Vascular Injury Panel 2 (human) Kits

SAA, CRP, VCAM-1, ICAM-1

V-PLEX®

Multiplex Kits
- V-PLEX®: K15198D
- V-PLEX Plus: K15198G

Individual Assay Kits
- Human SAA: K151SSD, K151SSG
- Human CRP: K151STD, K151STG
- Human VCAM-1: K151SRD, K151SRG
- Human ICAM-1: K151SUD, K151SUG

www.mesoscale.com®
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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Contact Information

MSD Customer Service
Phone: 1-240-314-2795
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

MSD Scientific Support
Phone: 1-240-314-2798
Fax: 1-240-632-2219 attn: Scientific Support
Email: ScientificSupport@mesoscale.com
**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 in accordance with 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. As a result of their association with such a wide spectrum of disease, these biomarkers are the subjects of drug discovery projects, diagnostics development, and basic research. The biomarkers constituting the panel are described below.

**Serum Amyloid A (SAA)** proteins are multifunctional, acute-phase proteins produced by hepatocytes in response to pro-inflammatory cytokines. The human SAA protein is polymorphic, comprising several related proteins (SAA1 to SAA4). SAA has been linked to functions related to inflammation, pathogen defense, HDL metabolism, and cholesterol transport. It has been implicated in several pathological conditions including atherosclerosis, rheumatoid arthritis, Alzheimer’s disease, and cancer. SAA is secreted as a 12 kDa, non-glycosylated polypeptide, almost exclusively bound to high density lipoproteins (HDL) in plasma; it requires release from HDL complexes in order to become active. SAA circulates at trace levels (1 –5 µg/mL) under normal conditions, but levels can increase 1000-fold (500–1000 µg/mL) within 4 to 6 hours after inflammatory stimuli such as infection, tissue injury, or trauma.

**C-Reactive Protein (CRP)** is a 23 kDa, acute phase protein and a critical marker of acute inflammation. It is produced mainly by the liver in response to pro-inflammatory cytokines and lipopolysaccharides (LPS). Upon infection, inflammation, or tissue damage, the level of CRP in human serum can increase >1,000-fold within 6 to 48 hours and return to basal levels (<1 µg/mL) very quickly, making this protein a key marker for innate host immune responses. In contrast to humans, CRP in mice is expressed at a very low level and is not an acute-phase reactant. Human CRP shares 71% and 64% sequence homology with mouse and rat, respectively. CRP has been used as a predictive biomarker for several pathological conditions, including cardiovascular disease, type 2 diabetes, and stroke, and is therefore a potentially useful biomarker in research concerning these conditions. CRP binds phosphocholine in the membranes of injured cells as well as nuclear components of necrotic and apoptotic cells that initiate the C1q-complement cascade and immune cell phagocytotic responses for clearance of damaged and apoptotic cells.
Vascular Cell Adhesion Molecule-1 (VCAM-1/CD106) is a 100-110 kDa, transmembrane protein expressed by activated endothelial cells, neurons, smooth muscle cells, fibroblasts, and macrophages. VCAM-1 expression is induced by IL-1β, IL-4, TNF-α, and IFN-γ. It binds to leukocyte integrins VLA-4 and α4β7, mediating attachment of leukocytes to the vascular endothelium and extravasation of immune cells from vessels to sites of inflammation. This binding may promote monocyte adhesion and accumulations on the vessel wall at sites that are prone to developing atherosclerotic lesions. Soluble VCAM-1 (sVCAM-1) is also present in plasma and cerebrospinal fluid at variable levels which may lead to a variety of pathological conditions. Other roles proposed for VCAM-1 include the regulation of osteoclastogenesis via a cell-to-cell contact mechanism and the induction of sickle cell adherence to vascular endothelial cells during hypoxemia.

