendix A for assay performance information using the reduced wash procedure.

Validation

MSD's V-PLEX products are validated following fit-for-purpose principles[1] and MSD design control procedures. V-PLEX assay components go through an extensive critical reagents program to ensure that the reagents are controlled and well characterized. Before the release of each V-PLEX panel, at least three independent kit lots are produced. Using results from multiple runs (typically greater than 50) and multiple operators, these lots are used to establish production specifications for sensitivity, specificity, accuracy, and precision. The COA provided with each kit outlines the kit release specifications for sensitivity, specificity, accuracy, and precision.

- ### Dynamic Range

  Calibration curve concentrations for each assay are optimized for a maximum dynamic range while maintaining enough calibration points near the bottom of the curve to ensure a proper fit for accurate quantification of samples that require high sensitivity.

- ### Sensitivity

  The lower limit of detection (LLOD) is a calculated concentration corresponding to the average signal 2.5 standard deviations above the background (zero calibrator). The LLOD is calculated using results from multiple plates for each lot, and the median and range of calculated LLODs for a representative kit lot are reported in the product insert. The upper limit of quantification (ULOQ) and lower limit of quantification (LLOQ) are established for each lot by measuring multiple levels near the expected LLOQ and ULOQ levels. The final LLOQ and ULOQ specifications for the product are established after assessment of all validation lots.

- ### Accuracy and Precision

  Accuracy and precision are evaluated by measuring calibrators and matrix-based validation samples or controls across multiple runs and multiple lots. For most assays, the results of control measurements fall within 20% of the expected concentration for each run. Precision is reported as the coefficient of variation (CV). Intra-run CVs are typically below 7%, and inter-run CVs are typically below 15%. Rigorous management of inter-lot reagent consistency and calibrator production results in typical inter-lot CVs of less than 10%. Validation lots are compared using controls and at least 40 samples in various sample matrices. Samples are well correlated with an inter-lot bias typically below 10%.

- ### Matrix Effects and Samples

  Matrix effects from serum, plasma, urine, and cell culture media are measured as part of development and validation. Dilution linearity and spike recovery studies are performed on individual samples rather than pooled samples to assess the variability of results due to matrix effects. The sample dilution suggested in the protocol gives an appropriate dilution factor for all assays in the multiplex. Some assays may benefit from lower or higher dilution factors depending on the samples and application (data is provided in the product insert). In addition to the matrices listed above, CSF samples were assayed, but spike recovery studies were not performed.

- **Specificity**

  The specificity of both capture and detection antibodies is measured during assay development. Antibody specificity is assessed by first running each assay using the multiplex plate with an assay-specific detection antibody and an assay-specific calibrator. These results are compared to the assay's performance when the plate is run with the multi-analyte calibrator and assay-specific detection antibodies as well as with assay-specific calibrator and all detection antibodies. For each validation lot and product release, assay specificity is measured using a multi-analyte calibrator and individual detection antibodies. The calibrator concentration used for specificity testing is chosen to ensure that the specific signal is greater than 50,000 counts.

- **Assay Robustness and Stability**

  The robustness of the assay protocol is assessed by examining the boundaries of the selected incubation times, evaluating the stability of assay components during the experiment, and evaluating the stability of reconstituted lyophilized components during storage. For example, the stability of the reconstituted calibrator is assessed in real time over 30 days. Assay component (calibrator, antibody, control) stability was assessed through freeze-thaw testing and accelerated stability studies. The validation program includes a real-time stability study with scheduled performance evaluations of complete kits for up to 54 months from the date of manufacture.

Representative data from the verification and validation studies are presented in the following sections. The calibration curve and measured limits of detection for each lot can be found in the lot-specific COA that is included with each kit and available for download at www.mesoscale.com.

Analysis of Results

The calibration curves used to calculate analyte concentrations were established by fitting the signals from the calibrators to a 4-parameter logistic (or sigmoidal dose-response) model with a $1/Y^2$ weighting. The weighting function provides a better fit of data over a wide dynamic range, particularly at the low end of the calibration curve. Analyte concentrations were determined from the electrochemiluminescence signals by back fitting to the calibration curve. These assays have a wide dynamic range (4 logs), which allows accurate quantification of samples without the need for multiple dilutions or repeated testing. The calculations to establish calibration curves and determine concentrations were carried out using the MSD DISCOVERY WORKBENCH® analysis software.

The best quantification of unknown samples will be achieved by generating a calibration curve for each plate using a minimum of two replicates at each calibrator level.

Typical Data

Data from the Vascular Injury Panel 2 (human) were collected over four months of testing by four operators (34 runs in total). Calibration curve accuracy and precision were assessed for three kit lots. Representative data from one kit lot are presented below. Data from individual assays are presented in Appendix B. The calibration curves were comparable (Figure 4). Calibration curves for each lot are presented in the lot-specific COA.

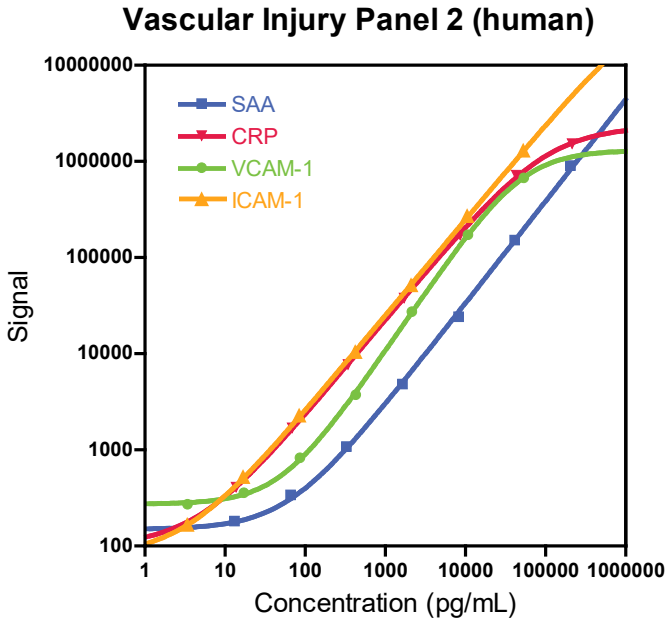

Figure 4. Typical calibration curves for the Vascular Injury Panel 2 (human) assay.

Sensitivity

The LLOD is a calculated concentration corresponding to the signal 2.5 standard deviations above the background (zero calibrator). The LLOD values shown below were calculated based on at least 71 runs across three kit lots.

The ULOQ is the highest concentration at which the %CV of the calculated concentration is <20% and the percent recovery of the calibrator is within 80–120% of the known value.

The LLOQ is the lowest concentration at which the %CV of the calculated concentration is <20% and the percent recovery of the calibrator is within 80–120% of the known value.

The quantitative range of the assay lies between the LLOQ and ULOQ (Table 5).

The LLOQ and ULOQ are verified for each kit lot and the results are provided in the lot-specific COA that is included with each kit and available at www.mesoscale.com.

Table 5. LLOD, LLOQ, and ULOQ for each analyte in the Vascular Injury Panel 2 (human) Kit

	Median LLOD (pg/mL)	LLOD Range (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
SAA	10.9	1.07–35.5	54.0	138,000
CRP	1.33	0.69–19.8	27.6	49,600
VCAM-1	6.00	0.93–35.8	37.6	32,000
ICAM-1	1.94	1.05-4.57	15.0	32,700

Precision

Controls were made by spiking calibrator into diluent at three levels within the quantitative range of the assay. Analyte levels were measured by four operators using a minimum of two replicates on 27 runs over four months. The results are shown below (Table 6). While the typical specification for precision is a concentration CV of less than 20% for controls on both intra- and inter-day runs, for this panel, the data shows most assays are below 15%.

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 27 runs of one kit lot.

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

Table 6. Intra-run and inter-run %CVs in the Vascular Injury Panel 2 (human) Kit

	Control	Average Conc. (pg/mL)	Average Intra-run %CV	Inter-run %CV	Inter-lot %CV
SAA	Control 1	42,586	4.7	9.6	10.6
	Control 2	4,211	3.6	14.6	8.3
	Control 3	494	4.6	15.6	6.8
CRP	Control 1	22,730	4.1	6.7	7.1
	Control 2	5,345	2.2	7.2	7.1
	Control 3	641	2.3	9.9	10.5
VCAM-1	Control 1	11,119	3.5	5.2	2.4
	Control 2	1,208	2.2	5.8	2.5
	Control 3	152	3.7	8.4	2.6
ICAM-1	Control 1	12,341	5.3	9.1	12.6
	Control 2	1,377	3.5	11.6	14.9
	Control 3	145	3.3	13.4	17.8