Intercellular Adhesion Molecule-1 (ICAM-1/CD54) is a nearly ubiquitous transmembrane glycoprotein that plays a key role in leukocyte migration and activation. ICAM-1 binds to leukocyte integrins LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18). At sites of inflammation, ICAM-1 is upregulated on endothelial and epithelial cells, where it mediates the adhesion and prolongs antigen presentation by dendritic cells and promotes T-cell proliferation and cytokine release. ICAM-1 activation also plays a role in angiogenesis, wound healing, and bone metabolism. Soluble forms of monomeric and dimeric ICAM-1 (sICAM-1) can be generated via proteolytic cleavage. Its presence has been reported in serum, cerebrospinal fluid, urine, and bronchoalveolar lavage fluid. Elevated levels of sICAM-1 in these fluids are associated with cardiovascular disease (as an indicator of vascular endothelial cell activation or damage), type 2 diabetes, organ transplant dysfunction, and certain malignancies.
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™) 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 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 CRP individual assays.
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 below 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

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Storage</th>
<th>Catalog #</th>
<th>Size</th>
<th>Quantity Supplied</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular Injury Panel 2 (human) Calibrator Blend (20X)*</td>
<td>≤-70 °C</td>
<td>C0198-2</td>
<td>20 µL</td>
<td>1 vial</td>
<td>Three recombinant human proteins and one protein from human pleural/ascitic fluid or plasma in diluent. Individual analyte concentration is provided in the lot-specific certificate of analysis (COA).</td>
</tr>
<tr>
<td>Diluent 100</td>
<td>2–8 °C</td>
<td>R50AA-4</td>
<td>50 mL</td>
<td>1 bottle</td>
<td>Diluent for initial-fold sample dilution</td>
</tr>
<tr>
<td>Diluent 101</td>
<td>≤-10 °C</td>
<td>R51AD-1</td>
<td>15 mL</td>
<td>1 bottle</td>
<td>Diluent for samples, calibrator, and detection antibody; contains protein, blockers, and preservatives.</td>
</tr>
<tr>
<td>Read Buffer T (4X)</td>
<td>RT</td>
<td>R92TC-3</td>
<td>50 mL</td>
<td>1 bottle</td>
<td>Buffer to catalyze the electro-chemiluminescence reaction.</td>
</tr>
</tbody>
</table>

*The CRP in this calibrator blend is derived from human source material which has been tested and found to be negative for HBsAg, HIV-1 and HIV-2 antibodies, and hepatitis C virus (HCV). This material should be handled and disposed of in accordance with local, state, and federal guidelines.

‡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

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Storage</th>
<th>Catalog #</th>
<th>Size</th>
<th>Quantity Supplied</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular Injury Control 1*</td>
<td>≤-70 °C</td>
<td>C4198-1</td>
<td>20 µL</td>
<td>1 vial</td>
<td>Multi-analyte controls in diluent ** The concentration of the controls is provided in the lot-specific COA.</td>
</tr>
<tr>
<td>Vascular Injury Control 2*</td>
<td>≤-70 °C</td>
<td>C4198-1</td>
<td>20 µL</td>
<td>1 vial</td>
<td>Multi-analyte controls in diluent ** The concentration of the controls is provided in the lot-specific COA.</td>
</tr>
<tr>
<td>Wash Buffer (20X)</td>
<td>RT</td>
<td>R61AA-1</td>
<td>100 mL</td>
<td>1 bottle</td>
<td>20-fold concentrated phosphate buffered solution with surfactant.</td>
</tr>
<tr>
<td>Plate Seals</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>Adhesive seals for sealing plates during incubations.</td>
</tr>
</tbody>
</table>

*Provided as components in the Vascular Injury Control Pack 1.

**The CRP in these controls is derived from human source material which has been tested and found to be negative for HBsAg, HIV-1 and HIV-2 antibodies, and HCV. This material should be handled and disposed of in accordance with local, state, and federal guidelines.
### Kit-Specific Components

**Table 3.** Components that are supplied with specific kits

<table>
<thead>
<tr>
<th>Plates</th>
<th>Storage</th>
<th>Part #</th>
<th>Size</th>
<th>Quantity Supplied</th>
<th>Quantity Supplied</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular Injury Panel 2 (human) Plate</td>
<td>2–8 °C</td>
<td>N45198B-1</td>
<td>4-spot</td>
<td>1</td>
<td>5</td>
<td>96-well plate, foil sealed, with desiccant.</td>
</tr>
<tr>
<td>Human SAA Plate</td>
<td>2–8 °C</td>
<td>N45198B-1</td>
<td>4-spot</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Human CRP Plate</td>
<td>2–8 °C</td>
<td>N451STB-1</td>
<td>4-spot</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Human VCAM-1 Plate</td>
<td>2–8 °C</td>
<td>N45198B-1</td>
<td>4-spot</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Human ICAM-1 Plate</td>
<td>2–8 °C</td>
<td>N45198B-1</td>
<td>4-spot</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>SULFO-TAG Detection Antibody</th>
<th>Storage</th>
<th>Catalog #</th>
<th>Size</th>
<th>Quantity Supplied</th>
<th>Quantity Supplied</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-hu SAA Antibody (50X)</td>
<td>2–8 °C</td>
<td>D21SS-2</td>
<td>75 µL</td>
<td>1</td>
<td></td>
<td>SULFO-TAG conjugated antibody</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D21SS-3</td>
<td>375 µL</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Anti-hu CRP Antibody (50X)</td>
<td>2–8 °C</td>
<td>D21ST-2</td>
<td>75 µL</td>
<td>1</td>
<td></td>
<td>SULFO-TAG conjugated antibody</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D21ST-3</td>
<td>375 µL</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Anti-hu VCAM-1 Antibody (50X)</td>
<td>2–8 °C</td>
<td>D21SR-2</td>
<td>75 µL</td>
<td>1</td>
<td></td>
<td>SULFO-TAG conjugated antibody</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D21SR-3</td>
<td>375 µL</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Anti-hu ICAM-1 Antibody (50X)</td>
<td>2–8 °C</td>
<td>D21SU-2</td>
<td>75 µL</td>
<td>1</td>
<td></td>
<td>SULFO-TAG conjugated antibody</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D21SU-3</td>
<td>375 µL</td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
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 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 # C4198-1 (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](http://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 20-26 °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 as directed without delay.
- Prepare calibrators, samples, and controls in polypropylene microcentrifuge tubes; use a fresh pipette tip for each dilution; 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 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.
- 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.
- No shaking is necessary after adding read buffer.
- If an incubation step needs to be extended, avoid letting the plate dry out by keeping sample or detection antibody solution in the plate.
- Remove the plate seals prior to reading the plate.
- If assay results are above the top of the calibration curve, dilute the samples and repeat the assay.
- Running a partial plate is not recommended with this panel.
Reagent Preparation

Bring diluents and buffers to room temperature.

Important: Upon 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 20-fold higher concentration than the recommended highest calibrator. Thaw the stock calibrator and keep on ice, then add to assay diluent to make the calibrator solutions as described below.

To prepare 7 calibrator solutions plus a zero calibrator for up to 6 replicates:

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.\textsuperscript{18-21} 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 2000g prior to using or freezing. Centrifuge plasma for 20 minutes at 2000g prior to 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 $\leq -10 \, ^\circ\mathrm{C}$ until needed. Repeated freezing and thawing of samples is not recommended. After thawing, centrifuge samples at 2000g for 3 minutes to remove particulates prior to 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 to dilute 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. Kit includes diluent sufficient enough for running samples in duplicates.

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 # C4198-1. (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 and are stable for up to three days at 2-8 °C. 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 prior to 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 Kit. 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 prior to sample addition can be omitted in order to reduce the total number of wash steps. See Appendix A for assay performance using this protocol.
Validation

MSD’s V-PLEX products are validated following fit-for-purpose principles1 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. Prior to 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 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 assay-specific detection antibody and 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 and with assay-specific calibrator and all detection antibodies. For each validation lot and for 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 and evaluating the stability of assay components during the experiment and the stability of reconstituted lyophilized components during storage. For example, the stability of reconstituted calibrator is assessed in real time over a 30-day period. 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 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 ECL 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.

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. 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](image-url)
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.

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

<table>
<thead>
<tr>
<th></th>
<th>Median LLOD (pg/mL)</th>
<th>LLOD Range (pg/mL)</th>
<th>LLOQ (pg/mL)</th>
<th>ULOQ (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAA</td>
<td>10.9</td>
<td>1.07–35.5</td>
<td>54.0</td>
<td>138,000</td>
</tr>
<tr>
<td>CRP</td>
<td>1.33</td>
<td>0.69–19.8</td>
<td>27.6</td>
<td>49,600</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>6.00</td>
<td>0.93–35.8</td>
<td>37.6</td>
<td>32,000</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>1.94</td>
<td>1.05–4.57</td>
<td>15.0</td>
<td>32,700</td>
</tr>
</tbody>
</table>
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. Results are shown below. 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 for cell culture supernatants. The average percent recovery shown below is based on samples within the quantitative range of the assay.