Dilution Linearity

To assess linearity, normal human serum, EDTA plasma, heparin plasma, citrate plasma, urine, cerebrospinal fluid, and cell culture supernatant were obtained from a commercial source. Percent recovery at each dilution was calculated by dividing the dilution-adjusted concentration by the expected concentration, i.e., the dilution-adjusted concentration at 1000-fold dilution for serum and plasma, 5-fold dilution for urine and cerebrospinal fluid, and 10-fold dilution for cell culture supernatants. The average percent recovery shown below is based on samples within the quantitative range of the assay (Table 7).

$$\%\ Recovery = \frac{measured\ concentration}{expected\ concentration} \times 100$$

Table 7. Analyte percent recovery at various dilutions in serum, EDTA plasma, heparin plasma, citrate plasma, urine, and cell culture supernatant samples

		SAA		CRP*		VCAM-1		ICAM-1	
Sample Type	Fold Dilution	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Serum (N= 15)	500	117	86–168	100	97–102	97	92–102	98	88–108
	1,000	100	–	100	–	100	–	100	–
	2,000	83	59–100	98	96–100	106	97–111	102	83–116
	4,000	74	51–100	96	94–99	112	100–122	100	80–116
EDTA Plasma (N= 18)	500	119	89–174	98	97–100	93	84–103	95	89–112
	1,000	100	–	100	–	100	–	100	–
	2,000	88	66–110	98	98–98	110	97–122	102	83–113
	4,000	77	47–103	98	96–99	119	104–136	101	80–112
Citrate Plasma (N= 3)	500	109	86–126	103	101–104	99	98–100	99	97–103
	1,000	100	–	100	–	100	–	100	–
	2,000	88	79–99	99	98–100	108	106–111	108	99–116
	4,000	82	69–100	97	95–98	117	113–122	103	96–112
Heparin Plasma (N = 15)	500	119	103–136	103	101–105	92	87–97	96	90–108
	1,000	100	–	100	–	100	–	100	–
	2,000	85	70–106	101	100–102	110	106–113	102	94–114
	4,000	79	55–104	100	100–100	122	119–127	105	96–115
Urine (N = 12)	5	ND	–	100	–	100	–	100	–
	10	ND	–	103	99–106	95	82–118	105	95–125
	20	ND	–	106	105–107	92	75–122	101	92–123
	40	ND	–	105	104–105	89	68-115	98	88–118
CSF (N = 8)	5	100	–	100	–	100	–	100	–
	10	98	89–106	102	95–109	107	99–115	102	91–113
	50	ND	–	106	102–110	124	104–136	103	91–116
	100	ND	–	102	97–106	135	114–153	102	87–118
Cell Culture Supernatant (N = 5)	10	100	–	100	–	100	–	100	–
	30	114	99–143	94	92–96	117	98–147	116	100–138
	90	113	98–129	92	89–94	110	96–125	108	98–127
	270	114	107–123	87	85–89	111	102–122	104	98–110

ND = not detected

*CRP was tested using N=2 for serum, EDTA plasma, citrate plasma, heparin plasma, urine, CSF, and cell culture supernatants.

Spike Recovery

Spike and recovery measurements of different sample types across the quantitative range of the assays were evaluated. Multiple individual human sera, EDTA plasma, heparin plasma, citrate plasma, urine, and cell culture supernatant samples from a commercial source were spiked with calibrators at three levels (high, mid, and low) (Table 8). The average % recovery for each sample type is reported along with %CV and % recovery range.

$$\%\ Recovery = \frac{measured\ concentration}{expected\ concentration} \times 100$$

Table 8. Analyte percent recovery at various dilutions in serum, EDTA plasma, heparin plasma, citrate plasma, urine, and cell culture supernatant samples

	Serum (N=11)			EDTA Plasma (N=13)			Heparin Plasma (N=14)		
	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range
SAA	103	8.2	92–126	101	9.3	81–137	103	8.5	88–124
CRP*	100	13.0	82–114	97	11.3	79–108	92	15.7	77–116
VCAM-1	104	6.1	90–122	93	6.9	77–105	97	7.1	84–120
ICAM-1	108	5.2	97–120	105	5.3	92–117	107	6.9	92–128