\[
\% \text{ Recovery} = \frac{\text{measured concentration}}{\text{expected concentration}} \times 100
\]

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

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Fold Dilution</th>
<th>SAA Average % Recovery</th>
<th>SAA % Recovery Range</th>
<th>CRP Average % Recovery</th>
<th>CRP % Recovery Range</th>
<th>VCAM-1 Average % Recovery</th>
<th>VCAM-1 % Recovery Range</th>
<th>ICAM-1 Average % Recovery</th>
<th>ICAM-1 % Recovery Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (n=15)</td>
<td>500</td>
<td>117</td>
<td>86–168</td>
<td>100</td>
<td>94–113</td>
<td>97</td>
<td>92–102</td>
<td>98</td>
<td>88–108</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>100</td>
<td>–</td>
<td>100</td>
<td>–</td>
<td>100</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>2,000</td>
<td>83</td>
<td>59–100</td>
<td>98</td>
<td>73–107</td>
<td>106</td>
<td>97–111</td>
<td>102</td>
<td>83–116</td>
</tr>
<tr>
<td></td>
<td>4,000</td>
<td>74</td>
<td>51–100</td>
<td>99</td>
<td>78–117</td>
<td>112</td>
<td>100–122</td>
<td>100</td>
<td>80–116</td>
</tr>
<tr>
<td>EDTA Plasma (n=18)</td>
<td>500</td>
<td>119</td>
<td>89–174</td>
<td>100</td>
<td>80–125</td>
<td>93</td>
<td>84–103</td>
<td>95</td>
<td>89–112</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>100</td>
<td>–</td>
<td>100</td>
<td>–</td>
<td>100</td>
<td>–</td>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>2,000</td>
<td>88</td>
<td>66–110</td>
<td>99</td>
<td>73–119</td>
<td>110</td>
<td>97–122</td>
<td>102</td>
<td>83–113</td>
</tr>
<tr>
<td></td>
<td>4,000</td>
<td>77</td>
<td>47–103</td>
<td>100</td>
<td>78–119</td>
<td>119</td>
<td>104–136</td>
<td>101</td>
<td>80–112</td>
</tr>
<tr>
<td>Citrate Plasma (n=3)</td>
<td>500</td>
<td>109</td>
<td>86–126</td>
<td>104</td>
<td>97–110</td>
<td>99</td>
<td>98–100</td>
<td>99</td>
<td>97–103</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>100</td>
<td>–</td>
<td>100</td>
<td>–</td>
<td>100</td>
<td>–</td>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>2,000</td>
<td>88</td>
<td>79–99</td>
<td>100</td>
<td>92–107</td>
<td>108</td>
<td>106–111</td>
<td>108</td>
<td>99–116</td>
</tr>
<tr>
<td></td>
<td>4,000</td>
<td>82</td>
<td>69–100</td>
<td>103</td>
<td>90–117</td>
<td>117</td>
<td>113–122</td>
<td>103</td>
<td>96–112</td>
</tr>
<tr>
<td>Heparin Plasma (n=15)</td>
<td>500</td>
<td>119</td>
<td>103–136</td>
<td>98</td>
<td>94–102</td>
<td>92</td>
<td>87–97</td>
<td>96</td>
<td>90–108</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>100</td>
<td>–</td>
<td>100</td>
<td>–</td>
<td>100</td>
<td>–</td>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>2,000</td>
<td>85</td>
<td>70–106</td>
<td>100</td>
<td>93–105</td>
<td>110</td>
<td>106–113</td>
<td>102</td>
<td>94–114</td>
</tr>
<tr>
<td></td>
<td>4,000</td>
<td>79</td>
<td>55–104</td>
<td>103</td>
<td>98–109</td>
<td>122</td>
<td>119–127</td>
<td>105</td>
<td>96–115</td>
</tr>
<tr>
<td>Urine (n=12)</td>
<td>5</td>
<td>ND</td>
<td>–</td>
<td>100</td>
<td>–</td>
<td>100</td>
<td>–</td>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>ND</td>
<td>–</td>
<td>106</td>
<td>86–116</td>
<td>95</td>
<td>82–118</td>
<td>105</td>
<td>95–125</td>
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<td></td>
<td>40</td>
<td>ND</td>
<td>–</td>
<td>102</td>
<td>89–120</td>
<td>89</td>
<td>68–115</td>
<td>98</td>
<td>88–118</td>
</tr>
<tr>
<td>CSF (n=8)</td>
<td>5</td>
<td>100</td>
<td>–</td>
<td>100</td>
<td>–</td>
<td>100</td>
<td>–</td>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>98</td>
<td>89–106</td>
<td>106</td>
<td>99–118</td>
<td>107</td>
<td>99–115</td>
<td>102</td>
<td>91–113</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>ND</td>
<td>–</td>
<td>107</td>
<td>96–115</td>
<td>124</td>
<td>104–136</td>
<td>103</td>
<td>91–116</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>ND</td>
<td>–</td>
<td>110</td>
<td>98–116</td>
<td>135</td>
<td>114–153</td>
<td>102</td>
<td>87–118</td>
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<tr>
<td>Cell Culture supernatant (n=5)</td>
<td>10</td>
<td>100</td>
<td>–</td>
<td>100</td>
<td>–</td>
<td>100</td>
<td>–</td>
<td>100</td>
<td>–</td>
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<tr>
<td></td>
<td>30</td>
<td>114</td>
<td>99–143</td>
<td>110</td>
<td>98–130</td>
<td>117</td>
<td>98–147</td>
<td>116</td>
<td>100–138</td>
</tr>
</tbody>
</table>