	Citrate Plasma (N=2)			Urine (N=11)			Cell Culture Supernatants (N=5)		
	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range
SAA	103	7.7	92–114	101	13.0	72–139	92	24.0	60–121
CRP*	97	14.6	84–124	83	4.0	77–87	86	5.3	80–96
VCAM-1	99	5.2	94–107	100	5.8	87–112	91	31.3	54–120
ICAM-1	105	6.8	95–114	102	7.7	85–116	103	18.1	79–129

*CRP was tested using N=4 for serum, heparin plasma and urine, and N=3 for EDTA plasma, citrate plasma, and cell culture supernatants.

Specificity

To assess specificity, each assay in the panel was tested individually. All assays exhibit <1% non-specific binding.

$$\%\ Nonspecificity = \frac{nonspecific\ signal}{specific\ signal} \times 100$$

Interference

Since murine monoclonal antibodies are used in the assays, different lots of human anti-mouse antibody (HAMA) positive and rheumatoid factor (RF) positive samples were tested with the Vascular Injury Panel 2 (human) kit. To assess HAMA interference, titrated concentrations of unrelated mouse antibodies were added to known HAMA-positive samples. To assess RF interference, known RF-positive and normal serum samples were blended in varying ratios. No cross-reactivity or interference from HAMA or RF samples was detected.

Stability

Kit components (calibrator, controls, and diluents) were tested for freeze-thaw stability. Results (not shown) demonstrated that kit components can tolerate three freeze-thaw cycles without significantly affecting the performance of the assay.

Calibration

All the assays in the panel are calibrated against a reference calibrator generated at MSD.

A National Institute for Biological Standards and Control (NIBSC) reference standard for CRP was tested against the MSD reference Vascular Injury Panel 2 (human) Calibrator Blend. The conversion ratio shown below was determined based on experiments run over four days (Table 9).

Table 9. Conversion Ratio of MSD Calibrators relative to NIBSC International Units

	NIBSC/WHO Standard*			
Assay	NIBSC code	Units/ampoule	Concentration/ ampoule	Conversion Ratio MSD Reference: NIBSC
CRP	85/506	0.049 IU	100 µg/mL	1 µg/mL of MSD = 0.758 mIU of NIBSC

*NIBSC standard is reconstituted and aliquoted per the World Health Organization (WHO) specification sheet.

Tested Samples

Normal Samples

Normal human serum, EDTA plasma, heparin plasma, citrate plasma, urine, and cerebrospinal fluid samples from a commercial source were diluted and tested at the recommended dilution. Results for each sample set follow (Table 10). Concentrations are corrected for sample dilution (Table 10).

Table 10. Normal human samples tested in the Vascular Injury Panel 2 (human) Kit

Sample Type	Statistic	SAA	CRP*	VCAM-1	ICAM-1
Serum (N = 42)	Median (ng/mL)	647	5,395	562	355
	Range (ng/mL)	77–190,349	617–16,394	97–904	120–617
	% Detected	100	100	100	100
EDTA Plasma (N = 38)	Median (ng/mL)	725	363	491	343
	Range (ng/mL)	132–35,439	120–16,960	167–688	214–756
	% Detected	100	100	100	100
Heparin Plasma (N = 36)	Median (ng/mL)	932	2,596	499	368
	Range (ng/mL)	ND*–18,561	375–11,118	225–789	260–803
	% Detected	94	100	100	100
Citrate Plasma (N = 3)	Median (ng/mL)	757	1,689	240	383
	Range (ng/mL)	441–1,660	382–5,774	214–1,831	371–412
	% Detected	100	100	100	100
Urine (N = 30)	Median (ng/mL)	6.19	1.00	9.99	7.56
	Range (ng/mL)	ND–7.50	ND–3.0	ND–5,927	0.35–1,740
	% Detected	7	62	93	100
CSF (N = 13)	Median (ng/mL)	2.52	1.0	11.1	3.59
	Range (ng/mL)	ND–167	ND–75	5.14–79.5	1.69–46.4
	% Detected	85	60	100	100

Median = median of detectable samples
% detected =% of samples with concentrations at or above the LLOD
ND = non-detectable
*CRP was tested using N=8 for serum, EDTA plasma, heparin plasma, citrate plasma and urine, and N=5 for CSF.