ND=Not detected
Spike Recovery

Spike and recovery measurements of different sample types across the quantitative range of the assays were evaluated. Multiple individual human serum, 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). The average % recovery for each sample type is reported along with %CV and % recovery range.

\[
\text{% Recovery} = \frac{\text{measured concentration}}{\text{expected concentration}} \times 100
\]

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

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Serum (N=11)</th>
<th>EDTA Plasma (N=13)</th>
<th>Heparin Plasma (N=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average % Recovery</td>
<td>%CV</td>
<td>% Recovery Range</td>
</tr>
<tr>
<td>SAA</td>
<td>103</td>
<td>8.2</td>
<td>92–126</td>
</tr>
<tr>
<td>CRP</td>
<td>105</td>
<td>8.1</td>
<td>93–126</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>104</td>
<td>6.1</td>
<td>90–122</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>108</td>
<td>5.2</td>
<td>97–120</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Citrate Plasma (N=2)</th>
<th>Urine (N=11)</th>
<th>Cell Culture Supernatants (N=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average % Recovery</td>
<td>%CV</td>
<td>% Recovery Range</td>
</tr>
<tr>
<td>SAA</td>
<td>103</td>
<td>7.7</td>
<td>92–114</td>
</tr>
<tr>
<td>CRP</td>
<td>108</td>
<td>9.7</td>
<td>96–127</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>99</td>
<td>5.2</td>
<td>94–107</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>105</td>
<td>6.8</td>
<td>95–114</td>
</tr>
</tbody>
</table>

Specificity

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

\[
\text{% Nonspecificity} = \frac{\text{nonspecific signal}}{\text{specific signal}} \times 100
\]

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, titrating 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. 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. Conversion Ratio of MSD Calibrators relative to NIBSC International Units

<table>
<thead>
<tr>
<th>Assay</th>
<th>NIBSC code</th>
<th>Units/ampoule</th>
<th>Concentration/ampoule</th>
<th>Conversion Ratio MSD Reference: NIBSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>85/506</td>
<td>0.049 IU</td>
<td>100 µg/mL</td>
<td>1 µg/mL of MSD = 964 IU of NIBSC</td>
</tr>
</tbody>
</table>

*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 factor. Results for each sample set follow. Concentrations are corrected for sample dilution.

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

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Statistic</th>
<th>SAA (Median ng/mL)</th>
<th>CRP (Median ng/mL)</th>
<th>VCAM-1 (Median ng/mL)</th>
<th>ICAM-1 (Median ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (N=42)</td>
<td>Median</td>
<td>647</td>
<td>1731</td>
<td>562</td>
<td>355</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>77–190,349</td>
<td>158–71,207</td>
<td>97–904</td>
<td>120–617</td>
</tr>
<tr>
<td></td>
<td>% Detected</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>EDTA Plasma (N=38)</td>
<td>Median</td>
<td>725</td>
<td>2122</td>
<td>491</td>
<td>343</td>
</tr>
<tr>
<td></td>
<td>% Detected</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Heparin Plasma (N=36)</td>
<td>Median</td>
<td>932</td>
<td>2343</td>
<td>499</td>
<td>368</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>ND–18,561</td>
<td>216–17,124</td>
<td>225–789</td>
<td>260–803</td>
</tr>
<tr>
<td></td>
<td>% Detected</td>
<td>94</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Citrate Plasma (N=3)</td>
<td>Median</td>
<td>757</td>
<td>509</td>
<td>240</td>
<td>383</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>441–1,660</td>
<td>337–556</td>
<td>214–1,831</td>
<td>371–412</td>
</tr>
<tr>
<td></td>
<td>% Detected</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Urine (N=30)</td>
<td>Median</td>
<td>6.19</td>
<td>1.42</td>
<td>9.99</td>
<td>7.56</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>ND–7.50</td>
<td>ND–1,993</td>
<td>ND–5,927</td>
<td>0.35–1,740</td>
</tr>
<tr>
<td></td>
<td>% Detected</td>
<td>7</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>CSF (N=13)</td>
<td>Median</td>
<td>2.52</td>
<td>18.8</td>
<td>11.1</td>
<td>3.59</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>ND–167</td>
<td>ND–1,037</td>
<td>5.14–79.5</td>
<td>1.69–46.4</td>
</tr>
<tr>
<td></td>
<td>% Detected</td>
<td>85</td>
<td>92</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Median = median of detectable samples
% Detected = % of samples with concentrations at or above the LLOD
ND = Non-detectable

Assay Components

Calibrators

The assay calibrator blend uses the following source of human proteins:

Table 11. Recombinant human proteins used in the Calibrators

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAA</td>
<td>In vitro wheat germ expression system</td>
</tr>
<tr>
<td>CRP</td>
<td>Human pleural/ascitic fluid or plasma</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Chinese hamster ovary cells expression system</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Mouse myeloma cells expression system</td>
</tr>
</tbody>
</table>
Table 12. Antibody source species

<table>
<thead>
<tr>
<th>Analyte</th>
<th>MSD Capture Antibody</th>
<th>MSD Detection Antibody</th>
<th>Assay Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAA</td>
<td>Mouse Monoclonal</td>
<td>Mouse Monoclonal</td>
<td>A</td>
</tr>
<tr>
<td>CRP</td>
<td>Mouse Monoclonal</td>
<td>Mouse Monoclonal</td>
<td>A</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Mouse Monoclonal</td>
<td>Sheep Polyclonal</td>
<td>B</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Mouse Monoclonal</td>
<td>Sheep Polyclonal</td>
<td>A</td>
</tr>
</tbody>
</table>

References

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

<table>
<thead>
<tr>
<th>Assay</th>
<th>Wash</th>
<th>Reduced Wash</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAA</td>
<td>6.16</td>
<td>12.2</td>
</tr>
<tr>
<td>CRP</td>
<td>0.93</td>
<td>1.04</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>5.50</td>
<td>6.74</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>0.64</td>
<td>1.05</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Assay</th>
<th>Multiplex</th>
<th>Individual Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAA</td>
<td>11.2</td>
<td>15.0</td>
</tr>
<tr>
<td>CRP</td>
<td>1.25</td>
<td>0.83</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>6.13</td>
<td>12.8</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>0.93</td>
<td>0.81</td>
</tr>
</tbody>
</table>
Summary Protocol

Vascular Injury Panel 2 (human) Kits

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to 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, 50X using Diluent 100 and 20X using Diluent 101 before adding 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

<table>
<thead>
<tr>
<th>Kit Name</th>
<th>V-PLEX</th>
<th>V-PLEX Plus*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-Plate Kit</td>
<td>5-Plate Kit</td>
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<tr>
<td>Multiplex Kits</td>
<td></td>
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</tr>
<tr>
<td>Single Assay Kits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human SAA</td>
<td>K151SSD-1</td>
<td>K151SSD-2</td>
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<tr>
<td>Human CRP</td>
<td>K151STD-1</td>
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<tr>
<td>Human VCAM-1</td>
<td>K151SRD-1</td>
<td>K151SRD-2</td>
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<tr>
<td>Human ICAM-1</td>
<td>K151SUD-1</td>
<td>K151SUD-2</td>
</tr>
</tbody>
</table>

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