Assay Components

Calibrators

The assay calibrator blend uses the following source of human proteins (Table 11).

Table 11. Recombinant human proteins used in the Calibrators

Calibrator	Source
SAA	In vitro wheat germ expression system
CRP	Mammalian cell expression system
VCAM-1	Chinese hamster ovary cells expression system
ICAM-1	Mouse myeloma cells expression system

Antibodies

The source species for the antibodies are shown in the table below (Table 12).

Table 12. Antibody source species

	Source Species		
Analyte	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
SAA	Mouse Monoclonal	Mouse Monoclonal	A
CRP	Mouse Monoclonal	Mouse Monoclonal	A
VCAM-1	Mouse Monoclonal	Sheep Polyclonal	B
ICAM-1	Mouse Monoclonal	Sheep Polyclonal	A

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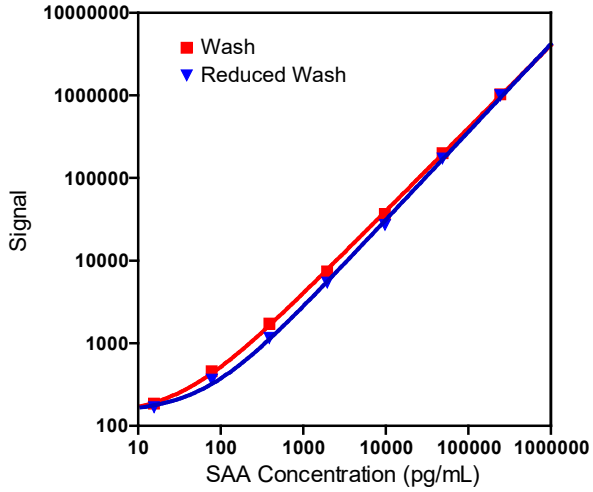
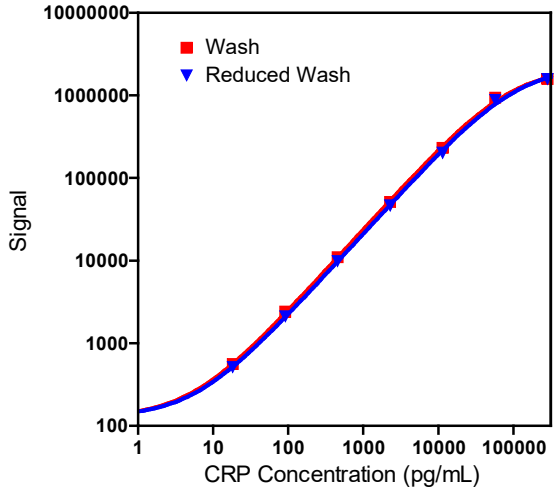
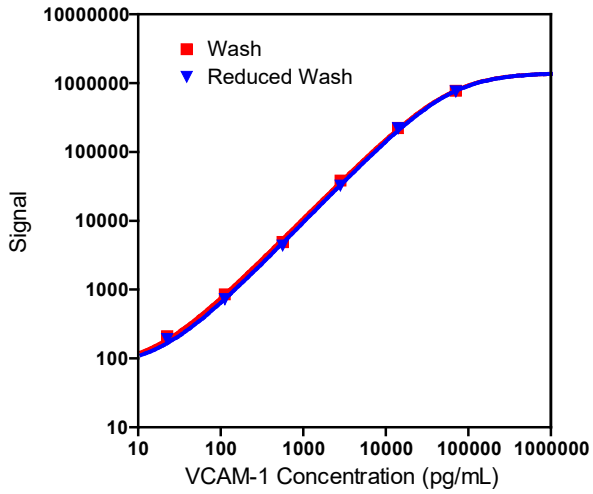
Appendix A

The calibration curves below illustrate the relative sensitivity of each assay under the **Alternate Protocol** (Reduced Wash; curves in blue) compared to the normal protocol (Wash; curves in red).







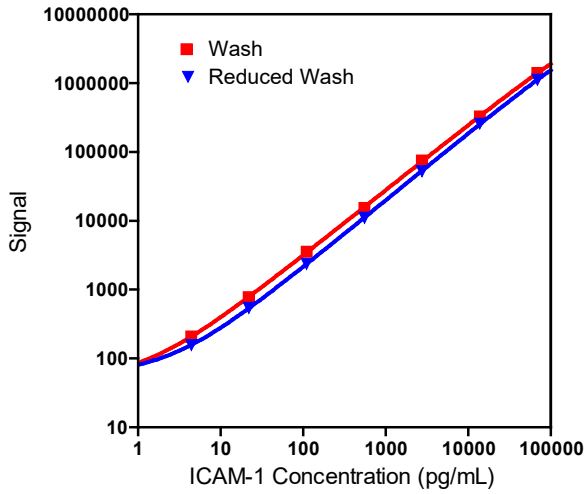
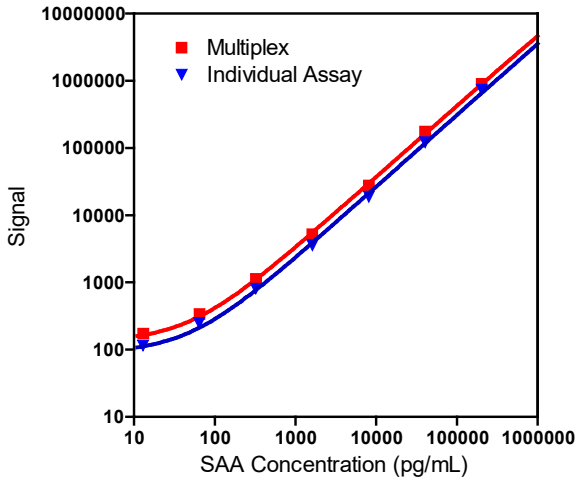


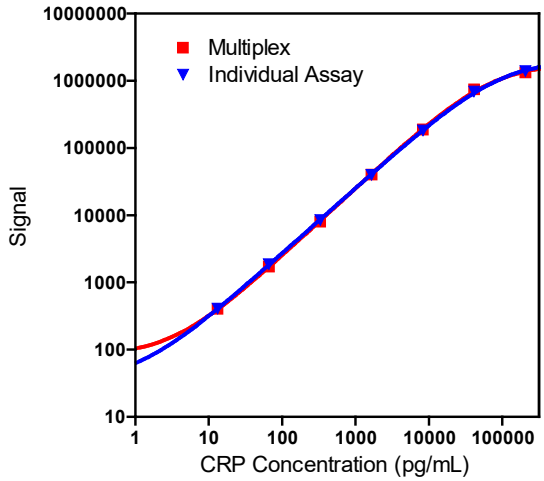
Table 13. Relative sensitivity when using alternate protocol

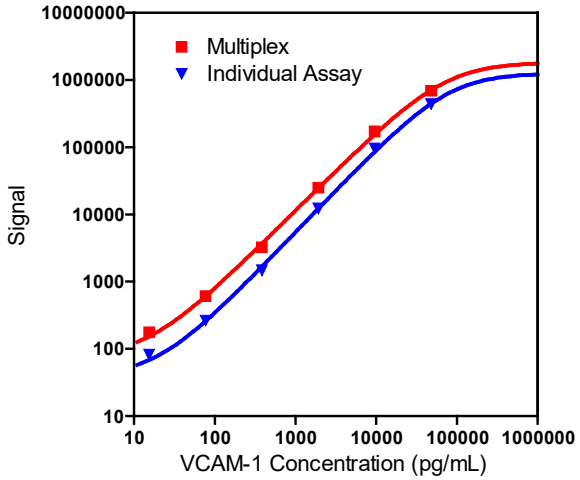
	LLOD (pg/mL)	
Assay	Wash	Reduced Wash
SAA	6.16	12.2
CRP	0.93	1.04
VCAM-1	5.50	6.74
ICAM-1	0.64	1.05

Appendix B

The calibration curves below compare assay performance when the assay is run as an individual assay (graphs in blue) vs. a multiplex (graphs in red).







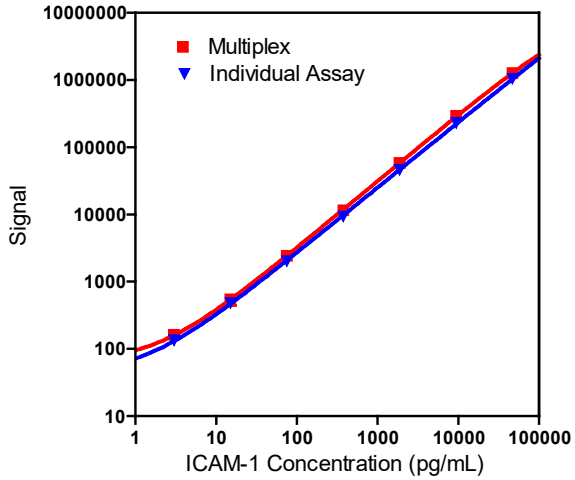


Table 14. Assay performance for multiplex and individual assays

	LLOD (pg/mL)	
Assay	Multiplex	Individual Assay
SAA	11.2	15.0
CRP	1.25	0.83
VCAM-1	6.13	12.8
ICAM-1	0.93	0.81

Summary Protocol

Vascular Injury Panel 2 (human) Kits

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol before performing the Vascular Injury Panel 2 (human) assays.

Sample and Reagent Preparation

- ❑ Bring all reagents to room temperature and thaw the calibrator on ice.
- ❑ Prepare calibration solutions in Diluent 101 using the supplied calibrator:
  - Dilute the stock calibrator 20-fold in Diluent 101.
  - Perform a series of 5-fold dilution steps and prepare a zero calibrator.
- ❑ Dilute serum and plasma samples 1000-fold, first 50X using Diluent 100, and then 20X using Diluent 101 before adding them to the plate.
- ❑ Controls do not require dilution.
- ❑ Prepare combined detection antibody solution by diluting each 50X detection antibody 50-fold in Diluent 101.
- ❑ Prepare 1X Read Buffer T by diluting 4X Read Buffer T 4-fold with deionized water.

STEP 1: Wash and Add Sample

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer.
- ❑ Add 25 µL/well of sample (calibrators, controls, or unknowns).
- ❑ Incubate at room temperature with shaking for 2 hours.

STEP 2: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer.
- ❑ Add 25 µL/well of 1X detection antibody solution.
- ❑ Incubate at room temperature with shaking for 1 hour.

STEP 3: Wash and Read Plate

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer.
- ❑ Add 150 µL/well of 1X Read Buffer T.
- ❑ Analyze the plate on the MSD instrument.

Catalog Numbers

Table 15. Catalog numbers for the V-PLEX and V-PLEX Plus Vascular Injury Panel 2 (human) multiplex and single assay kits*

Kit Name	V-PLEX			V-PLEX Plus*		
	1-Plate Kit	5-Plate Kit	25-Plate Kit	1-Plate Kit	5-Plate Kit	25-Plate Kit
Multiplex Kits						
Vascular Injury Panel 2 (human)	K15198D-1	K15198D-2	K15198D-4	K15198G-1	K15198G-2	K15198G-4
Single Assay Kits						
Human SAA	K151SSD-1	K151SSD-2	K151SSD-4	K151SSG-1	K151SSG-2	K151SSG-4
Human CRP	K151STD-1	K151STD-2	K151STD-4	K151STG-1	K151STG-2	K151STG-4
Human VCAM-1	K151SRD-1	K151SRD-2	K151SRD-4	K151SRG-1	K151SRG-2	K151SRG-4
Human ICAM-1	K151SUD-1	K151SUD-2	K151SUD-4	K151SUG-1	K151SUG-2	K151SUG-4

*V-PLEX Plus kits include controls, plate seals, and wash buffer. See **Kit Components** for details.

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL-01		Sample-01		Sample-09		Sample-17		Sample-25		Sample-33	
B	CAL-02		Sample-02		Sample-10		Sample-18		Sample-26		Sample-34	
C	CAL-03		Sample-03		Sample-11		Sample-19		Sample-27		Sample-35	
D	CAL-04		Sample-04		Sample-12		Sample-20		Sample-28		Sample-36	
E	CAL-05		Sample-05		Sample-13		Sample-21		Sample-29		Sample-37	
F	CAL-06		Sample-06		Sample-14		Sample-22		Sample-30		Sample-38	
G	CAL-07		Sample-07		Sample-15		Sample-23		Sample-31		Sample-39	
H	CAL-08		Sample-08		Sample-16		Sample-24		Sample-32		Sample-40	

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL-01		Control 1.1		Sample-06		Sample-14		Sample-22		Sample-30	
B	CAL-02		Control 1.2		Sample-07		Sample-15		Sample-23		Sample-31	
C	CAL-03		Control 1.3		Sample-08		Sample-16		Sample-24		Sample-32	
D	CAL-04		Sample-01		Sample-09		Sample-17		Sample-25		Sample-33	
E	CAL-05		Sample-02		Sample-10		Sample-18		Sample-26		Sample-34	
F	CAL-06		Sample-03		Sample-11		Sample-19		Sample-27		Sample-35	
G	CAL-07		Sample-04		Sample-12		Sample-20		Sample-28		Sample-36	
H	CAL-08		Sample-05		Sample-13		Sample-21		Sample-29		Sample-37	

Figure 5. Sample plate layout that can be used for the assays. Each sample, calibrator, and control (Plus Kit) is measured in duplicate in side-by-side wells.

Plate Diagram

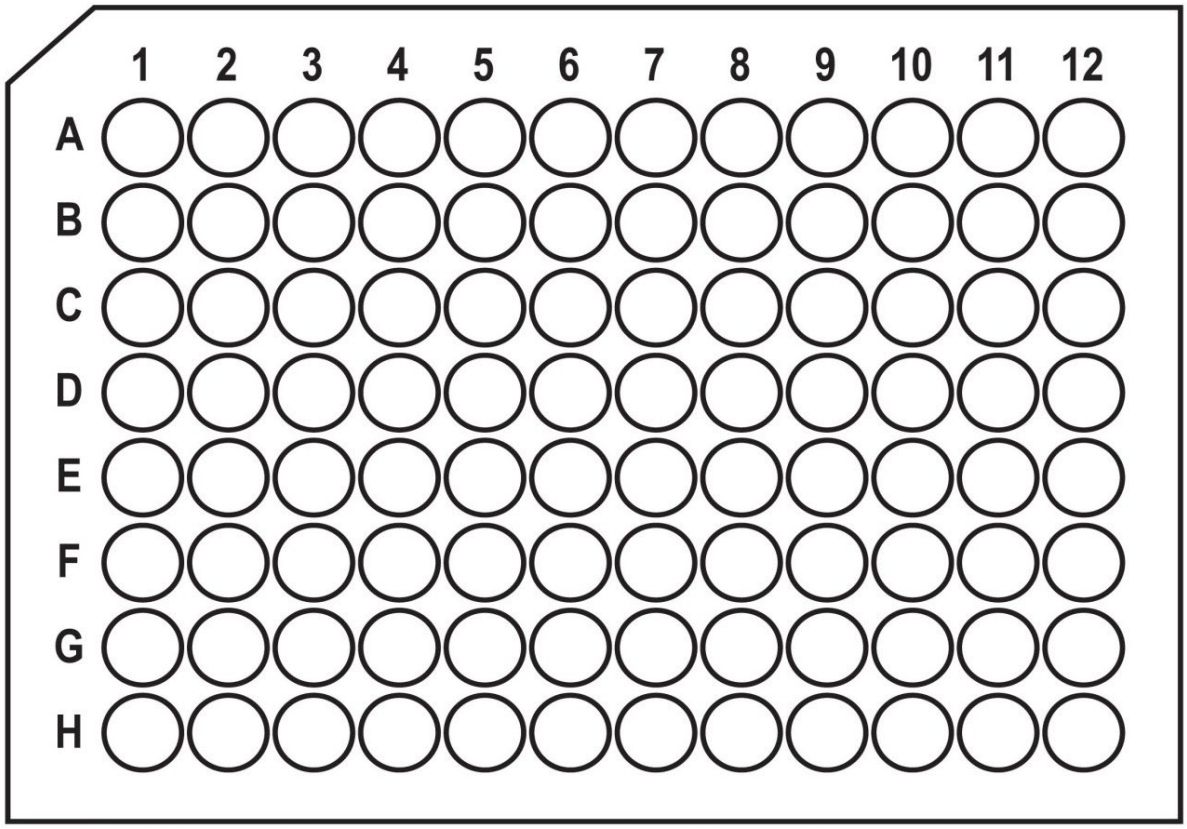


Figure 6. Plate diagram.